Microxpress™

Microbiology Manual

Second Edition, 2010

USER FEEDBACK*

Accumix

Mueller Hinton Agar
The medium was very good and gave very clear results when compared to the standard medium.

Plate Count Agar
Quantitative enumeration was carried out using Accumix medium, which gave satisfactory results as compared with other reputed brand.

TCBS Agar
The Accumix medium appears to show better retrievability for Vibrio like organisms with respect to the other compared medium.

Sabouraud Dextrose Agar, Endo Agar, Tryptone Glucose Extract Agar
Test results are at par with the brand currently used.

Violet Red Bile Agar
Quantitative examination was carried out using the Accumix medium, which gave satisfactory results as compared to the other media used.

Nutrient Agar, Sabouraud Chloramphenicol Yeast Glucose Agar, Violet Red Bile Agar
With reference to your dehydrated culture media samples under the brand name Accumix, I would like to congratulate for developing dehydrated culture media. The performance of Accumix media is excellent compared to others.

Brilliant Green bile Broth 2%, Fluid Lactose Medium
The medium powder is very well dehydrated. Good growth and gas production within 24 hours were observed.

EMB Agar, Levine
The medium allowed excellent, luxuriant growth of coliforms with characteristic metallic sheen within 24 hours.

Nutrient Agar
The growth of coliforms on the plates was luxuriant.

MacConkey Agar
Total coliform counts are at par with the standard brand used.

Nutrient Agar with 1% Peptone, Potato Dextrose Agar, Plate Count Agar
Quantitative examination was carried out using the above Accumix medium, which gave satisfactory results.

* Actual user comments across spectrum of laboratories; Data on file: Microxpress - A Division of Tulip Diagnostics (P) Ltd.

F O R E W O R D

Microxpress, a division of Tulip Diagnostics is a part of the Tulip Group of Companies renowned world over for its reliable immunodiagnostic products and platforms for clinical laboratory diagnostics.

Accumix Dehydrated Culture Media, Bases and Supplements are manufactured out of proven and well characterized ingredients in state of the art facilities under stringent product and process control specifications.

Microxpress Second Edition Manual also contains range of plant tissue culture medias and plant tissue culture chemicals. Also we are pleased to introduce accessories range required in microbiology laboratory for our esteemed customers.

This consolidated Microxpress Manual, lists out 740 products representing the Accumix Dehydrated Culture Media, Bases, Supplements, Ready to Use, Plant Tissue Culture Media, Plant Tissue Culture Chemicals and Accessories for Microbiology laboratory.

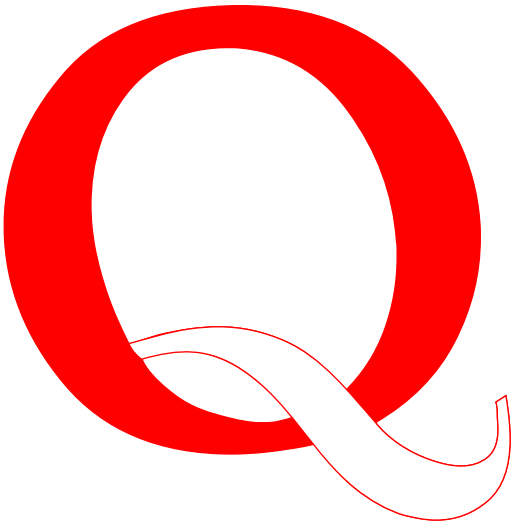
The company would add many more products to expand and consolidate its portfolio of excellent products with consistent quality and performance.

Accumix™

THE ONLY

TRUSTWORTHY ALTERNATIVE!

Quality Policy



The Quality Policy of Tulip Group of Companies is:

- To develop, manufacture and market state of the art, high quality, user friendly products.
- To design and manufacture devices in such a way that when used under the conditions and for the purpose intended, they will not compromise, directly or indirectly, the clinical conditions and safety of the products, the safety or health of the users or where applicable, other persons, or the safety of the property.
- To meet customer requirements and achieve customer satisfaction.
- To meet regulatory requirements.
- To be market leader and trend setter in diagnostic and laboratory testing.

Objectives:

- By periodically assessing customer and regulatory requirements and up grading products, processes and services.
- By adopting solutions for design and construction of device conforming to safety principles taking into account the generally acknowledged state of art.
- By emphasis on Research an Development of innovative and new products.
- By implementing Good Manufacturing Practices.
- By adopting and implementing Quality Management System adhering to international standards.
- By employing the best available personnel and training them to update the skill and knowledge.

contents

contents

INTRODUCTION

Since its inception in 1988, Tulip Group of Companies comprising of eight independent diagnostic companies, has emerged as a leading manufacturer and marketer of in vitro diagnostic reagents and kits, dehydrated culture media and high technology disinfectant products nationally and internationally.

Well known for its innovative approach, the companies are owned, managed and run by highly involved professionals.

The individual group companies specialize in research, development and designing of specific systems and platforms in diverse technological areas covering almost all areas of diagnostic relevance.

The Group believes in creating 'better systems for diagnosis and prevention' and sets trends by innovating continuously.

PRODUCT DEVELOPMENT

While Tulip Diagnostics (P) Ltd. focuses on assay systems for Immunohaematology, Haematology, Rheumatology, Infectious Diseases and Haemostasis, its division Microxpress focuses on dehydrated culture media, bases, supplements, reagents and tests kits for microbiology and mycobacteriology.

Orchid Biomedical Systems, Qualpro Diagnostics, Zephyr Biomedicals, focus on rapid membrane & ELISA based immunodiagnostic platforms for Fertility, Infectious Diseases, Parasitology, Cancer and Cardiac Markers. Coral Clinical Systems focuses on Clinical Biochemistry while its division BioShields focuses on high technology disinfectants.

MANUFACTURING

The products are manufactured in professionally set up modern facilities complying to relevant FDA guidelines.

The innovativeness is fuelled by an inventive streak with an accent on indigenous technology as a fundamental basis for product development and designing of viable technological platforms for diagnosis.

Production systems have been devised around process flows to achieve consistent product performance, batch to batch and stringent in coming, in process QA ensure adherence to expected performance parameters whereas finished QC benchmarked to standard reference materials ensures accuracy of products.

QUALITY ASSURANCE

The companies apply cGMP and GLP in force from time to time and all the companies are ISO 9001:2000, ISO 13485 (2003), NF EN ISO 13485 (2004) compliant. Most products are already CE marked.

HUMAN RESOURCES

The company places great importance to talent garnering and skill development. Inhouse training programmes are conducted at desired frequency to develop functional proficiency, understanding processes and imparting knowledge. Tulip Group team is constantly motivated to be responsible and responsive to its customers and business.

NATIONAL SALES

The Company's national business is built around twelve branch locations, nationwide with product flow all over the country through a diverse and efficient distributor network that guarantees product availability, maintenance of cool chain and customer responsiveness.

The Company has a professional sales team of around 325 sales / service professionals headquartered all over the country to carry forward its customer contact and sales programme; with a customer base of over 15000 customers and 300 distributors.

INTERNATIONAL PRESENCE

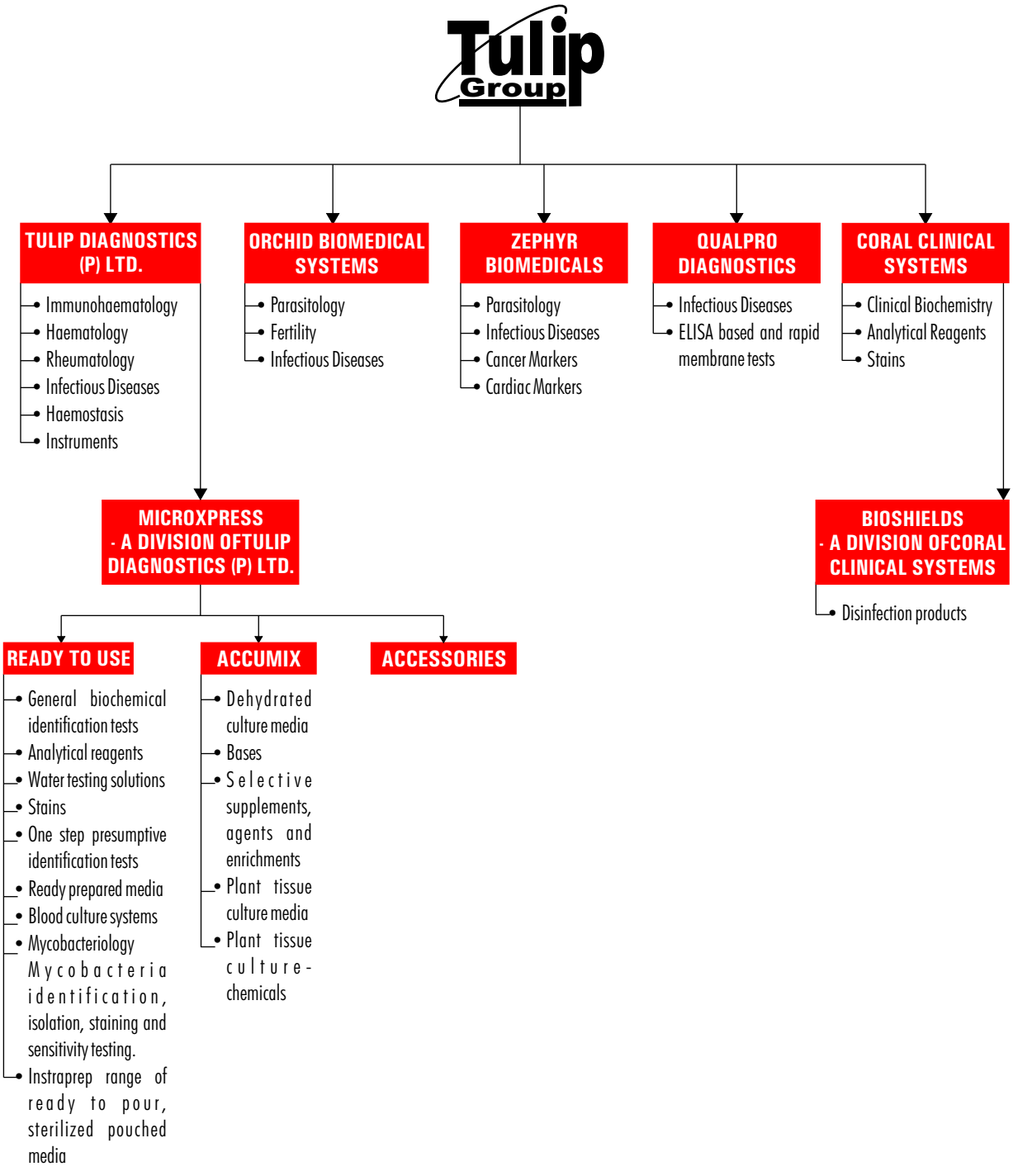
Internationally the company channelizes its products and technology through distributors, NGO's and arrangements with other international companies globally. The company also offers bulk, OEM and contract manufacturing facilities to various international companies. Currently, the company exports its products to over 50 countries worldwide, representing over 45% of its turnover.

OPPORTUNITIES FOR COLLABORATION

The company is constantly seeking distribution partners in un-represented countries. It also seeks competent vendors for various biomaterials, chemicals and instrumentation used in its manufacturing processes.

The company also seeks collaboration with like-minded companies who are looking to commercialize their products and technologies in India utilizing its deep resources and understanding of the Indian & International business environment.

Corporate Flowchart



TÜV SÜD Management Service

CERTIFICATE

The Certification Body
of TÜV SÜD Management Service GmbH
Trading as TÜV SÜD South Asia Private Limited

certifies that

MICROXPRESS
(A DIVISION OF TULIP DIAGNOSTICS (P) LTD.)
PLOT NO. S-126, PHASE III- B,
VERNA INDUSTRIAL ESTATE, SALCETE,
VERNA, GOA - 403 722, INDIA

has established and applies
a Quality Management System for

Design, Manufacture, Formulation,
Marketing & Sale of Diagnostic Reagents &
Kits Dehydrated Culture Media and Culture Media Bases

An audit was performed, Report No. **70024790**

Proof has been furnished that the requirements
according to

ISO 9001: 2008

are fulfilled. The certificate is valid until **2011-08-16**

Certificate Registration No. **12 100 16270/06 TMS**

Munich, 2009-08-05

ZERTIFIKAT ◆ CERTIFICATE ◆ 認証書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

LNE

Le progrès, une passion à partager

Certification Medical Device G MED

CERTIFICAT
CERTIFICATE OF REGISTRATION
N° 12421 Revision 2

Le LNE certifie que le système de management de la qualité développé par

LNE certifies that the quality management system developed by

MICROXPRESS
Plot No.S-126, Phase III B, Verna Industrial Estate
VERNA - GOA 403722 IND

pour les activités
for the activities

Conception, fabrication, formulation et ventes de réactifs et trousses de diagnostic in vitro, de milieux de culture de base et de milieux de culture déshydratés.

Design, manufacturing, formulation and marketing of in vitro diagnostic reagents and kits, dehydrated culture media and culture media bases.

réalisées sur le(s) site(s) de
performed on the location(s) of

MICROXPRESS
Plot No.S-126, Phase III B, Verna Industrial Estate, VERNA, GOA 403722 IND

est conforme aux exigences des normes internationales
complies with the requirements of the international standards

ISO 13485 : 2003 - NF EN ISO 13485 : 2004

Début de validité / *Effective date :* **December 12th, 2008 (included)**
Valable jusqu'au / *Expiry date :* **December 11th, 2011 (included)**
Etabli le / *Issued on :* **January 19th, 2009**

For the General Director
Laurence DAGALLIER
Deputy Director

cofrac

N° 4-0033
Portée disponible sur www.cofrac.fr

LNE N° 12421-2
Ce certificat est délivré selon les règles de certification G-MED / This certificate is issued according to the rules of G-MED certification
LNE/G-MED Organisme notifié pour les Dispositifs Médicaux / LNE/G-MED Notified Body for Medical Devices

Renouvelle le certificat 12421-1

Laboratoire national de métrologie et d'essais - Établissement public à caractère industriel et commercial
LNE/G-MED - Organisme notifié n° 0459
1, rue Gaston Boissier - 75724 Paris Cedex 15 - Tél. : 01 40 43 37 00 - Fax : 01 40 43 37 37 - www.lne.fr - www.gmed.fr

A Glance at Microxpress Manual

The Accumix Manual presents in one volume descriptions of Dehydrated Culture Media, Media Bases, Selective Supplements, Ready to use microbiology product and Plant Tissue Culture Media and Plant Tissue Culture Chemicals currently offered by Microxpress and is intended to provide information about other essential products used in clinical microbiology.

It includes both classical and modern media used for identification, cultivation and maintenance of diverse bacteria. The media are organized alphabetically. Each media includes the composition, directions for preparation, uses, etc. The composition section of each medium describes the ingredients that make up the medium, their amounts and their pH.

Microbiological testing with these products should be performed by a trained or professional microbiologist or under the supervision of microbiologists and staff qualified by training and experience to handle pathogenic microorganisms, specimens and samples suspected to contain them. It is also expected that the user will be thoroughly familiar with the intended use of the individual formulations and will follow test procedures outlined in the standard methods and official compendia or the procedure manual of the using laboratory.

The Microxpress Manual is organized into 7 sections

Section I : Microxpress Accumix Dehydrated Culture Media, Bases and Supplements-Monographs pertaining to the development, quality control and utilization of dehydrated culture media (Essentials...)

Section II : Microxpress Accumix Dehydrated Culture Media, Bases and Supplements-General technical information (User know how...)

Section III : Microxpress Accumix Dehydrated Culture Media, Bases and Supplements-Product description (Exploring...)

Section IV : Microxpress Accumix Dehydrated Culture Media, Bases and Supplements-Tables summarizing industrial and clinical applications (Applying...)

Section V : Microxpress Accumix Dehydrated Culture Media, Bases and Supplements-Picture Gallery

Section VI : Microxpress Ready to use Microbiology Products

- General biochemical identification tests
- Analytical reagents
- Water testing solutions
- Stains
- One step presumptive identification tests
- Ready prepared media
- Blood culture systems
- Mycobacteriology

  Mycobacteria identification, isolation, staining and sensitivity testing.

Section VII : Microxpress Plant Tissue Culture Media

Section VIII : Microxpress Plant Tissue Culture Chemicals

Section IX : Microxpress Accessories

Section X : Microxpress Bibliography

Great care has been taken so that the information and recommendations contained herein are accurate and compatible with the standards generally accepted at the time of publishing. However, it is difficult to ensure that all the information given is entirely accurate for all circumstances. Therefore Microxpress, a Division of Tulip Diagnostics (P) Ltd. makes no warranty with respect to its accuracy or its completeness nor assumes any liability resulting from its use.

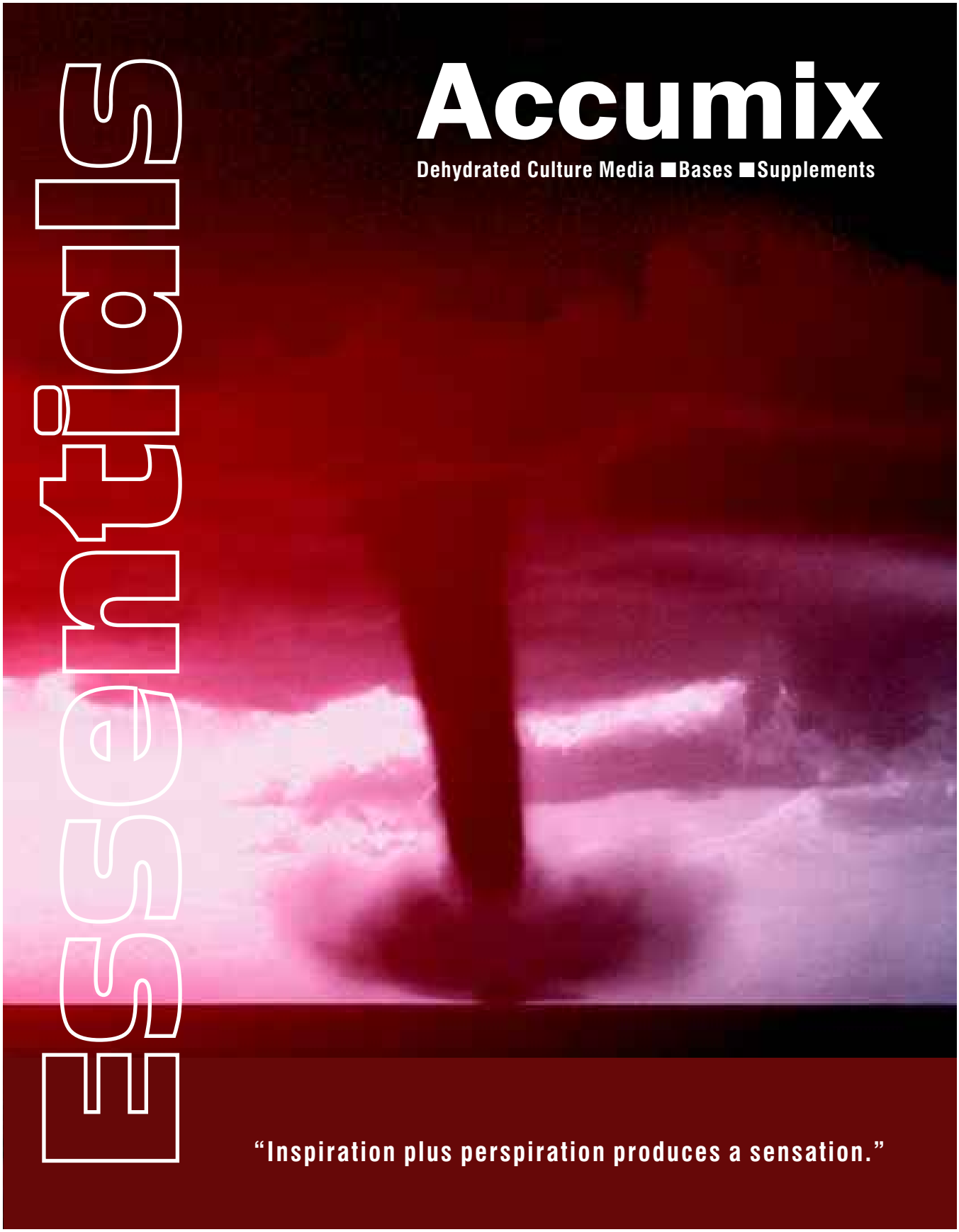
References for media listed in "official" and "standard methods" are provided. However, since procedures specified in these publications may differ from one another and from those included in this manual, these publications should be consulted when adherence to procedures is required.

For complete information regarding processing and inoculation of samples, procedures, results, detection of particular organisms, colony morphology and biochemical tests, counting of colonies and precautions and limitations, refer to appropriate references and official test procedures recommended in standard methods.

Essentials

Accumix

Dehydrated Culture Media ■ Bases ■ Supplements



"Inspiration plus perspiration produces a sensation."

Microorganism Growth Requirement

Cultivation of microorganisms depends on a number of important factors:

- Availability of proper nutrients
- Moisture for growth
- Availability of oxygen or other gases, as required
- Prevalence of proper temperature relations and appropriate pH
- Medium free from interfering bioburden
- Prevention of contamination

Basic Requirements of a Culture Media

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis and therefore media used in the laboratory for the cultivation of microorganisms must supply all the necessary nutrients for cellular growth and maintenance of these organisms.

A satisfactory culture medium, must therefore, contain:

A source of carbon and energy

Carbon required for synthesizing cellular components and energy required for metabolism, are both derived mainly from carbohydrates like glucose, maltose, lactose, sucrose, xylose, cellulose, glycogen etc and incorporated in the culture media.

A source of nitrogen

Nitrogen required for synthesizing cellular components like nucleic acids is derived from peptones and other extracts and incorporated in the culture media.

A source of phosphate and sulphur

Sulphur needed for synthesis of certain amino acids like cystine, methionine and other molecules while phosphorus required for synthesis of nucleotides, nucleic acids and phospholipids are both obtained from peptones and other extracts incorporated in the culture media.

Inorganic mineral salts and metals (macro and trace elements)

Inorganic salts and metals like sodium, potassium, magnesium, calcium, iron, phosphorus and traces of zinc, cobalt, manganese, copper, etc. required for bacterial growth are derived from various culture media ingredients. Inorganic salts are generally required in larger concentrations than trace elements.

A formulation may not have specific metals and minerals so listed. In such cases, it is assumed that all the factors required are present in the hydrolysates, buffers and agar components incorporated in the medium.

Water

Water is an essential ingredient of bacterial protoplasm and hence drying is lethal to cells. Free water must be available for transfer of nutrients and toxic waste products.

Enrichments and growth factors

Growth factors like vitamins, amino acids, purines, pyrimidines are supplied by the various ingredients incorporated in the culture media.

Factors that Influence Culture Media and their Growth Promoting Properties

The survival of microorganisms in the laboratory, as well as in nature, depends on their ability to grow under certain physical and chemical conditions. Growth is based on the replication of microbial cells, and corresponding increase in the density of these cells in the culture. Therefore, in addition to the use of an appropriate medium containing the nutrients and other growth factors required for replication and survival of microbes, a number of other parameters influence the growth of cultures.

1) pH

Microorganisms are susceptible to changes in acidity or alkalinity of the surrounding medium. This is true with regard to both, growth and survival. Whilst many bacteria show vigorous growth within a fairly wide range of acidity or alkalinity, there are others that require this to be adjusted within narrow limits before multiplication takes place. All organisms have a particular alkaline, acidic or neutral pH at which growth is optimum.

It is therefore essential to adjust the pH of the medium suitable for the optimum growth of the organism. Various types of pH indicators are incorporated in culture media to ascertain the pH of the medium e.g. thymol blue, methyl red, phenol red etc.

Furthermore, pH of a medium should not only be adjusted but also kept within the same range. Most microorganisms produce acids or alkalies, as a result of their metabolic activities and these must be prevented from altering the pH of the medium too radically. For example, bacteria when grown on a medium containing sugar generally produce acid intermediates or end products (formic, acetic, butyric and lactic acids). This is particularly true of fermentation under relatively anaerobic conditions. Accumulation of acid alters the pH and therefore becomes detrimental to the growth of the organism. It is therefore preferable and often essential to include buffers in culture media to resist the changes in pH of the medium. A buffer is a mixture of a weak acid and its conjugate base. e.g. citrate

buffer, acetate buffer, phosphate buffer.

2) Oxidation Reduction Potential (Eh)

In the medium, oxidation-reduction conditions are very important. Strict aerobes need oxygen while strict anaerobes require reducing conditions and hence absence of dissolved oxygen. This may be related to the metabolic characteristics of the organism. Strict aerobes obtain energy only through oxidation involving oxygen as the ultimate hydrogen acceptor; anaerobes utilize hydrogen acceptor other than oxygen while facultative anaerobes can act in both ways. In fact, anaerobes may be poisoned by the presence of oxygen because of the formation of toxic hydrogen peroxide which cannot be removed by catalase or superoxide free radicals which cannot be removed by superoxide dismutase or due to oxidation of certain essential groupings in the organism, e.g. the sulphydryl groups in proteins. It is possible to determine the intensity level of oxidizing or reducing conditions in a system by the net readiness of all the components in that system to take up, or part with electrons. This ability is usually expressed as the oxidation-reduction (redox) potential of the system. The redox potential can be obtained by adding dyes (oxidation-reduction indicators) and by observing the colour changes, to note how much they are reduced. Such changes are in intensity of colour, not changes from one colour to another, as in the case with the indicators used for the measurement of pH e.g. methylene blue, resazurin etc.

3) Temperature

Since all processes of growth are dependent on chemical reactions and the rate of these reactions are influenced by temperature, the pattern of bacterial growth is profoundly influenced by temperature. For each species there is a temperature range. The temperature that allows for most rapid growth in a short span of time is known as optimum growth temperature.

Most organisms of medical importance grow best at 37^{0}C (incubation temperature). Hence, it is important to maintain the exact temperature required for the optimum growth of the respective organism. An unsatisfactory temperature may inhibit or even kill the desired organisms.

4) Osmotic pressure

Osmotic pressure refers to the unbalanced pressure that gives rise to the phenomena of diffusion and osmosis, as in a solution where there are differences in concentration. It is related to the concentration of dissolved molecules and ions in a solution. Hypotonic solutions are low solute concentrated and the cells placed in these solutions may swell and burst. Hypertonic solutions have high solute concentration and the plasma membrane of cells placed in these solutions will shrink causing plasmolysis.

A variety of culture media may be used for the cultivation of organisms and these media should preserve, as far as possible, the cells in their original condition. Many a times, blood has to be incorporated in the media for culture of fastidious organisms and to exhibit activities like haemolysis. The media therefore should preserve the cells in their original condition and hence have an osmotic pressure nearly isotonic with the cells to be suspended in it to avoid lysis. Sodium chloride is many a times added to the culture medium to maintain osmotic balance.

5) Water availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends on its presence in the atmosphere (relative humidity) or its presence in a solution (water activity). Water activity is affected by the presence of solutes such as salts or sugar that are dissolved in the water. The higher the solute concentration of a solution the lower is the water activity (A_w) and vice versa. Organisms live over a range of A_w from 1.0 to 0.7. Organisms require an aqueous environment and must have "free" water.

Dehydrated Culture Media

One of the basic characteristics of an organism is its nutritional requirement. Any microbiological medium must provide everything the species under cultivation requires like sources of oxygen, nitrogen, carbon, water, energy, inorganic salts, trace elements and other growth factors. A dehydrated culture medium is described as a substance or a group of substances that satisfies these nutritional requirements. These media are available in the form of powder and are easy to reconstitute and labour saving.

Common media constituents

Media formulations are developed on the ability of bacteria to use media components. Some of the media constituents and their sources are outlined in the next table.

Constituents	Source
Amino-Nitrogen	Peptones, protein hydrolysates, infusions and extracts
Energy Sources	Sugars, carbohydrates and alcohols
Growth Factors	Blood, serum, amino acids, egg yolk, yeast extract or vitamins, NAD, hemin
Buffer Salts	Phosphates, acetates and citrates
Inorganic Mineral Salts and Metals	Phosphates, sulphate, iron Magnesium, potassium and calcium
Selective Agents	Antimicrobials, dyes and chemicals
Indicator Dyes	Phenol red, neutral red and bromothymol blue
Gelling Agent	Agar

Media Formulation

Media for the cultivation of microorganisms contain the substances necessary to support the growth of microorganisms. Due to the diversity of microorganisms and their diverse metabolic pathways and requirement of certain physical and chemical conditions there are numerous media components. The components of a medium can be divided into various roles or functions.

1) Nutrients (Peptones, Protein Hydrolysates, Infusions and Extracts)

Peptones are the major source of nitrogen and vitamins in culture media. They are water-soluble ingredients derived from proteins by hydrolysis or digestion of the source material; e.g. meat, milk, soya, etc. with enzymes or acids. Depending on the protein substrate and the enzyme used, the resulting nitrogen containing compounds exist in different qualitative and quantitative relations.

Naegali is credited with the earliest publications describing the requirements of microorganisms for a protein component which he called 'peptone'. Peptones contain mixtures of polypeptides, oligopeptides, amino acids, organic nitrogen bases, salts and trace elements to support the growth of a variety of bacteria. The variety of peptones produced reflects the differing demands of microorganisms for amino acids and peptides.

Tryptone (Casein hydrolysate) with its pale colour and high tryptophan content and soya peptone with its high carbohydrate content are valuable vegetable peptones in addition to meat peptones. Meat infusions contain water-soluble fractions of protein (amino acids and small peptides) along with other water-soluble products such as vitamins, trace elements, minerals and carbohydrates (glycogen). Chemoorganotrophs generally require amino-nitrogen compounds as essential growth factors.

Infusions or extracts have low amino-nitrogen content to sustain the growth of large numbers of bacteria. (The infusions of materials such as muscle, liver, yeast cells and malt are usually low in peptides but contain valuable extractives such as vitamins, trace metals and complex carbohydrates). Proteins hydrolyzed with acids or enzymes (peptones) generally have high concentrations of water-soluble protein fractions (peptides) to support large bacterial growth. Therefore, it is a common practice to combine infusions and peptones to obtain the best of both nutrition sources.

The nutrient components of culture media are carefully selected to recover the required spectrum of organisms in the sample. General purpose media such as Blood Agar in its various forms will often contain mixtures of peptone to ensure that peptides of sufficient variety are available for the great majority of organisms likely to be present. However, more fastidious organisms will require additional growth factors to be added.

2) Energy (Carbohydrates)

The most common carbohydrate added to culture media as a source of energy is Dextrose. Others may be used as required. (Carbohydrates incorporated in culture media as energy sources may also be used for differentiating genera and identifying species. Carbohydrates added to the media at 5-10 grams per liter are usually present as biochemical substrates to detect the production of specific enzymes in the identification of organisms). It is also usual to add pH indicators to such formulations.

3) Buffering agents

Buffering agents are added to maintain the pH of culture media. It is important that the pH of a culture medium is maintained around the optimum necessary for growth, of the desired organism. The use of buffering compounds at specific pK values is especially necessary when fermentable carbohydrates are added as **energy sources.**

However, a side effect of such compounds is their ability to chelate (bind) divalent cations (Ca^{++}, Mg^{++}). Polyphosphate salts, sometimes present in sodium phosphate, are compounds, which can bind essential cations so firmly that they are not available to the organisms. The effect of these binding agents will be seen in culture media as diminished growth, unless care has been taken to supplement the essential cations in the formulation. Opacity forming in a medium, after heating or on standing at $50^{0}C$ for several hours, is commonly caused by phosphate interaction with metals. Such phosphate precipitates can very effectively bind iron and lower the available amount of this essential metal in the medium.

4) pH and Oxidation Reduction Indicators

The pH indicator has the task of revealing the formation of acids from carbohydrates and the formation of bases from peptones, single amino acids or amines by changing colour. This is a very effective way of detecting fermentation of specific carbohydrates in a culture medium. Such compounds are expected to change colour distinctly and rapidly at critical pH values.

The oxidation-reduction indicators take on particular colourations in their oxidized state, when there is oxygen present in the medium. For e.g. methylene blue changes from colourless to blue, and resazurin, from colourless to pink.

5) Selective agents and Supplements

Chemicals and antimicrobials are added to culture media to make them selective for certain organisms. The selective agents are chosen and added at specific concentrations to suppress the growth of unwanted microorganisms in a polymicrobial sample. It is, of course, essential to have established that the selective agents, at the appropriate concentration, will allow uninhibited growth of the desired organisms. A few selective agents are listed below: -

a) Bile salts

Among the substances of biological origin, bile salts are the most widely used selective agents. They are present as both, bile salt mixtures or bile salts, and as pure substances (sodium deoxycholate, sodium taurocholate). Bile derivatives like bile salts and bile salt mixtures are used as selective agents to differentiate between those organisms that are adapted for survival in the gut and those, which cannot live in that environment. (Mainly to inhibit gram-positive organisms, non-intestinal organisms and spore formers).

Bile is derived from the liver and its composition varies according to its animal source and its state of preservation. It contains bile pigments, bile acids in free and conjugate form, fatty acids, cholesterol, mucin, lecithin, inorganic salts, glycuronic acids, urea and porphyrins. The liver detoxifies bile salts by conjugating them to glycine or taurine. A bile salt is the sodium salt of a conjugated bile acid. Bile Salts and Bile Salt Mixture contain extracts standardized to provide inhibitory properties for selective media. Bile Salts consist mainly of sodium glycocholate and sodium taurocholate. Bile salt mixture is a modified fraction of bile acid salts, providing a refined bile salt (increased selectivity) and is generally effective at less than one-third concentration of bile salts. Bile salt mixture incorporated into certain media (SS Agar, Violet Red Bile Agar) gives a very sharp differentiation between lactose fermenters and non-lactose fermenters of enteric origin, permitting the detection of scanty non-lactose fermenters in the presence of numerous coliforms. Sodium deoxycholate is the sodium salt of deoxycholic acid and sodium taurocholate is the sodium salt of taurocholic acid. Since Sodium Deoxycholate is a salt of a highly purified bile acid, it is used in culture media in lower concentrations than naturally occurring bile. Sodium Taurocholate contains about 75% sodium taurocholate in addition to other naturally occurring salts of bile acids. (Although bile containing media are expected to suppress gram-positive organisms and allow only bile tolerant gram-negative organisms to grow, some bile salts (free from bile acids) will allow staphylococci and streptococci to grow. Particular advantage can be taken of this characteristic where for e.g. MacConkey Agar without Crystal Violet, NaCl and with 0.5% Sodium Taurocholate (or 0.5% bile salts) is used as a general-purpose medium for clinical bacteriology. The growth of staphylococci and faecal streptococci would be looked for on this medium when it is used for the culture of urine, faeces and purulent material obtained from patients. The suppression of swarming growth of Proteus in such specimens is a particular advantage of this medium).

When incorporated into culture media, bile salts should not affect the colour of the indicator dyes or their subsequent change in colour. It is a normal practice to titrate the level of bile salts into culture media by measuring the growth of appropriate test organisms as an indicator.

b) Antimicrobials

Antimicrobials are used in culture media as selective agents to restrict the growth of certain organisms and promote the growth of others. For e.g. chloramphenicol and cycloheximide are incorporated to inhibit a wide range of bacteria and fungi respectively.

c) Organic and inorganic salts

The organic and inorganic salts may also be used as selective agents. Sodium chloride at a high concentration inhibits both the gram-positive and gram-negative flora, with the exception of staphylococci and some other halophilic organisms. Sodium azide at different concentrations is used for the selective isolation of streptococci and enterococci. Sodium selenite and tetrathionate stimulates the growth of Salmonella while inhibiting the growth of most gram-positive organisms and restricting the growth of normal intestinal flora. Sodium citrate, sodium tellurite and sodium lauryl sulphate also belong to this group of ingredients.

d) Dyes

Dyes are essential in the preparation of differential and selective culture media. In these formulations, dyes act as bacteriostatic agents, inhibitors of growth and sometimes also as pH or Eh indicators. Dyes when used as selective agents may interact with other components in the formulation. Thus, the same sample of brilliant green may show different inhibition titres against the same sample of *E.coli* when tested in different peptone solutions. Bile salts interact with brilliant green and reduce the toxicity of the dye and therefore the toxicity levels of such dyes must be ascertained. Most of the compounds used e.g. phenol red, neutral red, fuchsin, etc; are toxic and therefore it is essential to use low concentrations of pre-screened material.

e) Readymade selective supplements

Readymade selective supplements are added to the basal media for selective isolation of organisms. They are freeze dried, accurate preparations of antimicrobials which are added, normally one vial per 500 ml. of sterile cooled medium, to base nutrient media to create specific, selective media. The result of the use of these supplements is that it is possible for non-specialized laboratories to isolate unusual but important organisms from contaminated clinical samples or food raw materials. Legionella, Listeria, Campylobacter, Haemophilus, Yersinia, Brucella, Bordetella, Neisseria species as well as many other difficult organisms can be isolated and confident statements made about their presence or absence in the material examined. They are more specific in their selective action than the chemical agents. Hence, they demand special care and post-sterilization addition. However, the wide variety of organisms and their almost infinite ability to adapt to changing conditions makes a truly selective medium unlikely.

6) Gelling or solidifying agents

Gelling or solidifying agents are added to a liquid medium in order to change the consistency to a solid or semi solid state. The outstanding gel forming substance used in culture media is agar. Its inertness to microbial action, the unique setting and melting temperatures, the high gel strength, the ability to allow diffusion of compounds while interlocking water in a rigid gel, its clarity and low toxicity has made it the substance of choice. Its ability to retain its gel structure at 60^0C makes agar of special value to culture media, which have to be incubated at this temperature to isolate thermophilic organisms. Agar is obtained from agarophyte sea-weeds mainly Gelidium, Gracilaria and Pterocladia species and extracted as an aqueous solution at greater than 100^0C, decolourized, filtered, dried and Milled to a powder. Agar is not an inert gelling agent; it contributes nutrients and/or toxic substances to culture media, depending on the chemical process adopted in its manufacture.

7) Enrichments and growth factors

To improve the fertility properties of the culture media for the cultivation of auxotrophs (fastidious organisms like Neisseria, Haemophilus, etc.) various enrichments are added normally after autoclaving and cooling the base medium. Blood, serum, haemoglobin, albumin, egg yolk, whole eggs, NAD, hemin, etc. are some of the enrichments added. Growth factors like vitamins, amino acids, purines, pyrimidines are supplied by the various ingredients of culture media.

Environmental Factors in Culture Media

1) Atmosphere

Most bacteria are capable of growth under ordinary conditions of oxygen tension. Obligate aerobes require oxygen, while anaerobes grow only in the absence of oxygen. The microaerophiles develop best under partial anaerobic conditions, and the facultative anaerobes can grow in either.

Anaerobic conditions for growth of organisms are obtained in a number of ways:

- Absorption of oxygen by chemicals.
- Displacement of the air by hydrogen or nitrogen.
- Addition of small amounts of agar to liquid media.
- Addition of fresh tissue to the medium.
- Addition of a reducing substance to the medium; for example sodium thioglycollate, L-cystine.

Many organisms require an environment of 5-10% CO_2. Levels greater than 10% are often inhibitory due to decrease in pH as carbonic acid forms. Culture media vary in their susceptibility to form toxic oxidation products if exposed to light and air.

2) Water Activity

Proper moisture conditions are necessary for continued luxuriant growth of microorganisms. Organisms require an aqueous environment and must have "free" water not bound in complex structure necessary for transfer of nutrients and toxic waste products. Evaporation during incubation or storage, results in loss of free water and reduction of colony size or total inhibition of organism growth.

3) Protective Agents

Calcium carbonate, sodium thioglycollate, starch and charcoal are a few of the protective agents used in culture media to neutralize and absorb toxic metabolites produced by bacterial growth. Surfactants, such as polysorbate 80, lower the interfacial tension around bacteria suspended in the medium. This permits more rapid entry of desired compounds into the bacterial cell to increase growth.

Types of Media

The composition of a particular culture medium formulation determines the classification of a medium as basal (general-purpose), enriched, selective, differential or indicator or a combination of these types.

1) Basal media

A basal medium is the one that supports the growth of a wide variety of microorganism types and lacks inhibitory properties. Such a medium may be enriched with a supplement, usually animal blood, so as to cultivate fastidious microbial species e.g. Nutrient Agar which can be enriched by the addition of blood.

2) Enriched media

An enriched media is devised to meet the nutritional requirements of more exacting bacteria (auxotrophs) like Neisseria and Haemophilus by addition of growth promoting substances like blood, serum, egg, NAD, hemin and other growth factors. e.g. Soya Bean Casein Digest Medium enriched by the addition of 5% blood, Blood Agar, Loeffler Serum Agar.

3) Differential media

Differential media incorporate certain chemical constituents or substances that enable presumptive identification of a specific genus or species either from a pure or mixed culture. This helps to characterize different bacteria, by their special colonial appearance. e.g. TSI Agar is a differential medium for gram-negative enteric organisms on the basis of their ability to ferment dextrose, lactose and sucrose and to produce hydrogen sulphide.

4) Selective media

Selective media are the ones that inhibit or poison all but a few types of bacteria either by containing enrichment that selectively favours it or by containing inhibitory substances that suppress others. Selectivity is usually achieved with a chemical agent, dye or an antibiotic e.g. MacConkey Agar with bile salts and crystal violet is used to inhibit gram-positive bacteria while stimulating the growth of gram-negative enterics. Mannitol Salt Agar with a high concentration of salt is selective for staphylococci while inhibiting most other bacteria. Deoxycholate Citrate Agar containing deoxycholate and citrate selectively inhibits gram-positive organisms and normal intestinal flora while allowing the growth of enteric pathogens Salmonella and Shigella. Bile Esculin Azide Agar containing azide inhibits most of the gram-negative organisms allowing the growth of streptococci. Cetrimide Agar Base containing cetrimide inhibits most of the gram-positive and gram-negative organisms while allowing only Pseudomonas species to grow. Sodium selenite in Selenite F Broth and tetrathionate in Tetrathionate Broth selectively stimulates the growth of Salmonella while inhibiting gram-positive and normal intestinal organisms.

5) Indicator media

These media incorporate certain substances, which are changed visibly as a result of the metabolic activities of the particular organisms e.g. the incorporation of ferrous salts like ferric citrate and a source of sulphite like sodium thiosulphate in SS Agar helps in the detection of H_2S production and thereby helps to differentiate between Salmonella (H_2S production is indicated as a black dot in the center of the colony) and Shigella (without black center).

Many culture media formulations combine the properties of selectivity and differentiation due to the inclusion of a variety of chemicals. The isolation of microorganisms from clinical and industrial materials frequently requires the use of enriched broth media in addition to the selective, differential and non-selective plated media.

Culture Media Ingredients

AGAR

INTRODUCTION:

The revolution in the microbiology industry has created a demand for increasingly innovative and valuable media for growth of cultures. As microbiologists endeavor to meet this demand, the need for effective use of hydrocolloids, one of the industry's most significant raw materials, is of prime importance.

Agar is one such hydrophilic colloid extracted from certain seaweeds. Agar has been defined in various ways namely; a gelatinous solidifying agent used in culture media to grow microorganisms, a complex polysaccharide derived from certain marine algae, a vegetable gelatin made from various kinds of algae or seaweed, a tasteless dried seaweed that is used as a thickening agent. . . the list is endless.

Bacteriological agar is incorporated into culture media as a solidifying agent for the isolation of bacteria and fungi as well as the differentiation of strains and the study of their susceptibility to chemotherapeutic agents and antibiotics. Today, agar is utilized around the world in bacteriological culture media as the only gelling agent of choice.

Agar is mainly used as a solidifying agent and by itself has no nutritional value. It is soluble in hot water, a 1% neutral solution of which sets at 45^{0}C to a firm gel, melting at 95^{0}C. The amount of agar needed to gel broths varies but 10 to 20 grams (1-2%) per liter of water gives a good gel consistency and is commonly used. Smaller quantities of agar (0.05-0.5%) are used for motility studies and growth of anaerobes and (0.1%) microaerophiles.

Agar usually does not alter the pH of the medium but if it contains free acids they must be neutralized before autoclaving. Heat sensitive ingredients, which are damaged by autoclaving, should be prepared sterile separately and then added.

HISTORICAL BACKGROUND:

Agar is known to be the phycocolloid of most ancient origin. According to a Japanese legend the original manufacturing method of agar was first discovered in 1658 by Minora Tarazaemon. It was made and sold as an extract in solution (hot) or in gel form (cold), to be used in areas near factories and the product was then called "tokoroten". Later in the beginning of the 18th century, as the product underwent industrialization to a dry and stable form, it came to be known as "kanten" meaning "cold weather". The word "agar-agar", however, has a Malayan origin. "Agar" became the most commonly accepted term, although in French and Portuguese speaking countries it is called "gelosa".

Agar production by modern techniques of industrial freezing was initiated in California. Until World War II, America and Japan were the only producers of this phycocolloid. "Gelidium Pacificum" known as the genuine agar was used by the Japanese industry. During the Second World War, shortage of available agar ignited the production opportunity for those countries with coastal resources of "Gelidium Sesquipedale" an agaroid similar to "Gelidium Pacificum" used by the Japanese industry. In succession to this, Portugal, Spain and other European countries, which did not have agarophyte seaweeds, followed suit.

The significant use of agar in bacteriology was introduced by a woman named Fanny Angelina Eilshemius, the wife of a German doctor Walther Hesse who was involved in the study of public health and bacterial metabolism. One hot summer, when Dr. Walther wasattempting to do counts on bacterial air contaminants, he was having trouble with his gelatin plates melting in the heat and being digested

by many of the bacteria he was trying to grow on them. In frustration, he asked his wife as to how her puddings and jellies stayed solid in warm weather. She explained to him that she used agar-agar, a complex polysaccharide extracted from seaweed, to keep them solid in hot weather. Dr. Hesse evaluated the possibility of using agar-agar to prepare microbial media and found it to be a perfect medium for the growth of microorganisms on solid medium as it was non-toxic to most microbes, melts only at 95^0 C, but solidifies at 45^0 C (a temperature most bacteria can survive), was non-toxic to other forms of life, stable to sterilization temperatures and physiologically inert. In 1882, Robert Koch was the first to use agar-agar as a culture medium in his experiments on tuberculous bacteria. By the early 1900's agar became the agent of choice instead of gelatin. Agar was found more suitable because it remained solid at the temperature required for growth of human pathogens and was resistant to breakdown by bacterial enzymes.

SOURCES OF AGAR:

Agar is a phycocolloid, water-soluble polysaccharide extracted from a group of marine algae. Agarophytes, the red seaweeds used as the raw material for manufacturing agar, are mainly the genera Gelidium (Gelidiaceae), Gracilaria (Gracilariaceae), Pterocladia (Gelidiaceae), and Ahnfeltia (Phyllophoraceae) in different countries. Gelidium yields the best quality of agar, but its cultivation is difficult and its natural resource is less than Gracilaria, which is being cultivated in several countries and regions on a commercial scale. Pterocladia and Ahnfeltia grow only in a few regions and are utilized only in New Zealand and U.S.S.R., respectively.

CHEMISTRY OF AGAR:

Chemically, agar consists of D-galactose, 3, 6-anhydro-L-galactose and sulfate. Agar can be separated into two polysaccharides, agarose (70%) and agaropectin (30%) using the acetylation method. The agarose is a neutral polymer, while the agaropectin is an acidic polymer. Acid hydrolysis and enzymic degradation of agar isolated the agarobiose and neoagarobiose respectively. This revealed that agarose is composed of agarobiose repeating disaccharide units alternating with 1,3-linked-b-D-galactopyranose and 1,4-linked-3, 6-anhydro-b-L-galactopyranose. Agaropectin has the same background structure as agarose, but contains considerable amount of acid groups such as sulfate, pyruvate and glucuronate groups.

Recent fractionation studies by Yaphe et. al. (1971) indicated that agar is not only made up of one neutral and one charged polysaccharide, but is composed of a complex series of related polysaccharides which range from a virtually neutral molecule to a highly charged (sulfated) galactan. The neutral polysaccharide has a gelling ability and approaches the structure of an ideal agarose, which contains a trace of polar residues such as sulfate (0.1 to 0.5%) and pyruvic acid (0.02%). These sulfate and pyruvate groups remain linked in small quantities to the agarose structure, depending on the seaweed used in agar production.

PROPERTIES OF AGAR:

- **Gelation**

  Agar has a great gelling power due to which it can form gels, which are more resistant (stronger) than those of any other gel-forming agent. A 1.5% aqueous solution gels at 45^0 C and does not melt below 95^0 C, a property solely unique to agar.

- **Gel Strength**

  Agar has the ability to form gels upon cooling of a hot solution at 45^0 C and to melt to solution upon heating at 95^0 C. At temperatures above the melting point of the gel, thermal agitation overcomes the tendency to form helices and the polymer exists in solution as a random coil. On cooling, these helices form a three-dimensional network of junction points of polymer chains. Further cooling leads to aggregation of these junction points.

- **Gelling and Melting Temperatures**

  The gelling and melting temperatures of agars correlate with their methoxyl content. The higher the methoxyl content in agarose, the higher is the gelling temperature. For example, gelling temperatures of agars from Gelidium species range from 28 to 31^0 C and melting temperatures from 80 to 90^0 C, the difference between the two temperatures being 51 to 60^0 C. The difference between the gelling and melting temperatures is called as 'hysteresis'. Higher the hysteresis better is the agar-agar quality.

- **Viscosity and Molecular Weight**

  The viscosity of an agar solution at constant temperature and concentration is a direct function of the average molecular weight. Usually, the viscosity is lower as the gel strength is greater for the agar solution. The average molecular weight of agar ranges from 8,000 to greater than 100,000.

- **pH and Sterilization**

  Agar can be used over a wide range of pH, from 5 to 8. In some cases, it can also be used beyond these limits. Agar withstands thermal treatments very well above 100^0 C, which allows for good sterilization.

- **Compatibility**

  Agar is usually compatible with most other polysaccharides and with proteins in neutral conditions.

APPLICATIONS OF AGAR :

- Bacteriological agar has been developed to address the needs of large-scale commercial and industrial applications, where good quality agar is required at a competitive price. It is incorporated into culture media for the isolation of bacteria and fungi as well as in the differentiation of strains and the study of their susceptibility to chemotherapeutic agents and antibiotics.

- It is also used as a solidifying agent in microbiological culture media where good clarity and toxic inhibition is required for the growth of bacteria, yeasts and moulds.
- Bacteriological agar is widely employed in immunology, cell culture, marine biology, clinical microbiology, mycology and protozoology. It is used in culture media for isolation of pathogens that cause infections. The growth of microorganisms in specific media helps to identify and diagnose the cause of diseases such as urinary infection, throat infection etc. The bacteria do not use the agar for growth; in fact the agar merely provides a semi- solid surface to grow the bacteria on.
- Agar is the first phycocolloid to be used in food industry. In food industry, agar is mainly used as a gelling agent and a stabilizing agent for controlling viscosity. Its satiating and regulating characteristics make it an ideal fiber additive in preparation of low calorie food products.
- Agar has proved to be most valuable in microbiology and is being widely used as a solidifying agent in culture medium for growing practically all pathogenic and non- pathogenic bacteria, yeasts and moulds.
- In prosthetic dentistry, it is necessary to make accurate casts of intricate objects. Agar, mixed with other substances, serves as the ideal dental impression material.
- Agar is used as a laxative in the prevention of constipation. Agar is a hygroscopic substance and can absorb water and expand considerably. This increases the bulk and stimulating recovery of the intestine, facilitating waste elimination.
- Agar is used in cosmetics, lotions, shaving creams, fertilizers, rubber, shoe polish and paints. It is also a major ingredient in dairy products, ice creams, chocolates, ointments, bread, pet food, air-fresheners, soft drinks and such other industrial products.
- Agarose is an ideal gel matrix for diffusion and electrokinetic movement of biopolymers and its gel is biologically inert with controlled ionic properties. This property of agarose has a wide usage in biomedicine and biotechnology.
- Agar gel electrophoretic media have been used for many years to separate and identify serum and spinal fluid proteins and other biological mixtures, facilitating the diagnosis of illnesses of patients. Separation of nucleic acids, lipoproteins, lactic dehydrogenase isoenzymes, serum proteins, glycoproteins, heparin, bacterial proteins, plant viruses, etc. are done using agarose gels. Agarose gel particles are used for separations of molecular weight and artificial mixtures of ribosomes, proteins and viruses.

Agar is suitable in Bacteriology because of its properties like

- Good transparency in solid and gel forms which allows identification of colony type
- Consistent gelling and melting temperatures, called hysteresis
- Consistent gel strength that withstands the rigors of streaking
- Freedom from metabolically useful substances such as peptides, proteins and fermentable hydrocarbons
- Freedom from toxic substances (bacterial inhibitors) and not degraded by bacterial enzymes
- Freedom from haemolytic substances that might interfere with normal haemolytic reactions of bacteria
- Freedom from contamination by thermophilic spores
- Ability to allow diffusion of compounds although locking water into a rigid gel, a property exploited in microbiological assays.

PEPTONES AND HYDROLYSATES

Peptone was originally formulated in 1880 by Naegeli. Peptones are rich in amino acids and nitrogen compounds readily utilized by bacteria and are one of the most important constituents of culture media.

Protein Hydrolysates (Peptones), Infusions and Extracts

Protein Biochemistry

Proteins consist of amino acids joined together by means of the covalent peptide bond linkage. When the bonds are hydrolyzed, proteins yield polypeptides of varying molecular sizes, proteoses and peptides down to the level of simple amino acids. Bacterial peptones are mixtures of various products of protein hydrolysis, organic nitrogen bases, inorganic salts and trace elements.

Preparation of Peptones

The composition of peptones varies with the origin and the method of preparation. Some common sources of peptone are meat, beef, fish, casein, gelatin, soyabean meal, etc. Proteins may be broken down into amino acids and peptides by hydrolysis using strong acids or proteolytic enzymes such as pepsin, papain or pancreatin, which contains trypsin and chemotrypsin. These protein hydrolysates are called peptones. Microbial proteoses and proteolytic enzymes secreted by microorganisms are becoming more widely used in peptone production.

Manufacture

Peptone manufacture includes hydrolysis / digestion, centrifugation, filtration, concentration and drying. Animal tissues are chopped in order to prepare for digestion and demineralized water is added to the starting material to form a thick suspension. The digestion process follows with an acid or an enzyme hydrolysis. Acid and alkaline hydrolysis are performed by boiling the protein with acids or strong alkalies at increased pressure to raise the temperature of the reaction. This procedure can however, decrease the vitamin content of the protein and a portion of the amino acid content. (Tryptophan is usually totally lost; cystine, serine and threonine are partially broken down and asparagine and

reaction. This procedure can however, decrease the vitamin content of the protein and a portion of the amino acid content. (Tryptophan is usually totally lost; cystine, serine and threonine are partially broken down and asparagine and glutamine are converted to their acidic form).

Digestion with proteolytic enzymes is performed at lower temperatures and normal atmospheric pressure. The resulting material from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths. This process is often less harmful to the protein and amino acids. The proteolytic enzymes are specific to the peptide bonds they will break. The enzyme pepsin will cut an amino acid chain where there is a phenylalanine or leucine bond. Papain will cut the chain adjacent to arginine, lysine and phenylalanine and pancreatin will show activity at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds. Microbial proteoses and proteolytic enzymes secreted by microorganisms are becoming more widely used in peptone production. Before digestion with enzymes, a base or acid is added to bring the pH of the protein suspension to the optimum for the specific enzyme being used. For e.g. pepsin is most effective at pH 2.0 and trypsin at pH 8.5. The enzyme is added when the pH and temperature are optimal. The amount of enzyme necessary, the time for digestion, and control of pH and temperature are dependent on the degree of hydrolysis intended.

Once protein digestion is complete, the suspension may be heated to inactivate the enzymes. The protein / enzyme slurry is then centrifuged and filtered to remove the insoluble materials and to clarify and concentrate the material. The suspension is then concentrated to approximately 67% total solids and the product now appears as a syrup, which is spray dried and packaged. Peptones could be of different types based on the source it is derived from.

Peptone Performance

Ingredients used for peptone manufacture, agents of hydrolysis, buffering agent used, conditions of hydrolysis (amount of enzyme used, time of digestion, pH and temperature), determine the degree of hydrolysis and the quality of the hydrolysate and therefore must be carefully controlled. Purification, concentration and drying steps must also be carefully regulated due to their effect on the characteristics of the peptone. The essential requirements of a good peptone include the ability to support the growth of moderately exacting bacteria from small inocula, the absence of fermentable carbohydrate and a very low content of copper.

MEAT PEPTONES

Meat peptones are proteins from animal sources that have been hydrolyzed, or broken down to amino acids and peptides, to provide nitrogen for organisms. Sources of animal protein include meat from muscular tissue or offal and gelatin. Muscular tissue and offal are utilized fresh, frozen or dried. Gelatin is extracted by boiling collagen, the fibrous protein found in connective tissue, bone and cartilage.

A variety of proteolytic enzymes, or proteoses may be used to accomplish enzymatic hydrolysis of animal protein. Pepsin and trypsin are widely used for animal peptone manufacture. Pepsin is isolated from porcine or other animal stomach. Trypsin, along with chemotrypsin, carboxypeptidase A, carboxypeptidase B, and elastase, are enzymes isolated from animal pancreas.

CASEIN PEPTONES

Casein peptones are derived from milk. Milk contains water, carbohydrates (lactose), proteins, lipids and salts. The protein portion of the milk is the starting material for casein peptones. It is separated out by hydrolysis after the removal of cream. The soluble protein (whey protein) is discarded. The insoluble portion, which precipitates out, consists of a group of heterogeneous phospho-proteins (micelle) collectively called as casein. Casein is one of the most nutritive of milk proteins, as it contains all of the common amino acids and is rich in the essential ones. Hydrolysis is affected either with hydrochloric acid or with a proteolytic enzyme.

Casein Acid Hydrolysate (Acid Hydrolysate) is poor nutritionally because tryptophan is destroyed during the hydrolysis and some other amino acids are reduced in amount (cystine is destroyed, serine and threonine are reduced, aspargine and glutamine undergo hydrolysis to yield aspartic and glutamic acid) Tryptophan must therefore be added to the medium to make it suitable for **tryptophan requiring bacteria.**

In the enzymatic hydrolysis of casein, enzymes from the pancreas are utilized to manufacture these peptones. The proteoses found in the pancreas consist of trypsin, chymotrypsin, carboxypeptidase A, carboxypeptidase B and elastase and are used for this purpose. One of the characteristics of pancreatic digest of casein, in contrast to the acid hydrolysis of casein, is a peptone that contains greater amount of peptide and tryptophan.

Tryptone (enzymic hydrolysate) contains abundant tryptophan and the full range of amino acids and does not require such supplementation. Casein hydrolysate may be substituted for peptone in broth and other media. Different grades of milk peptones are available.

SOY PEPTONES

Soy peptones are derived from enzymatic digestion of soy flour. Before digestion, the soy flour is thoroughly washed to eliminate the tryptic inhibitor present in it. Soy flour is rich in high quality protein, carbohydrates, calcium and B vitamins. The enzymes used in the digestion process must be from animal free sources or obtained from organisms that have been grown in animal free media. Sometimes the organism from which the enzyme has been sourced has been stored with bovine serum albumin (BSA). All these factors should be taken into consideration when selecting for animal free peptones.

FISH PEPTONES

Fish Peptone is a non-mammalian, non-animal peptone used as a nitrogen source in microbiological culture media. This is a non-bovine origin peptone, to reduce Bovine Spongiform Encephalopathy (BSE) risk. It is suitable for pharmaceutical and vaccine production and can replace any peptone, depending on the organism and production application.

YEAST PRODUCTS

The main sources of yeast extract are "brewer's" yeast and "baker's" yeast.

Brewers yeast is a byproduct from the brewing industry, and requires de-bittering before it is suitable for fermentation use. The process of manufacturing bakers yeast to obtain yeast extract is different compared to the manufacture of other peptones. Yeast extract is an autolysate. Cell hydrolysis is performed by the endogenous enzymes of the Sacchoromyces organisms. Autolysis is done either by controlled temperature shock or osmotic shock. After autolysis, soluble material is separated from insoluble material by means of centrifugation followed by several filtration steps. The final filtration product is concentrated and then spray dried, or is left in the concentrated paste form, which contains approximately 60-80% solids.

INFUSIONS AND EXTRACTS

(Liver extract, Malt extract, Yeast extract, Beef Heart for infusion, Beef extract, etc)

The water-soluble fractions of materials such as muscle, liver, yeast cells and malt are usually low in peptides but contain valuable extractives such as vitamins, trace metals and complex carbohydrates. It is a common practice to combine infusions and peptones to obtain the best of both nutrients.

It is our policy to minimize the theoretical risk of transmitting agents responsible for causing animal disease when selecting starting materials to manufacture our microbial culture media. The culture media range does not contain, and are not derived from, specified risk materials. The countries of origin of raw materials and enzymes are not included by USDA on the list of countries where Bovine Spongiform Encephalitis (BSE)is reported.

Culture Media Quality Assurance Programme

Culture Media Quality Assurance

The development of dehydrated culture media is a process leading to the large-scale manufacture of a reproducible, stable product. At Microxpress, the individual ingredients and the final dehydrated product are carefully quality-controlled to ensure consistent, high quality performance and obviate the need for media to be prepared in the laboratory from raw materials.

Quality Assurance Programme

All manufacturing operations are conducted according to protocols and standards which describe such procedures as the monitoring, maintenance, cleaning and calibration of equipment; plant sanitation, warehouse control of incoming materials and materials under test; labeling control and handling, storage and distribution of finished goods. The master formula and accompanying documents for each lot/ batch of product includes manufacturing control and packaging information pertaining to the product.

The finished product requires statistical evaluation of each ingredient and measurement of the interaction of each ingredient on the other. Various quality control measures are adopted prior to and during manufacturing processes so that desired end products with predictable reproducible results are obtained. All the components of the medium, including special protein hydrolysates, which may have to be specially manufactured, are assembled and a laboratory mix tested to see that it meets the performance specification. Finally, the components are milled, mixed and blended to produce a fine homogeneous, free flowing powder which is held in containers for further testing before release. Stability trials begin only after ascertaining that the desired end product has been achieved. All this, plus literature, labels and pack inserts is carried out under the aegis of R&D/Marketing. Further production lots are manufactured under surveillance which include GMP monitoring and end-product testing by the Quality Control Department.

Quality Assurance Programme pertains to Raw Material Testing, In-process Sample Testing and Finished Product Testing.

Raw Materials

It is very important that the quality of products is maintained at the highest level and lot-to-lot variation is minimal. Raw materials may vary considerably in composition and the extent to which the protein components have been denatured during any processing procedures, therefore the conditions of manufacturing should be carefully controlled to minimize the variations inherent in biological materials so as to maintain quality.

Quality tests on raw materials include identity tests, tests for performance and compatibility with other ingredients in a pre production laboratory mix of the medium components. Bought-in components have buying specifications (each raw material is analyzed for grade, quality and purity as per standards) and in-house components have manufacturing specifications and standard-operating processes produced. Peptones are examined physically, chemically and microbiologically, agar is tested for clarity, gel strength, diffusion characteristics, etc. Additional tests are also done on other media ingredients like carbohydrates, inorganic minerals, selective agents, dyes and pH indicators, growth factors, etc.

Dehydrated media mixtures are examined for appearance, homogenicity, and moisture content.

Peptones

Protein hydrolysates or peptones are a major component of culture media. Besides in-house specifications, peptones are analyzed as specified in U.S. Pharmacopoeia under the title "Peptic Digests of Animal Tissue" and "Pancreatic Digest of Casein" (107). To ensure that the product confirms to predetermined specifications, tests are carried out routinely to analyze peptones and the **following criteria monitored :**

1. Degree or extent of digestion
2. Loss at 100^{0}C
3. Total nitrogen content
4. Alpha amino nitrogen content
5. Residue on ignition
6. Salt content
7. Phosphate content
8. Freedom from nitrites
9. Trace metals
10. Lipids
11. Fermentable carbohydrates
12. Vitamins
13. Incompatibility with other ingredients at 121^{0}C for 15 minutes
14. Microbiological evaluation

(a) Growth Response

Microbial growth is measured in liquid media by change in absorbance and solid media are used to study colony characteristics.

(b) Production of desired results

(1) Indole production

(2) Acetyl methyl carbinol production

(3) Hydrogen sulphide production

Carbohydrates

Carbohydrates commonly used in culture media are:

Adonitol

Aesculin

Cellulose

Dextrin

Dextrose

Fructose

Galactose

Glycerol

Glycogen

Inositol

Lactose

Maltose

Mannitol

Mannose

Rafinose

Rhamnose

Salicin

Sorbitol

Starch

Sucrose

Trehalose

Xylose

Organisms can be identified and differentiated by their oxidative or fermentative assimilation of test carbohydrates added to the medium. Growth supportive properties of carbohydrates are tested by preparing liquid and solid culture media along with known standard carbohydrates. After sufficient incubation, characteristics like size, shape, colour and enzyme reactions with indicators are compared between reference and test carbohydrate on solid media. Carbohydrates are also tested to ensure their identity and purity.

Selective Agents (Bile Salts and Bile Salt Mixture)

Bile salts are analyzed chemically as described in National Formulary for Sodium cholate, Sodium taurocholate and Sodium deoxycholate (107). Bile salts when incorporated in culture media should not affect the colour of the indicator dye(s) or their subsequent change in colour as desired in the medium. Bile salts should not form a surface scum or deposit in the medium after storage. Bile functional test is carried out using standard bile salts as control. Growth and recovery test is carried out with a standard test organism when a bile salt of known performance is used as a control.

Dyes and Indicators

Dyes are used in the preparation of differential and selective culture media. In these formulations dyes act as bacteriostatic agents, inhibitors of growth, and sometimes as pH or Eh indicators. When used as selective agents, interaction can occur between the dye and other components of the medium. Thus, the same sample of a particular dye may show different inhibition titers against an organism like *E. coli* when tested in different peptone solutions. Bile salts interact with the dyes and reduce their toxicity. The toxic levels of the dyes are therefore

ascertained by adding to bile salts medium. Aniline dyes are more toxic when fully oxidized and therefore a change in its toxicity is possible as reduction of the dye takes place when it is sterilized in the presence of other media components like peptone or tryptone. Hence, the dyes are analyzed as specified by H. J. Conn's (Biological stains, 9th edition). Microbiological suitability is also carried out by adding dyes in culture media tested with various test organisms compared with standard known dyes, besides ascertaining chemical purity of dyes as per biological stains.

Solidifying Agent

The clarity of agar gel in culture media is of utmost importance. Ideally, the molten solution should be crystal clear without any haziness or deposit. The agar should also allow the diffusion of compounds although locking water in a rigid gel. All agars must be free from toxicity to microorganisms and free from impurities such as nitrogenous compounds, insoluble salts, non-agar gums, free sugar compounds, dead microorganisms and live thermophilic organisms. Microbiological agar is specially processed to yield a low toxicity, high clarity and high diffusion gel.

Agar is routinely analyzed for the following :

1. Ash content
2. Acid insoluble ash
3. Sulphate
4. Chloride
5. Calcium
6. Magnesium
7. Iron
8. Total nitrogen
9. Gelling strength
10. Setting temperature
11. Moisture content
12. pH at 1.2%
13. Agar gel
14. Melting point
15. Total microbial count

Toxicity test is also carried out to ascertain the presence of toxic and inhibitory substances by growing fastidious organisms on culture media containing agar and various parameters like growth, colony size, shape, pigments, etc. are compared with known standard agar.

Vitamins

Vitamins and growth factors are analyzed as per the U.S. Pharmacopeia (107) for chemical purity and other tests. Besides, potency is also confirmed by microbiological methods.

Inprocess Sample

A sample batch is prepared from pre-tested and approved components and analyzed. All tests are performed in parallel with a previously approved reference batch of the medium. This use of a control standard medium with each test ensures uniformity in reading the results. Specifications like colour, clarity, solubility, pH, gelling and cultural characteristics are ascertained before large scale batch is approved for manufacturing. Samples are withdrawn at intermediate stages to ascertain purity. The lot in process is continuously recorded under controlled conditions of moisture, humidity, temperature, etc.

Quality Control of Final Medium

Representative samples are reconstituted and examined for appearance, odour, colour, clarity, moisture, solubility, gelling temperature, pH, gel strength and compatibility with post sterilization additives. Microbiological performance is accessed by testing with small inocula of well defined cultures to measure recovery of growth, colony size and morphology, colour reactions, biochemical performance, differentiation and selectivity to ascertain intended or desired results of the product. Special procedures such as antimicrobial succeptibility tests are performed where appropriate for the recommended use of the medium. All tests are performed in parallel with a previously approved reference batch of the medium.

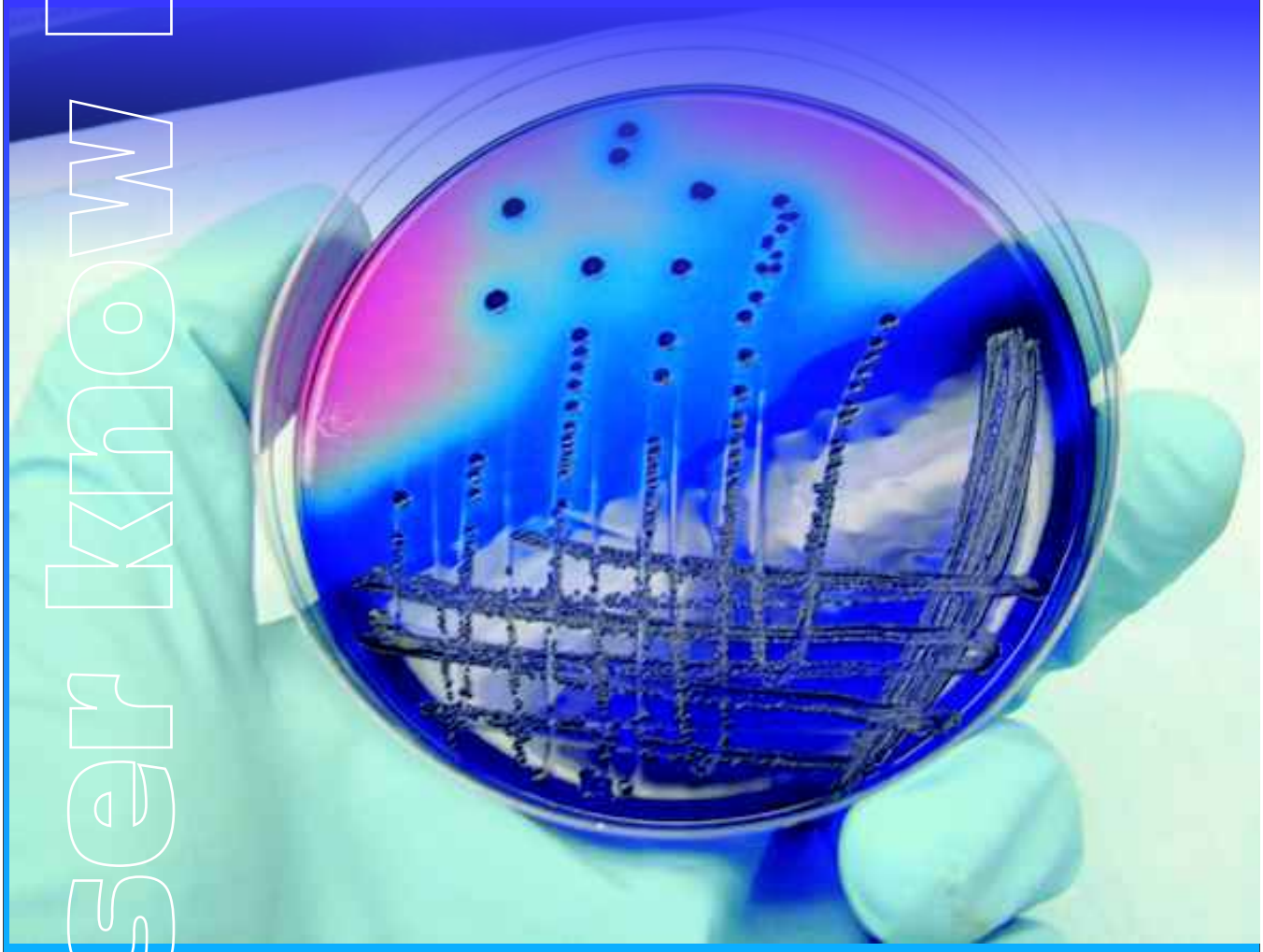
Stability Study to Evaluate the Shelf Life of the Final Product

Samples of each manufactured lot/ batch are retained for the total shelf life of the product. Stability tests and lot-to-lot uniformity tests are carried out using these retained samples.

user know how

Accumix

Dehydrated Culture Media ■ Bases ■ Supplements



"It does not take great men to do great things,
just those who are greatly dedicated to doing them."

General Guidelines to Users

APPROPRIATE USE OF DEHYDRATED CULTURE MEDIA

Dehydrated culture media are subject to strict physical, chemical and bacteriological quality control measures on both the raw materials and finished products. Generally, dehydrated media should be sterile, able to support the growth of the respective organism and produce appropriate biochemical reactions. The preparation of culture media from dehydrated media requires accuracy and attention to preparation. The following points help in successful and reproducible preparation of culture media.

DEHYDRATED MEDIA AND INGREDIENTS

Dehydrated media ingredients are hygroscopic and sensitive to moisture and therefore the storage and other conditions mentioned must be strictly followed. The media must be stored in a cool (15-30°C), dark and dry area unless otherwise specified. The date of opening the container must be noted. Expiry must be checked prior to opening the container (applies to intact container). It requires to be verified that the physical characteristics of the powder are typical.

Culture media have a tendency to form lumps due to the following conditions: -

1. High humidity during storage
2. Container left open for too long
3. Container not tightly sealed every time after opening
4. Medium is too old

Glassware and Plastic ware (Media preparation utensils)

High quality, low alkali borosilicate glass must preferably be used. Detergent residues must be avoided. Large enough vessels, at least 2-3 times the volume of the medium must be used to allow proper mixing. Chipped glassware must not be used.

Dilution bottles or tubes

Use glass bottles or tubes of resistant glass, preferably borosilicate glass, closed with glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic compounds on sterilization. Try and avoid cotton plugs as closures. Plastic bottles of nontoxic material and acceptable size may be used provided they are sterilized properly.

Petri dishes

For the plate count, use glass or plastic petri dishes about 100x15 mm. Use dishes, the bottoms of which are free from bubbles and scratches and flat so that the medium will be of uniform thickness throughout the plate. For membrane filter technique, use loose-lid glass or plastic dishes, 60x15 mm, or tight-lid dishes, 50x12 mm. Sterilize petri dishes and store in metal cans (aluminum or stainless steel), or wrap in paper-preferably best quality sulfate pulp.

Fermentation tubes and vials

Use fermentation tubes of any type, if their design permits conforming to medium and volume requirements for concentration of nutritive ingredients. Where tubes are used for testing gas production, enclose a vial, inverted. Use tube and vial of such size that the vial will be completely filled with the medium, at least partly submerged in the tube, and large enough to make gas bubbles easily visible.

Sample bottles

For bacteriological samples, use sterilizable bottles of glass or plastic of any suitable size and shape. Use bottles capable of holding a sufficient volume of sample for all required tests and an adequate air space permitting proper washing, and maintaining samples uncontaminated until examinations are completed. Metal or plastic screw cap closures with liners can be used on sample bottles provided no toxic compounds are produced on sterilization.

Before sterilization, cover tops and necks of sample bottles having glass closures with aluminium foil or heavy craft paper.

Water

Distilled or deionized water that meets the specifications for purified water, pH 5.5-7.5 must be used. It must be free from dissolved metals and bactericidal or inhibitory compounds. Distilled water should also be free of contaminating nutrients. Store distilled water out of direct sunlight to prevent growth of algae and turn supplies over as rapidly as possible. Aged distilled water may contain toxic volatile organic compounds absorbed from the atmosphere if stored for prolonged periods in unsealed containers. Good housekeeping practices usually eliminate nutrient contamination.

Equipment

Measuring devices, pH meters, scales, optical counting devices and other equipment that is frequently used need to be accurately calibrated.

Incubators

Incubators must contain a uniform and constant temperature at all times in all areas, that is they must not vary more than 0.5 in the areas used. Obtain such accuracy by using a water jacketed or anhydric-type incubator with thermostatically controlled low-temperature electric heating units properly insulated and located in or adjacent to the walls or floor of the chamber and preferably equipped with mechanical means of circulating air. Maintain an accurate thermometer. It is also desirable, in addition, to maintain a maximum and minimum-registering thermometer within the incubator on the middle shelf to record the gross temperature range over a 24-hour period. Install a recording thermometer whenever possible, to maintain a continuous and permanent record of temperature.

Autoclaves

Use autoclaves of sufficient size to avoid internal crowding; constructed to provide uniform temperatures within the chambers (up to and including the sterilization temperature of 121^{0}C); equipped with an accurate thermometer the bulb of which is located properly on the exhaust line so as to register minimum temperature within the sterilizing chambers, equipped with pressure gauge and properly adjusted safety valves connected directly with saturated-steam supply lines equipped with filters to remove particulates and oil droplets.

pH equipment

Use electronic pH meters, accurate to at least 0.1 pH units, for determining pH values of media.

Balances

Use balances providing a sensitivity of at least 0.1 g at a load of 150 g, with appropriate weights. Use an analytical balance having a sensitivity of 1 mg under a load of 10 g for weighing small quantities of materials. Single pan rapid-weight balances are most convenient.

Temperature monitoring devices

Use glass or metal thermometers graduated to 0.5^{0}C to monitor most incubators and refrigerators. Use thermometers graduated to 0.1^{0}C for incubators operated above 40^{0}C.

Inoculating equipment

Use wire loops made of 22- or 24- gauge nickel alloy or platinum-iridium for flame sterilization. Use loops of at least 3 mm in diameter. Sterilize by dry heat or steam.

Optical counting equipment

1. Pour and spread plates: Use Quebec-type colony counter, dark field model preferred, or one providing equivalent magnification (1.5 diameters) and satisfactory visibility.
2. Membrane filters: Use a binocular microscope with magnification of 10X to 15X provide day light fluorescent light source at angle of 60-80^0 above the colonies; use low-angle lighting for non-pigmented colonies.

Membrane filtration equipment

Use filter funnel and membrane holder made of seamless stainless steel, glass or autoclavable plastic that does not leak or corrode.

Reconstitution / Rehydration

The procedure employed for dissolving dehydrated culture media very often determines the clarity and performance of the finished product. Homogenicity of the solution and minimal direct heat exposure are important factors. Complete instructions for the preparation of culture media are given on the label of each bottle. For rehydration, as mentioned; clean, undamaged glassware and distilled or deionized water must be used.

Place approximately weighed amount of the medium in a clean dry flask, 2-3 times larger than the final volume of the prepared medium to allow adequate mixing. Add part of the required amount of distilled water and swirl to dissolve. After thorough mixing, add the remainder of the water with care being taken to wash down the sides of the container. Allowing agar preparations to stand for 5 minutes with occasional agitation prior to boiling enhances dissolution. Majority of broth media are clear and dissolve easily at this stage. To completely dissolve the powder, it is heated using an open flame, hot plate or boiling water bath.

When heating is required, heat should be applied gently and the preparation agitated as required to prevent scorching. However, care must be taken to avoid media eruptions. Boil as briefly as possible to obtain solution; 1 minute is usually sufficient. Care must be taken not to over boil the culture media as this could result in pH drift, darkening, precipitation, poor gel strength and reduced bacteriological performance.

Complete dissolution of the medium is indicated by perfect transparency of the solution that runs down the walls of the flask. In no case should powdered medium be added to water and immediately placed into an autoclave since layering and separation of ingredients, precipitate formation and darkening are likely to occur with diminition of performance. The culture media should not be used at a temperature higher than 50^{0}C and should not be remelted as far as possible.

Sterilization

After preparation, most culture media require sterilization in an autoclave. Although sterilization of culture media is best carried out in a steam autoclave at temperatures around 121^{0}C it has to be recognized that damage is caused to the medium by the heating process. Heat- treatment of complex culture media, which contain peptides, sugars, minerals and metals results in nutrient destruction, either by direct thermal degradation or by reaction between the medium components. Toxic products caused by chemo oxidation can also be formed during heat treatment.

It is important therefore, to optimize the heating process so that the medium is sterile after heating but minimal damage is caused to the ingredients. As a general rule, it is accepted that short duration, high temperature processes are more lethal to organisms and less chemically damaging than longer, lower temperature processes.

The completely dissolved medium is dispensed as desired and sterilized as directed on the label. Generally sterilization is done at 121^{0}C for 15 minutes in an autoclave. The recommended 15-minute sterilization assumes a volume of 1 liter or less. For larger volumes, the sterilization time should be extended but the temperature should not be raised. When larger volumes are used, validation studies should be performed to determine the optimum sterilization cycle for each

unique container size / volume combination. Autoclave media containing carbohydrates at a temperature not exceeding 116-118°C to avoid caramalization of the carbohydrate. Do not autoclave media that should not be heat- sterilized.

Temperature pressure relation is listed below.

Pressure of saturated steam P.S.I (inside the autoclave)	Temperature attained in °C	Temperature attained in °F
5 lbs	108	226
10 lbs	116	240
12 lbs	118	244
15 lbs	121	250
20 lbs	127	260
25 lbs	131	267
30 lbs	134	274

Efficiency of autoclaving should be ascertained from time to time. Temperature conditions inside autoclave may not be uniform. This can be tested by putting 2 bottles containing 2% w/v glucose in 2% w/v disodium phosphate in the autoclave at different places. Heat produces browning of solution. Bottle near steam inlet will be browner than the one away from it.

Overheating Effects

Overheating is a common cause of drift in pH, darkening, precipitation, poor gel strength and reduced bacteriological performance. These effects can also be produced if a concentrated pool of ingredients at the bottom of the container is heated. All culture media should be in solution before sterilization. This will reduce the occurrence of Maillard-type reactions (non enzymatic browning) taking place in the medium.

Overheating effects will occur if agar media are allowed to gel in bottles and later steamed to melt the agar. It will also occur if molten medium is held at 50°C for more than three hours before use. Agar media, with pH values below 5.0 are very sensitive to overheating in any form because the agar is hydrolyzed and the gel strength fails.

Adding Enrichments and Supplements

Enrichments and supplements tend to be heat sensitive and therefore must be sterilized separately and then added. Heat labile supplements must be added to the medium that has been cooled to 45-50°C. The sterile supplement must be brought to room temperature before it is added to the medium. Adequate mixing of the supplements with the basal medium by thorough mixing must be ensured. Sterile broths may be cooled to room temperature before adding enrichment.

To prepare Blood Agar, defibrinated blood is recommended rather than blood containing an anticoagulant. Sterile blood should be stored at 2-8°C, and brought to 35-37°C before adding to the medium, which has been cooled to 40-45°C.

pH Adjustment

Commercial dehydrated media are designed to fall within the specified pH range after steam sterilization. (The pH value of reconstituted dehydrated culture media prepared with distilled or deionized water shall produce the equivalent value as prescribed on the label at a temperature of 25°C). The pH tends to fall approximately 0.2 units during steam sterilization. For filter sterilization, the pH must be adjusted if necessary, prior to filtering. pH measurement and correction of the pH should be carried out at 25°C. For liquid culture media, pH measurement after sterilization should be carried out by cooling the medium to 25°C. Take an aliquot of liquid medium aseptically and measure pH. For solid culture media, pH should be measured after cooling the medium to 40°C. Take an aliquot of the cooled medium aseptically and measure pH. The pH should be adjusted to the value specified and it is corrected by adding 1N or 0.1 N HCl or NaOH solution to a sample of known volume taken from the reconstituted culture medium. Finally after calculation, the required acid or alkali is added in the remaining culture medium.

Dispensing Culture Media

The medium is generally dispensed into petri plates or tubes in a laminar flow cabinet with the surface disinfected. Heat labile supplements are generally added at this stage. Gentle mixing during dispensing is required. Sterile media should be poured into plates at about 45-50°C to reduce water evaporation and to avoid the formation of condensed water in the lids of the petri plates.

Dispensing should be done quickly without bubble formation and can be done manually or automatically. The tubes should be recapped immediately to avoid chances of contamination. Petri dish covers are left slightly open for 1-2 hrs to obtain a dry surface, (before inoculation, agar surface should be dried at 30-40°C to avoid microbial swarming).

Storage of Prepared Media

If the medium is not used on the same day it is prepared, it should be stored properly in moisture proof containers to prevent drying of the medium. In general, store steam sterilized plated media inverted in a plastic bag or other container in a dark refrigerator at 2-8°C(remains for several weeks). However, media should not be stored below 0°C as this destroys their gel structure. It is recommended to use fresh media than stored media. Agar containing media should not be stored at higher temperatures (40-50°C) for longer time, as agar tends to clump. Media containing labile beta-lactum selective agents have a very short active life and therefore should be used within a few days of preparation. Loss of water can result in precipitation and crystallization of certain substances in culture media.

Prepared broth media can be stored at 2-8°C, without allowing to freeze. Liquid media in test tubes or flasks should also be provided with airtight seals. Prepared plates are often vulnerable to contamination, dehydration and chemical

degradation. Proper storage at 2-8°C prevents water loss of the medium. Most media, and especially those containing dyes or indicators, should be protected from light during storage. Prepared media that have been refrigerated should be removed from refrigeration and brought to room temperature prior to inoculation to allow water of condensation to evaporate and to avoid temperature shock to the inoculum.

However, before inoculation the plates should be carefully examined for contamination, uneven filling or bubbles on agar surface, colour changes, haemolysis and cracks due to shrinking and loss of volume.

Precautions in the use and disposal of culture media

Inoculation of culture media with bacteria, deliberately or accidentally, leads to a very great number of organisms being produced. High concentrations of any organisms are potentially hazardous and must be disposed off safely. Therefore, after use, prepared plates, samples, sample containers or other contaminated material must be sterilized or incinerated before discarding. All autoclaved biohazards should be disposed off in accordance with state and local environmental regulations.

Only qualified personnel who have been trained in microbiological procedures should handle all infected specimens and inoculated culture media. User should ensure that any machinery or apparatus used and by chance contaminated must be safely disinfected or sterilized. The environment in which microbiological cultures are handled must also be taken into account. Most countries have categories of organisms, which are divided into those, which may be handled in the general microbiological laboratory, those which require special laboratory conditions and for the most dangerous organisms a totally contained and highly protected environment.

The United States Department of Health and Human Services has published guidelines for handling infectious agents and materials. The guidelines describe four-biosafety levels.

Biosafety Level 1 is applicable when work is done with defined and characterized strains of viable organisms known to consistently cause disease in healthy adult humans.

Biosafety Level 2 practices are applicable to laboratories where work is done with the broad spectrum indigenous moderate risk agents that are associated with human diseases; activities can be performed on the open bench provided the potential for producing splashes or aerosol is low.

Biosafety Level 3 practices are applicable to laboratories working with agents with a potential for respiratory transmission and which may cause potential and seriously lethal infection. All laboratory manipulations must be performed in a biological safety cabinet or other enclosed equipment to protect personnel and the environment from the exposure to potentially infectious aerosol.

Biosafety Level 4 practices are applicable for work with highly dangerous agents, which may be transmitted via the aerosol route, for which there is no available vaccine or therapy and for which specialized equipments and facilities are required.

Consult the reference for specific recommendations on the practices, equipment and facilities of the four-biosafety levels.

Handling of Dehydrated Culture Media

Most of the products supplied have no known risks except those usually associated with fine powder. These products are meant for carrying out bacteriological work in the laboratory as specified under the use of individual medium and should not be used for human or animal consumption. Powder may cause irritation of the upper respiratory tract. To avoid mild skin rashes, prevent prolonged contact with the powder and ensure that excessive dust is not produced. Any residue should be washed off with ample cold water. To prevent the risk of inhaling fine dust it is recommended that approved masks should be worn while handling dehydrated media.

Hazardous and Toxic Products

There are a number of products, which contain toxic substances. These must be used in accordance with the product specific "material safety data sheet". All relevant risk and safety phrases are on the product label e.g. media containing sodium azide, bismuth, cycloheximide, lithium chloride, basic fuchsin, sodium hydrogen selenite, etc. The material safety data sheet is available for all products, a hard copy of which can be obtained from the company on request.

Sodium azide

Generally, the products contain less than 1% of sodium azide and have low toxicity. However, some individuals have enhanced sensitivity to azide and therefore precaution must be taken to prevent ingestion or inhalation of the dust. Sodium azide has a tendency to react with many metals, particularly copper and lead which produce explosive metal azides. It is recommended to observe strict regulation of local and national legislation of disposal. Flush using sufficient water to prevent the powder remaining in contact with pipeline and drains.

Basic fuchsin

Basic fuchsin is a potential carcinogen. Care should be taken to avoid inhalation of the powdered dye and contact with skin.

Lithium chloride

Lithium chloride is harmful hence all bodily contacts and inhalation of vapours must be avoided.

Sodium hydrogen selenite

Sodium hydrogen selenite is highly toxic, terratogenic and corrosive. It must be handled with great care. On contact with skin, it is necessary to wash with plenty of water.

Chloramphenicol

Chloramphenicol is a potent carcinogen and must be handled with care. Avoid inhalation. On contact with skin, it is necessary to wash with plenty of water.

Potassium Hydroxide

Potassium Hydroxide is corrosive. It must be handled with care. On contact with skin, wash immediately with plenty of water.

The following hazard symbols are mentioned on the respective product labels as well as warnings in the manual.

Xn

Harmful

T

Toxic

N

Dangerous for the environment

C

Corrosive

First Aid Measures

The following first aid precautions should be taken in case of accidents while handling any of the toxic or hazardous products: -

Inhalation

Remove person from area of exposure. Rest and keep warm and seek medical advice.

Skin Contact

Remove all contaminated clothing, wash the effected area thoroughly and seek medical advice.

Ingestion

Wash out mouth with water, give water to drink immediately and seek medical advice.

Eye Contact

Irrigate thoroughly with water and seek medical advice.

Spillage

Wash away with large volumes of running water; wear protective overalls, gloves, eye protection and facemask. Collect the material in a suitable container, seal and dispose off.

Storage of Dehydrated Culture Media

It is essential to store all the products at specified conditions to achieve optimum performance. Products should not be stored beyond the allocated shelf life. The storage conditions and expiry dates of each product are shown on the respective label of the container. Generally products should be used in order of their lot / batch numbers. (Provision should be made for rotating the stock of dehydrated media to ensure product freshness).

Environmental conditions like light, humidity and temperature are the frequent causes of instability of the product

Light

All the dehydrated culture media should be stored away from light and exposure to direct sunlight.

Humidity and Temperature

It is important not to leave bottles open to atmosphere since dehydrated powder will be affected by humidity. Hot or media preparation rooms are not suitable to store containers of culture media, particularly containers that are frequently opened and closed. They should be stored in a dry, dark place at room temperature.

Recommended Storage Temperature and Time

Dehydrated Culture Media

Sealed, unopened containers Temperature (RT) below 30^{0}C, for a few storage at 2-8^{0}C is required. Unless mentioned, media described in this manual should be stored at Room Temperature (RT) below 30^{0}C. Such media stored under optimal conditions have a shelf life of 2-5 years. When the container is opened for the first time, the date should be noted on the container. The lid should be replaced quickly after the media has been taken out and the cap is to be closed tightly to protect from hydration.

Media Bases

Sealed unopened containers should be stored at Room Temperature (RT) below 30^{0}C and they generally have a shelf life of 3-5 years.

Selective Supplements

Selective Supplements should be stored at 2-8^{0}C. They generally have a shelf life of 1-3 years. Horse serum should be stored at -20^{0}C.

Packing

Dehydrated Culture Media are filled in HDPE, double sealed containers in 100 grams and 500 grams pack with respective package inserts for instructions, individually encased in shrink film packs. The catalogue numbers AM1XXX and AM5XXX indicates 100 gm and 500 gm packaging respectively where X denotes a number.

All Microxpress products are CE compliant and the following symbols are mentioned in the respective packaging.

	Store at 22-30^{0}C
REF	Catalogue Number
IVD	In vitro Diagnostic Medical Device
	Consult Instructions for use
	Manufacturer
	Hygroscopic keep container tightly closed
DCM	Dehydrated Culture Media
EC REP	Authorised Representative

	Store at 2-8^{0}C
LOT	Batch Number
DCB	Dehydrated Culture Bases
DCS	Dehydrated Culture Supplements
RO	Received on
OO	Opened on
	Use by

Expiry

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance. Use the product before expiry date on the label.

Product Deterioration

Verify that the physical characteristics of the powder are typical. Hydration can lead to deterioration, which render the medium unusable.

Factors that Influence the Quality of Dehydrated Culture Media

Effects of the media on the colony growth may be too poor or too strong because of residues of growth inhibiting substances present in improperly washed glassware, overheating of the medium and shift in pH. Also, if the surface of the media remains moist as a result of improper media preparation, the colonies of certain microorganisms tend to liquefy or swarm inhibiting the growth of bacteria that requires testing. Atypical growth of organisms may be observed if the dehydrated culture media is old or if residues of foreign substances are present in the glassware or water used. Pouring of plates correctly and aseptic culturing methods are very essential so as to avoid contamination on plates.

Faults	Possible Causes
Clumping of the media	Container was not tightly sealed after use Container was left open for a long time Humidity was high during storage Culture medium was too old
Drift in pH	Overheating due to excessive sterilization, heterogeneous mixing, repeated remelting and inadequate storage Impure water and glassware used Hydrolysis of ingredients pH determined when medium was hot
Incomplete solubility	Inadequate water usage Incomplete mixing which causes overheating
Turbidity, precipitation, contamination, toxicity	Water not adequately demineralized Lab ware used for preparation not clean Incorrect value of pH, insufficient sterilization Overheating Loss of water due to evaporation Poor technique in adding enrichments and pouring plates
Solidification point too high	Too much dehydrated media weighed out Unsuitable agar used
Darkening of the medium or colour change	Overheating or repeated remelting Incorrect pH
Incomplete or soft gelling	Glassware not clean Incorrect proportions of product to volume of water Agar not in solution Incomplete mixing Repeated remelting Acid hydrolysis of agar
Loss of growth promoting properties or differentiating properties	Repeated remelting or overheating Incomplete mixing Disturbance in the formula by the inoculum carriers

Limitations of the Procedure

Detergent residues in lab ware / glassware can compromise quality of the media. Quantities of media in excess of one litre may require an extended autoclave time to achieve sterilization. Longer sterilization cycles can cause nutrient concentration changes and generation of inhibitory substances. Since the nutritional requirements of organisms vary, a single medium is rarely adequate for detecting all organisms of potential significance in a clinical specimen or industrial sample. Colour differentiation and the growth of colonies may not be optimal if the heat sensitive carbohydrates and other enrichments are added to the medium when hot.

The selective agents may inhibit some strains of the organisms to be cultivated and permit the growth of organisms they were supposed to inhibit if the unwanted organisms are present in large numbers in the specimen. Culture of specimens grown on selective media should, therefore, be compared with specimens cultured on non-selective media to obtain additional information and help recovery of potential pathogens and other significant organisms.

Though some of the media formulations are developed for isolation of specific organisms, it is recommended that biochemical and / or serological tests be performed on colonies from pure cultures for complete identification.

Comparative Chart

APHA PRODUCTS VIS A VIS ACCUMIX PRODUCTS

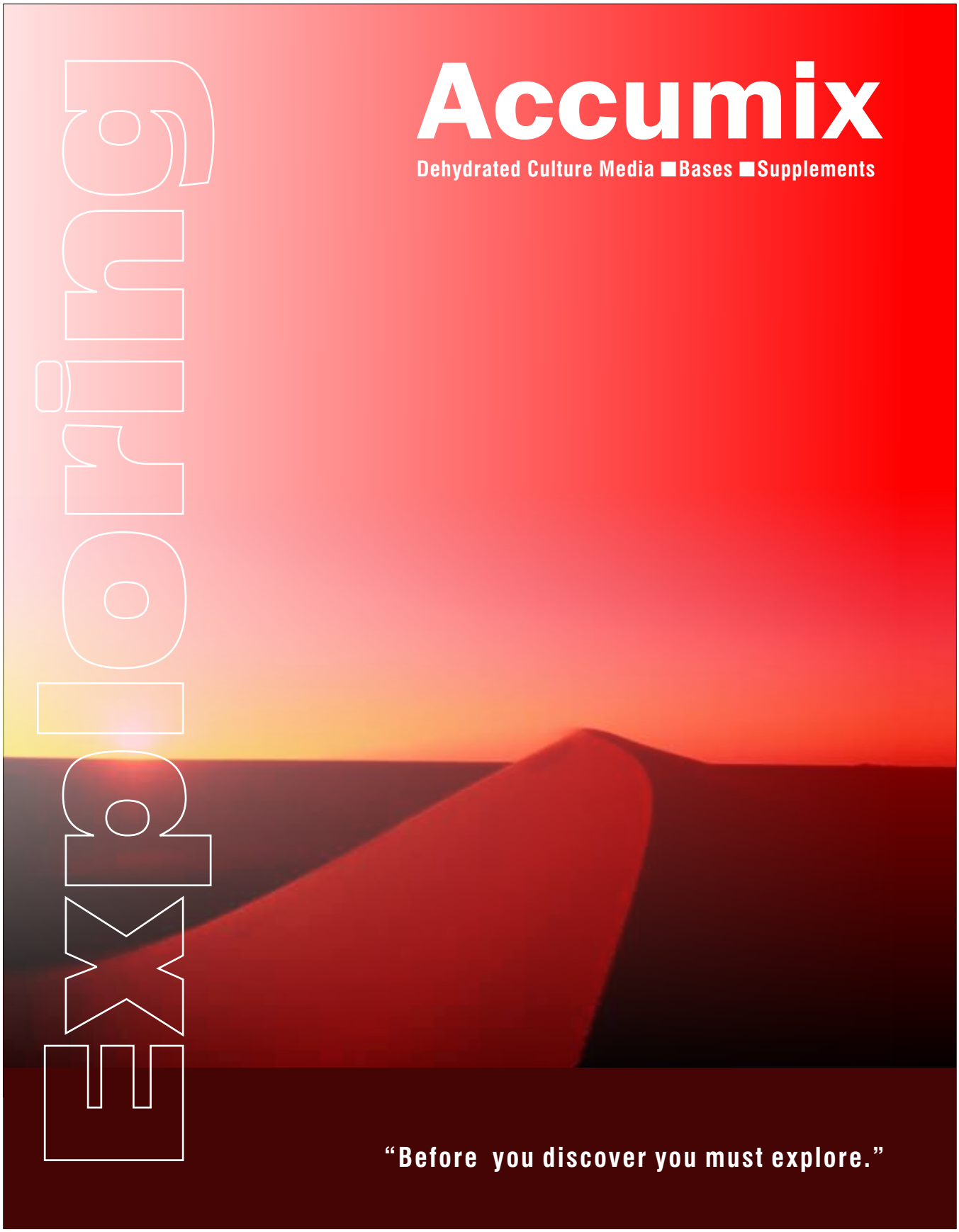
APHA	ACCUMIX CAT. NO.	ACCUMIX	FOOD	DAIRY	WATER
Baird Parker Medium	AM1011/AM 5011	Baird Parker Agar Base			
	AS010	Egg Yolk Emulsion			
	AS011	Egg Yolk Tellurite Emulsion		*	*
Bile Esculin Agar	AM1012/AM5012	Bile Esculin Agar			*
Bismuth Sulphite Agar	AM1013/AM5013	Bismuth Sulphite Agar	*	*	*
Brain Heart Infusion Agar	AM1017/AM5017	Brain Heart Infusion Agar	*	*	*
Brain Heart Infusion Broth	AM1018/AM5018	Brain Heart Infusion Broth	*	*	*
Brilliant Green Agar	AM1018/AM5018	Brilliant Green Agar, Modified	*	*	*
Brilliant Green Lactose Bile Agar	AM1019/AM5019	Brilliant Green Bile Agar	*		*
Brilliant Green Lactose Bile Broth 2%	AM1020/AM5020	Brilliant Green Bile Broth 2%	*	*	*
Cefsulodin Irgasan- Novobiocin (CIN) Agar	AM1116/AM5116	Yersinia Selective Agar Base			
	AS029	Yersinia Selective Supplement	*		
Christensen's Urea Agar	AM1105/AM5105	Urea Agar Base, Christensen's			
	AS028	Urea, 40%	*	*	
Citrate Agar, Christensen	AM1025/AM5025	Christensen Citrate Agar	*		
Columbia Blood Agar Base	AM1029/AM5029	Columbia Blood Agar Base	*		
Cooked Meat Medium	AM1030/AM5030	Cooked Meat Medium	*		
Deoxycholate Citrate Agar	AM1031/AM5031	Deoxycholate Citrate Agar	*		*
Dextrose Tryptone Bromo Cresol Purple Agar	AM1036/AM5036	Dextrose Tryptone Agar	*	*	
Dextrose Tryptone Brom Cresol Purple Broth	AM1037/AM5037	Dextrose Tryptone Broth	*		
EC Broth	AM1039/AM5039	EC Broth	*	*	*
Eosin Methylene Blue Agar, Levine	AM1040/AM5040	EMB Agar, Levine	*	*	*
Lactose Broth	AM1042/AM5042	Fluid Lactose Medium	*	*	*
Kligler Iron Agar (KIA)	AM1050/AM5050	Kligler Iron Agar	*		
Lactose Broth	AM1052/AM5052	Lactose Broth	*	*	*
Lauryl Sulphate Tryptose Broth (LST)	AM1053/AM5053	Lauryl Sulphate Broth	*	*	*

APHA	ACCUMIX CAT. NO.	ACCUMIX	FOOD	DAIRY	WATER
MR-VP Medium	AM1070/AM5070	MR-VP Medium	*	*	*
MacConkey Agar	AM1059/AM5059	MacConkey Agar with Crystal Violet, NaCl and			
		0.15% Bile Salts	*	*	*
MacConkey Broth	AM1063/AM5063	MacConkey Broth Purple with			
		Bromocresol Purple	*	*	*
Malonate Broth, Modified	AM1065/AM5065	Malonate Broth, Ewing Modified	*	*	*
Mueller Hinton Agar	AM1071/AM5071	Mueller Hinton Agar	*		
Nutrient Agar	AM1075/AM5075	Nutrient Agar pH 6.8		*	*
Nutrient Broth	AM1077/AM5077	Nutrient Broth		*	
Plate Count Agar	AM1081/AM5081	Plate Count Agar	*	*	*
Potato Dextrose Agar (Acidified)	AM1082/AM5082	Potato Dextrose Agar	*	*	
Salmonella Shigella Agar	AM1093/AM5093	SS Agar	*	*	*
Selenite Cystine Broth	AM1044/AM5044	Fluid Selenite Cystine Medium	*	*	*
Simmons Citrate Agar	AM1090/AM5090	Simmons Citrate Agar	*	*	*
Tetrathionate Broth	AM1096/AM5096	Tetrathionate Broth Base, Hajna	*	*	
Thiosulphate Citrate Bile Salt Sucrose Agar	AM1095/AM5095	TCBS Agar	*		*
Triple Sugar Iron Agar	AM1099/AM5099	Triple Sugar Iron Agar	*	*	*
Trypticase Soya Agar	AM1091/AM5091	Soyabean Casein Digest Agar	*	*	
Trypticase Soya Broth	AM1092/AM5092	Soyabean Casein Digest Broth	*	*	*
Tryptone (Tryptophan) Broth	AM1104/AM5104	Tryptone Water	*		*
Tryptone Phosphate Broth	AM1103/AM5103	Tryptone Phosphate Broth	*		
Tryptone Glucose Extract Agar	AM1101/AM5101	Tryptone Glucose Yeast Extract Agar	*	*	*
Urea Broth	AM1106/AM5106	Urea Broth Base			
	AS028	Urea 40%	*	*	
Violet Red Bile Agar	AM1107/AM5107	Violet Red Bile Agar	*	*	
XLD Agar	AM1112/AM5112	Xylose Lysine Deoxycholate Agar	*	*	*
Yeast Malt Agar	AM1114/AM5114	Yeast Malt Agar		*	*

Exploring

Accumix

Dehydrated Culture Media ■ Bases ■ Supplements



"Before you discover you must explore."

Acetate Differential Agar AM5908

Use

Acetate Differential Agar is used to differentiate species of *Shigella* from *Escherichia coli* and non-fermentative gram-negative bacilli.

Summary

Bacteria that are capable of growing on a simple, chemically defined medium can readily be tested for their ability to use a given compound as their sole source of carbon and energy. A defined medium is prepared with the test compound substituted for the normal carbon and energy source. Acetate Differential Agar is used to test the ability of an organism to utilize acetate as the sole carbon and energy source. Organic acids have been widely used in media containing organic nitrogen sources as a means of differentiating *Enterobacteriaceae*. Most bacteria can use citrate and acetate in the presence of organic nitrogen. Trabulsi and Ewing developing Acetate Differential Agar by replacing sodium citrate by sodium acetate made a new approach. This medium differentiates the members of the genus of *Shigella* species from *Escherichia coli*. Differentiation is based on the organism's ability to utilize acetate.

Principle

Acetate Differential Agar consists of a mixture of salts and sodium acetate, as a sole source of carbon. Bromothymol blue acts as an indicator. *Shigella* species are unable to utilize acetate and fail to grow;

and therefore the medium remains unchanged. Most strains of *Escherichia coli* grow well within 24-48 hours, but some strains grow more slowly. Growth indicates the utilization of acetate, which results in

the production of alkaline products. The colour of the indicator changes to blue due to the increase in pH.

Formula*

Ingredients in grams per liter

Sodium acetate	2.00
Magnesium sulphate	0.10
Sodium chloride	5.00
Mono-ammonium phosphate	1.00
Dipotassium phosphate	1.00
Bromothymol blue	0.08
Agar	20.00

Final pH (at 25°C) 6.7 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 29.18 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool in slanted position for use as slants.

Quality Control

Dehydrated Appearance

Greenish yellow, homogeneous, free flowing powder.

Prepared Appearance

Emerald green coloured, clear to slightly opalescent gel form in tubes as slants.

Cultural Response

Cultural characteristics up to 1-7 days at 35-37°C.

Organisms (ATCC)	Growth	Acetate utilization	RGI
Citrobacter freundii (8090)	Good-luxuriant	+	More than 70%
Enterobacter cloacae (23355)	Good-luxuriant	+	More than 70%
Escherichia coli (25922)	Good-luxuriant	+	More than 70%
Klebsiella pneumoniae (13883)	Good-luxuriant	+	More than 70%
Salmonella serotype Arizonae (13314)	Good-luxuriant	+	More than 70%
Salmonella serotype Typhi (19430)	Poor	_	0%
Shigella sonnei (25931)	None	_	0%
Proteus vulgaris (13315)	None	_	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Key: + = Colour change of the medium from green to blue.

– = No change, medium remains green.

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle and streak on the surface of the agar slant.
2. Incubate aerobically at 35-37°C for at least 7 days with intermittent observation.
3. Observe for a colour change from green to blue along the slant.

Interpretation of Results

1. Bacteria capable of utilizing acetate as the sole carbon source will grow on the medium and produce an alkaline reaction
2. Colour of the medium changes to blue. There is no change of colour in negative reaction.

Precautions / Limitations

1. Some strains of *E. coli* utilize acetate slowly or not at all and may give a

false-negative reaction.

2. Some biotypes of *Shigella flexneri* utilize acetate and thereby may give false- positive result.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label

Acetamide Agar AM59081

Use

Acetamide Agar is recommended for confirmation *Pseudomonas aeruginosa* in water samples.

Summary

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms (34.1 & 104.1). However, few organisms are reported to deaminate acetamide. A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification (42.3 & 85.1.2). The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater*

Principle

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa, Comamonas* (Pseudomonas) *acidovorans, Achromobacter xylosoxidans* subsp. *xylosoxidans (Alcaligenes xylosoxidans)* and *Alcaligenes faecalis* (odorans). Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

Formula

Ingredients in Grams/Litre

Part A	
Acetamide	10.00
Part B	
Sodium chloride	5.00
Dipotassium hydrogen phosphate	1.39
Potassium dihydrogen phosphat	0.73
Magnesium sulphate	0.50
Phenol red	0.012
Agar	15.00

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 22.63 grams of part B in 1000 ml distilled water.
2. Add 10.0 grams of Part A.
3. Heat to boiling to dissolve the medium completely.
4. Dispense in tubes or as desired.
5. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
6. Cool the tubes in a slanted position.

Quality Control

Dehydrated Appearance

Part A :Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Prepared Appearance

Orange coloured clear to slightly opalescent gel forms in tubes as slants.

Cultural Response

Cultural characteristics after 4-7 days at 35-37°C.

Organisms (ATCC)	Growth	Deamination	RGI
Pseudoomonas maltophila (13637)	Luxuriant	Negative reaction, no purplish red colour within 7 days	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Positive reaction, purplish red colour within 7 days	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Limitations of the procedure

1. Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.
2. Only about 37% of apyocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label

Acetamide Broth AM59082

Use

Acetamide Broth is recommended for confirmation of non-fermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

Summary

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms (34.1 &

104.1). However, few organisms are reported to deaminate acetamide. A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification (42.3 & 85.1.2). The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater*

Principle

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa, Comamonas (Pseudomonas) acidovorans, Achromobacter xylosoxidans* subsp. *xylosoxidans (Alcaligenes xylosoxidans) and Alcaligenes faecalis (odorans).* Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

Formula*

Ingredients in Grams/Litre

Part A	
Acetamide	10.00
Part B	
Sodium chloride	5.00
Dipotassium hydrogen phosphate	1.39
Potassium dihydrogen phosphat	0.73
Magnesium sulphate	0.50
Phenol red	0.012

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 7.63 grams of part B in 1000 ml distilled water.
2. Add 10grams of Part A.
3. Heat to boiling to dissolve the medium completely.
4. Dispense in tubes or as desired.
5. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes

Quality Control

Dehydrated Appearance

Part A :Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Prepared Appearance

Orange coloured, clear to slightly opalescent gel forms in the tube as slants solution

Cultural Response

Cultural characteristics after 4-7 day at 35-37°C.

Organisms (ATCC)	Growth	Deamination
Pseudomonas maltophila (13637)	Luxuriant	Negative, no reaction, no purplish red colour within 7 days.
Pseudomonas aeruginosa (27853)	Luxuriant	Positive reaction, purplish red colour within 7 days.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label

AK Agar No. 2 (Sporulating Agar) (Arret and Kirshbaum Medium) AM5909

Use

A K agar No. 2 is a culture medium recommended for the production of spores of *Bacillus subtilis* ATCC 6633, which are used for detection of penicillin and other antibiotic residues in milk and diary products.

Summary

A K Agar No. 2 was devised by Arret and Kirshbaum for specific use in the production of spores of *Bacillus subtilis* ATCC 6633 for use in the Penicillin Milk Test Procedure. This medium was formerly specified in the spore preparation phase of the American Public Health Association disc assay procedure for the detection of sulfa drugs and antibiotics in milk.

Principle

Pancreatic digest of Gelatin, casein enzymic hydrolysate and beef extract are sources of nitrogen, sulfur, amino acids and essential trace ingredients. Yeast extract is a rich source for bacterial replication. Manganous sulfate plays an important role in the sporulation process.

Formula

Ingredients Grams/Litre

Pancreatic digest of Gelatin	6.0
Casein enzymic hydrolysate	4.0
Yeast extract	3.0
Beef extract	1.50
Dextrose	1.0
Manganous sulphate	0.30
Agar	15.00

Final pH (at 25°C) 6.6 ± 0.2

Directions

1. Suspend the 30.8 gms of powder in 1000 ml distilled water.
2. Boil with stirring to dissolve the medium completely.
3. Dispense in 300 ml amounts in Roux on other suitable bottles.

4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

NOTE: Do not autoclave until the medium has been completely dissolved.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 5 days at 35°C.

Organisms(ATCC)	Growth	RGI
Bacillus subtilius (6633)	Luxuriant with sporulation	More than 70%
Bacillus megaterium (9855)	Luxuriant with sporulation	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

For preparation of spore suspensions for use in the FDA procedure for the Penicillin Milk Test.

1. transfer cultures of *Bacillus subtilis* ATCC 6633 monthly to fresh slants.
2. Wash the growth from the fresh slant culture with sterile physiological saline onto the surface of a Roux bottle containing 300 ml of AK agar NO. 2.
3. Incubate the bottles for 5 days at 35 $\pm$ 2°C and wash off the resulting growth into 50 ml of sterile physiological saline.
4. Centrifuge the suspension and decant and discard the supernatant fluid.
5. Resuspend the sediment in sterile saline and heat shock the suspension at 70°c for 30 min.
6. The resultant spore suspension can be stored for several months.

For preparation of spore suspensions for use in the APHA procedure for detection of sulfa drugs and antibiotics in milk.

1. Transfer cells of *Bacillus megaterium* ATCC 9855 by streaking the entire surface of sterile AK agar NO. 2 contained in a prescription (180 ml capacity) or Roux bottle.
2. Incubate the bottles at 35 $\pm$ 2°C for 48 hours.
3. After incubation, wash the spores and vegetative cells from the agar surface with buffered MS (microbiologically suitable) water.
4. Sediment the spores and cells by centrifugation at 5,000x g for 15 minutes at 3°C.
5. Store the spore suspension in buffered MS water under refrigeration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label

Alkaline Peptone Water AM1910/AM5910

Alkaline Peptone Water ISO AM5911

Alkaline Peptone Water BIS AM1912/AM5912

Use

Alkaline Peptone water is used as an enrichment medium for the cultivation of *vibrio s* pecies from clinical and nonclinical specimen.

Summary

Alkaline peptone water is recommended by APHA for isolation of *vibrio* species from food. *Vibrio* species grow best in alkaline conditions and this medium provides a favorable environment for the growth of *vibrios* (1). is recommended by ISO committee under the specifications ISO 8914: 1990. It is also useful for preliminarily enrichment of *vibrios* from faeces or other contaminated materials (89.2).

Principle

Peptic digest of animal tissue provide carbon, nitrogen and essential nutrients necessary for growth. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium.

Formula*

Ingredients in grams/ liter	Alkaline Peptone Water	Alkaline Peptone Water ISO	Alkaline Peptone Water BIS
Peptic digest of animal tissue	10.0	20.0	10.0
Sodium Chloride	10.0	30.0	5.0
Final pH (at 25°C)	8.4 ±0.2	8.6 ±0.2	8.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend then powder in 1000 ml distilled water.

   Alkaline Peptone Water - 20 gms

   Alkaline Peptone Water ISO - 50 gms

   Alkaline Peptone Water BIS - 15 gms
2. Mix thoroughly.

3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C(15lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C

Organisms(ATCC)	Growth
Vibrio parahaemolyticus (17802)	Luxuriant
Vibrio cholerae (15748)	Luxuriant
Vibrio fischeri (1738)	Luxuriant

Procedure

For Clinical Sample

1. Swab specimen inserts directly into medium.
2. For faecal specimen inoculate1g faeces into 10ml APW, or about 2g into 20 ml.
3. Incubate at 37°C for 18-12 hrs.
4. Inoculate a loopful from the top of the peptone water on to a selective plating medium such as TCBS Agar (Cat. No.AM5095).

For Food Sample

1. Add 10gms of seafood to 90 ml of APW.
2. Incubate at 37°C for 18-20 hrs.
3. Inoculate a loopful from the peptone water on to a selective plating medium such as TCBS Agar (Cat. No.AM5095).

Interpretation of Results

Growth in the medium is indicated by the presence of turbidity compared to an uninoculated control.

Precautions / Limitations

1. It is preferable that biochemical and / or serological tests be performed on colonies from pure culture for complete identification.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label

Alternative Thioglycollate Medium — AM1900/AM5900

Use

Alternative Thioglycollate Medium (NIH Thioglycollate Broth) is recommended for sterility testing of certain biological products, which may be turbid or viscous.

Summary

Alternative Thioglycollate Medium (NIH Thioglycollate Broth) recommended by USP is the Fluid Thioglycollate Medium without agar and indicator components. It is used for the same sterility tests as that of Fluid Thioglycollate Medium except that anaerobic incubation is recommended rather than aerobic incubation. This medium also meets the requirements of the USP growth promotion test (111).

Principle

Tryptone, Yeast extract and L-cystine provide sources of nitrogen, carbon and other growth factors while dextrose is the carbohydrate source. Sodium chloride provides essential ions and maintains the osmotic balance. Sodium thioglycollate is a reducing agent, which prevents the accumulation of peroxides that is lethal to bacterial growth and neutralizes the antibacterial effect of mercurial preservatives. L-cystine is also a reducing agent, since it contains sulphydryl groups that inactivate heavy metal compounds, which exert a bacteriostatic effect in the materials under examination, and also maintains a low redox potential, thereby maintaining anaerobiosis. Absence of agar makes it a suitable medium for sterility testing of viscous materials and devices having tubes with small lumina.

Formula*

Ingredients in grams per liter

Tryptone	15.0
Dextrose	5.5
Yeast Extract	5.0
Sodium Chloride	2.5
Sodium Thioglycollate	0.5
L-Cystine	0.5

Final pH (at 25° C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29 grams of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for one minute to dissolve the powder completely.
4. Dispense as desired into containers.
5. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
6. Tighten lids of the containers immediately (while still warm) to reduce oxidation.

7. Cool to 25^0 C and store in a cool dark place preferably below 25^0 C.

Note: If more than the upper one third of the medium is pink prior to use, reheat once (100^{0}C) in a water bath to drive off absorbed oxygen (till pink colour disappears).

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24 to 72 hours at 35^0 C.

Organisms (ATCC)	Growth
**Bacillus subtilis* (6633)	Luxuriant
**Bacteroides vulgatus* (8482)	Luxuriant
**Candida albicans* (10231)	Luxuriant
**Clostridium sporogenes* (11437)	Luxuriant
**Micrococcus luteus* (9341)	Luxuriant
Streptococcus pyogenes (19615)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant
Neisseria meningitidis (13090)	Luxuriant
Bacteroides fragilis (25285)	Luxuriant

*These cultures may be incubated at 25-30^0 C for 2-7 days.

Procedure

1. Aseptically inoculate each tube with the sample for testing.
2. Incubate the tubes anaerobically at 35-37^0 C.
3. Observe for growth after 24 hours and up to 72 hours.

Interpretation of Results

1. Upon incubation, growth is indicated by the presence of turbidity compared to an uninoculated control.
2. Subcultures to appropriate solid media should be made to obtain pure cultures of isolates, which can then be further tested and identified.

   Note: Inspect the tubes for clarity before inoculation. Ensure that the tubes are labeled correctly with the respective sample.

Precautions / Limitations

1. Some dextrose fermenting organisms, which are able to reduce the pH of the medium to a critical level, may not survive in this medium. Early subculture is required to isolate these organisms.
2. In test samples, the proper surface to volume ratio of the medium must be maintained to avoid oxidation of the medium, which is unsuitable for microaerophilic and anaerobic growth.
3. Anaerobes can be overgrown by more rapidly growing facultative organisms. Gram stain and examine the broth if plating medium reveals no growth.
4. The growth of some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
5. Do not rely on broth cultures exclusively for isolation of anaerobes.
6. Do not reheat the medium more than once as it may give rise to toxicity.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label

Anaerobic Agar — AM1000/AM5000

Use

Anaerobic Agar is recommended for the cultivation of anaerobic microorganisms.

Summary

Anaerobic bacteria cause a variety of infections in humans including ottis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma and bacteremia. Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body. Anaerobes vary in their sensitivity to oxygen and nutritional requirements. Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.

Brewer described a special petri dish cover that allows surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of brewer's original formula. The medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.

Principle

Tryptone in the medium provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing a source of carbon and energy. Sodium Chloride maintains the osmotic equilibrium. Sodium Thioglycollate and Sodium Formaldehyde Sulfoxylate are reducing agents generating a low oxidation-reduction potential, thus maintaining good anaerobic conditions. Methylene Blue serves as an indicator of anaerobiosis with a blue colour indicating the presence of oxygen.

Formula*

Ingredients in grams per liter

Tryptone	20.0
Dextrose	10.0
Sodium Chloride	5.0
Sodium Thioglycollate	2.0
Sodium Formaldehyde Sulfoxylate	1.0
Methylene Blue	0.002

Agar	20.0

Final pH (at 25° C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 58 grams of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for one minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light beige coloured, homogeneous free flowing powder.

Prepared Appearance

Light green, slightly opalescent.

Cultural Response

Cultural characteristics after 48-72 hours at 35°C when incubated anaerobically.

Organisms (ATCC)	Growth	RGI
Clostridium butyricum (9690)	Luxuriant	More than 70%
Clostridium perfringens (12914)	Luxuriant	More than 70%
Clostridium sporogenes (11437)	Luxuriant	More than 70%
Bacteroides fragilis (25285)	Good	More than 70%
Fusobacterium mortiferum (9817)	Good	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

For Standard Petri Dishes

1. Inoculate a properly obtained specimen onto the medium using the pour plate technique.
2. Incubate anaerobically at 35° C.
3. If the plates are incubated in an anaerobic chamber, examine at 24 hours. If the plates are incubated in an anaerobic jar or anaerobic pouch, examine at 48 hours.
4. The incubation period may be extended if necessary to recover some anaerobes.

For Brewer Anaerobic Agar Plates

1. Dispense 50-60 ml of Anaerobic Agar into a standard petri dish. For best results use porous tops during solidification to obtain a dry surface.
2. Inoculate the surface of the medium by streaking or smearing.
3. Replace the petridish lid with a sterile Brewer petri dish cover. Note: The cover should not rest on the petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. The sealing ring inside the cover must be in contact with the medium. The seal must not be broken before the end of the incubation period. A small amount of air may be caught over the surface of the medium, which will react with the reducing agents to form an anaerobic environment.
4. Incubate aerobically as desired.

Interpretation of Results

1. Refer to U.S.P. and other appropriate references for interpretation of results.

Precautions / Limitations

1. Methylene Blue is inhibitory to some anaerobic microorganisms.
2. Clinical specimens must be obtained properly and transported to the laboratory in suitable anaerobic transport container.
3. It is essential to determine the environment of the medium so as to ascertain whether is anaerobic.
4. To ascertain whether an organism is an anaerobe, it is essential to perform aerotolerance testing on each isolate recovered.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Andrade Peptone Water — AM1001/AM5001

Andrade Peptone Water BIS — AM10011

Use

Andrade Peptone Water is a base to which various carbohydrates may be added to study fermentation reactions, particularly of members of the *Enterobacteriaceae*.

Summary

Andrade Peptone Water is used for studying the various carbohydrate fermentation patterns of different organisms.

Principle

Peptone used in this medium is free from fermentable carbohydrates and nitrates, which may interfere with gas production. Andrade indicator is a solution of acid fuchsin which when titrated with sodium hydroxide, changes colour from pink to yellow. As the pH decreases, the colour changes from yellow to pink. The medium is pink when hot but becomes straw coloured on cooling.

The biochemical identification of organisms capable of growing in this medium is made by observing the catabolism of various carbohydrates added to separate tubes of peptone water. To detect fermentation, which is the production of acid and gas, a small inverted Durham's tube is inserted into the peptone water to trap any gas produced by the organism. A single bubble in the tube is sufficient to label the organism positive for gas formation. Some organisms will utilize the sugar to produce acid only, without gas formation.

Formula*

Ingredients in grams per liter

Peptone	10.00
Sodium Chloride	5.00
Andrade Indicator	0.10

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15.1 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense desired quantity in test tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Cool to room temperature. Aseptically add sterile stock solution of carbohydrate to a final concentration of 0.5% to 1.0% (w/v).

Quality Control

Dehydrated Appearance

Light yellow coloured with pink tinge, homogeneous, free flowing powder.

Prepared Appearance

Light pink coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Acid*	Acid**
Escherichia coli (25922)	Luxuriant	-	+
Salmonella serotype typhi (6539)	Luxuriant	-	+
Shigella sonnei (25931)	Luxuriant	-	+

Key:

*= Acid in absence of added Dextrose.

**= Acid in the presence of added Dextrose.

Procedure

1. Aseptically inoculate each tube of Andrade Peptone Water containing an inverted Durham's tube and the respective carbohydrate with a single, well separated colony or with a colony from a plate.
2. Incubate the tubes at 35-37°C aerobically. If anaerobic incubation is required then fresh indicator should be added to the tubes after incubation at the time of examination.
3. Observe for bubbles or gas and any colour change in the medium. Most reactions are complete within 18-24 hours, however it may be necessary to look for late reactions after prolonged incubation.

Interpretation of Results

1. Acid is produced when the carbohydrate is fermented and this is indicated by a yellow color change in the medium.
2. Gas production is indicated by the presence of gas bubbles in inverted Durham's tube.

Precautions / Limitations

1. Inspect the tubes for clarity before inoculation. Ascertain that there is no colour or only a slight pinkness in the medium and that the tubes contain no air trapped in the inverted Durham's tube.
2. Check whether each individual tube is correctly labeled for the contained sugar.
3. Andrade Peptone Water is reddish-pink when hot, it should return to a colourless or slightly pink coloured solution when cooled to room temperature.
4. Some sugar solutions may affect the pH of the medium. Care should be taken to avoid alterations in pH.
5. Mixed or contaminated cultures may give false reactions.
6. Andrade indicator may fade on prolonged storage and should not be used beyond the expiry date.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Antibiotic Assay Medium A (No. 1, Seed Agar) AM1002/AM5002

Antibiotic Assay Medium C (No. 3, Assay Broth) AM1003/AM5003

Antibiotic Assay Medium No. 11 (Neomycin, Erythromycin Assay Agar) AM1004/AM5004

Use

Antibiotic Assay Media are used for determining antibiotic potency by microbiological assay techniques.

Summary

Antibiotic Assay Media are used in the performance of antibiotic assays and conforms to the specifications of USP (113) and FDA. The activity or potency of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. Antibiotic assays are performed by the cylinder plate method, punched-hole method, paper disc method, serial dilution method and the turbidimetric assay methods. Antibiotic Assay Media are identified numerically with names assigned by Grove and Randall in the Assay Methods of Antibiotics.

Principle

Peptone, tryptone, yeast extract and beef extract provide nutrients and growth factors. Sodium chloride maintains the osmotic balance. Dipotassium phosphate and potassium dihydrogen phosphate provide buffering action. Dextrose is the carbon and energy source.

Formulae*

Ingredients in grams per liter	Antibiotic Assay Medium A (No.1)	Antibiotic Assay Medium C (No.3)	Antibiotic Assay Medium No.11
Peptone	6.0	5.0	6.0
Tryptone	4.0	-	4.0
Yeast Extract	3.0	1.5	3.0
Beef Extract	1.5	1.5	1.5
Dextrose	1.0	1.0	1.0
Sodium Chloride	-	3.5	-
Dipotassium Phosphate	-	3.68	-
Potassium Dihydrogen Phosphate	-	1.32	-
Agar	15.0	-	15.0
Final pH (at 25°C)	6.6 ± 0.2	7.0 ± 0.2	8.3 ± 0.1

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

Antibiotic Assay Medium A (No. 1)	30.5 gms.
Antibiotic Assay Medium C (No. 3)	17.5 gms.
Antibiotic Assay Medium No. 11	30.5 gms.

2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C. Pour into sterile petri plates as desired.

Quality Control

Antibiotic Assay Media	Dehydrated Appearance	Prepared Appearance	Cultural Response
Antibiotic Assay Medium A (No. 1)	Light yellow coloured, homogeneous, free-flowing powder	Light yellow coloured, slightly opalescent gel	Cultural characteristics after 18-24 hours at 35-37°C
Antibiotic Assay Medium C (No. 3)	Light yellow coloured, homogeneous, free-flowing powder	Light yellow coloured, clear solution, without any precipitate	Cultural characteristics after 18-24 hours at 35-37°C
Antibiotic Assay Medium No. 11	Light yellow coloured, homogeneous, free-flowing powder	Light yellow, very slightly to slightly opalescent gel	Cultural characteristics after 18-24 hours at 35-37°C

Antibiotic Assay Medium A (No. 1)

Organisms (ATCC)	Growth
Bacillus subtilis (6633)	Good to luxuriant
Escherichia coli (25922)	Good to luxuriant
Micrococcus luteus (10240)	Good to luxuriant
Staphylococcus aureus (29737)	Good to luxuriant
Staphylococcus epidermidis (12228)	Good to luxuriant
Klebsiella pneumoniae (10031)	Good to luxuriant

Antibiotic Assay Medium C (No. 3)

Organisms (ATCC)	Growth	Serial Dilution test with
Escherichia coli (10536)	Luxuriant	Chloramphenicol, Spectinomycin
Staphylococcus aureus (29737)	Luxuriant	Amikacin, Chlortetracycline

Organisms (ATCC)	Growth	Serial Dilution test with
Klebsiella pneumoniae (10031)	Luxuriant	Streptomycin, Capreomycin
Streptococcus faecium (10541)	Luxuriant	Gramicidin, Tyrothricin

Antibiotic Assay Medium No. 11

Organisms (ATCC)	Growth	Inhibition Zones with
Bacillus pumilus (14884)	Luxuriant	Neomycin, Framycetin
Micrococcus luteus (10240)	Luxuriant	Ampicillin, Clindamycin, Erythromycin
Staphylococcus aureus (25923)	Luxuriant	Kanamycin, Neomycin
Staphylococcus epidermidis (12228)	Luxuriant	Paromomycin, Neomycin, Netilmycin

Procedure

1. Maintain stock cultures of test organisms on agar slants and make transfers at 1 or 2 week intervals.
2. Prepare the inoculum for assay by suspending growth from a fresh 24-48 hour agar slant using sterile purified water or saline.

3. Dilute the culture to obtain desired concentration of the test organism.

Note: When *Bacillus subtilis* is used as the test organism;

- Inoculate it on Antibiotic Assay Medium A (No. 1).
- Incubate at 37°C for 1 week.
- Wash spores from the agar surface and heat the spores at 56°C for 30 minutes.
- Wash the spores three times in purified water and heat again at 65°C for 30 minutes.
- Dilute to the required optimal concentration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Assay Methods

Cylinder Plate Method

This method depends upon diffusion of the antibiotic from vertical steel cylinders placed on the surface of the inoculated agar medium. This produces a zone of inhibition around the cylinder containing antibiotic solution depending upon the concentration of the antibiotic.

For the assay, use 20 x 100 mm glass or plastic petri plates with sufficient depth so that the cylinders used in the assay will not be pushed into the medium by the cover. Use stainless steel or porcelain assay cylinders having the following dimensions : 8 mm outside diameter, 6 mm inside diameter and 10 mm long (0.1 mm). Carefully clean the cylinders to remove all residues, using an occasional acid bath (approximately 2N nitric acid or chromic acid). Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius. To assure accurate assays, work on a level surface to obtain uniformly thick base and seed layers in the petri plate. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics with most assays specifying a 21 ml base layer and a 4 ml seed layer. In any case, plates with flat bottoms are required to assure complete coverage of the bottom of the plate when a small amount of basal medium is used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Punched-Hole Method

In this method, holes are punched out of the inoculated culture medium and the antibiotic solutions are then pipetted into them. Rest of the procedure is similar to that of the cylinder plate method.

Paper-Disc Method

Paper discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. Antibiotic can also be applied to the disc after it has been placed on the medium. Use plates containing a single layer of medium with 2 mm thickness for these tests. Rest of the procedure is similar to the cylinder plate method.

Serial Dilution Method

Minimum Inhibitory Concentration (MIC) of an antibiotic can be expressed by determining the antibiotic activity quantitatively. It is done using the known sensitivity of a test organism towards a particular antibiotic. First, serial dilutions of the antibiotic to be tested are pipetted out into the antibiotic broth. A defined quantity of the test organism is then inoculated in this medium. The last tube that does not show any turbidity due to suppression of microbial growth indicates the presence of active antibiotic at a concentration corresponding to the minimum inhibitory concentration.

Turbidimetric Assay Method

The turbidimetric method depends upon the inhibition of the test organism in a medium containing uniform solution of an antibiotic. The advantage of this method over that of cylinder plate method is that it requires a shorter incubation period of 3-4 hours. Use glass or plastic test tubes (16 x 125 mm or 18 x 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes. Tubes that will be placed in the spectrophotometer should be matched and free from scratches or blemishes. Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution. Sterilize previously used tubes prior to subsequent use.

Prepare working dilutions of the antibiotic reference standards in specific concentrations. To a 1 ml quantity of each solution, add 9 ml of inoculated broth, as required. Prepare similar solutions of the sample containing approximately the same amount of antibiotic activity.

Incubate the tubes in a water bath for 3-4 hours at the required temperature. At the end of the incubation period add 0.5 ml of 1:3 formalin to stop growth. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.

Precautions /Limitations

1. The use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics in order to achieve satisfactory test results.

2. The assay of antibiotics is a highly skilled process, which requires very close attention to the details specified in the official publications, and these must be consulted.

Selection of Method, Media and Test Organism for Antibiotic Assay

Antibiotic Assay	Method	Organism (ATCC)	Antibiotic Medium for				
			Maintenance	Inoculum	Cylinder Plate		Turbidimetric Assay
					Base Layer	Seed Layer	
Amikacin	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Ampicillin	Cylinder plate	*Micrococcus luteus (9341)*	1	1	11	11	-
Bacitracin	Cylinder plate	*Micrococcus luteus (10240)*	1	1	2	1	-
Capreomycin	Turbidimetric	*Klebsiella pneumoniae (10031)*	1	1	-	-	3
Cephalexin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Cephalothin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Cephapirin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Cephradine	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Chloramphenicol	Turbidimetric	*Escherichia coli (10536)*	1	1	-	-	3
Chlortetracycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Clindamycin	Cylinder plate	*Micrococcus luteus (9341)*	1	1	11	11	-
Cloxacillin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Cycloserine	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Demeclocycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Demethylchlortetracycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Dicloxacillin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Dihydrostreptomycin	Turbidimetric	*Bacillus subtilis (6633)*	1	1	-	-	3
Doxycycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Erythromycin	Cylinder plate	*Micrococcus luteus (9341)*	1	1	11	11	-
Gentamicin	Cylinder plate	*Staphylococcus epidermidis (12228)*	1	1	11	11	-
Gramicidin	Turbidimetric	*Streptococcus faecium (10541)*	3	3	-	-	3
Kanamycin	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Lincomycin	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Methacycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Methicillin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Minocycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Nafcillin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Neomycin	Turbidimetric	*Klebsiella pneumoniae (10031)*	1	1	11	11	-
Netilmycin	Cylinder plate	*Staphylococcus epidermidis (12228)*	1	1	11	11	-
Novobiocin	Cylinder plate	*Staphylococcus epidermidis (12228)*	1	1	2	1	-
Oxacillin	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Oxytetracycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Paromomycin	Cylinder plate	*Staphylococcus epidermidis (12228)*	1	1	11	11	-
Penicillin-G	Cylinder plate	*Staphylococcus aureus (29737)*	1	1	2	1	-
Rolitetracycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Sisomycin	Cylinder plate	*Staphylococcus epidermidis (12228)*	1	1	11	11	-
Spectinomycin	Turbidimetric	*Escherichia coli (10536)*	1	1	-	-	3
Streptomycin	Turbidimetric	*Klebsiella pneumoniae (10031)*	1	1	-	-	3
Tetracycline	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Tobramycin	Turbidimetric	*Staphylococcus aureus (29737)*	1	1	-	-	3
Troleandomycin	Turbidimetric	*Klebsiella pneumoniae (10031)*	1	1	-	-	3
Tyrothricin	Turbidimetric	*Streptococcus faecium (10541)*	3	3	-	-	3
Viomycin	Turbidimetric	*Klebsiella pneumoniae (10031)*	1	1	-	-	3

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) AM50013

Use

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) is used for detection of Penicillin-G in milk samples.

Summary

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) is suitable for plate counts in pharmaceutical and related products and for the microbial assay and detection of antibiotics like penicillin in milk. These medium is formulated in accordance to the specifications and procedures listed by the Food and Drug Administration (110.3).This medium is identical numerically with name assigned by Grove and Randall (36.2).

Principle

Peptic digest of animal tissue, yeast and beef extract provides nutritional requirement for growth of the indicator organisms like Bacillus stearothermophilus , Micrococcus luteus . This medium is similar to Antibiotic assay medium no. 2 except for the additional ingredient dextrose. Dextrose in the medium serves as easily available source of carbon stimulating luxuriant growth of the test organims. Generally presence of penicillin in milk is detected by the cylinder plate method, using Micrococcus luteus as the test organism, and a paper disk method, using Bacillus stearothermophilus .

Freshly prepared plates should be used for antibiotic assays. The use of this medium assures well defined zones of the test organism.

Formula*

Ingredients in Grams/Litre

Peptic digest of animal tissue (Peptone)	6.00
Beef extract	1.50
Yeast extract	3.00
Dextrose	1.00
Agar	15.00

Final pH (at 25°C) 6.6±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 26.5 grams in 1000 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 55°C for 18-24 hours.

Organisms (ATCC)	Growth
Bacillus stearothermophilus (7953)	Luxuriant
Micrococcus luteus (10240)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Antibiotic Assay Medium E (No. 5) (Streptomycin Assay Agar with Yeast Extract) AM50031

Antibiotic Assay Medium F (No. 8) (Base Agar with low pH) AM50032

Antibiotic Assay Medium G (No. 19) AM500411

Antibiotic Assay Medium No. 32 AM500412

Use

Antibiotic Assay Media are used for determining antibiotic potency by microbiological assay techniques.

Summary

Antibiotic Assay Media are used in the performance of antibiotic assays and conforms to the specifications of USP (113) and FDA. The activity or potency of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. Antibiotic assays are performed by the cylinder plate method, punched hole method, paper disc method, serial dilution method and the turbidimetric assay methods. Antibiotic Assay Media are identified numerically with names assigned by Grove and Randall in the Assay Methods of Antibiotics.

Principle

Peptone, yeast extract and beef extract provide nutrients and growth factors. Sodium chloride maintains the osmotic balance. Dextrose is the carbon and energy source.

Formula*

Ingredients in grams per liter	Antibiotic Assay Medium E (No.5)	Antibiotic Assay Medium F (No.8)	Antibiotic Assay Medium G (No.19)	Antibiotic Assay Medium (No.32)
Peptic digest of animal tissue	6.0	6.0	9.4	6.0
Yeast extract	3.0	3.0	4.7	3.0
Beef extract	1.5	1.5	2.4	1.5
Dextrose	-	-	10.0	1.0
Sodium chloride	-	-	10.0	-
Manganese sulphate	-	-	-	0.3
Casein enzymic hydrolysate	-	-	-	4.0
Agar	15.0	15.0	23.5	15.0
Final pH (at 25°C)	7.9 ±0.2	5.9 ±0.2	6.1 ±0.2	6.6 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.
   Antibiotic Assay Medium E (No. 5) 25.5 gms.
   Antibiotic Assay Medium F (No. 8) 25.5 gms.
   Antibiotic Assay Medium G(No. 19) 60.0 gms.
   Antibiotic Assay Medium (No. 32) 30.8gms.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C. Pour into sterile petri plates as desired.

Quality Control

Antibiotic Assay Media	Dehydrated Appearance	Prepared Appearance	Cultural Response
Antibiotic Assay Medium E (No. 5)	Light yellow coloured, homogeneous, free-flowing powder	Medium amber coloured, slightly opalescent gel	Cultural characteristics after 18-24 hours at 35-37°C
Antibiotic Assay Medium F (No. 8)	Light yellow coloured, homogeneous, free-flowing powder	Light amber coloured, slightly, opalescent gel	Cultural characteristics after 18-24 hours at 35-37°C
Antibiotic Assay Medium G (No. 19)	Light yellow coloured, homogeneous, free-flowing powder	Yellow coloured, clear to slightly, opalescent gel	Cultural characteristics after 18-24 hours at 25-30°C
Antibiotic Assay Medium (No. 32)	Light yellow coloured, homogeneous, free-flowing powder	Yellow coloured, slightly, opalescent gel	Cultural characteristics after 18-24 hours at 35-37°C

Antibiotic Assay Medium E(No. 5)

Organisms (ATCC)	Growth	Inhibition zone with
Bacillus subtilis (6633)	Good to luxuriant	Dactinomycin, Dihydrostreptomycin, Kanamycin B, Rifampicin, Streptomycin

Antibiotic Assay Medium F(No. 8)

Organisms (ATCC)	Growth	Inhibition zone with
Bacillus subtilis (6633)	Luxuriant	Mitomycin, Vancomycin, Plicamycin
Staphylococcus aureus (29737)	Luxuriant	

Antibiotic Assay Medium G (No. 19)

Organisms (ATCC)	Growth	Inhibition zone with
Saccharomyces cerevisiae (9763)	Luxuriant	Amphotericin B, Netamycin, Nystatin
Saccharomyces cerevisiae (2601)	Luxuriant	

Antibiotic Assay Medium (No. 32)

Organisms (ATCC)	Growth	Inhibition zone with
Bacillus subtilis (6633)	Good to luxuriant	Dihydrostreptomycin, Vancomycin

Procedure

1. Maintain stock cultures of test organisms on agar slants and make transfers at 1 or 2 week intervals.
2. Prepare the inoculum for assay by suspending growth from a fresh 24-48 hour agar slant using sterile purified water or saline.
3. Dilute the culture to obtain desired concentration of the test organism.

   Note: When Bacillus subtilis is used as the test organism;

   - Inoculate it on Antibiotic Assay Medium (No. 5 & 32).
   - Incubate at 37 for 1 week.
   - Wash spores from the agar surface and heat the spores at 56 for 30 minutes.
   - Wash the spores three times in purified water and heat again at 65 for 30 minutes.
   - Dilute to the required optimal concentration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Antibiotic Assay Medium No. 10 AM50034

Use

Antibiotic Assay Medium No. 10 is used as seed layer medium for assaying the products containing Polymyxin B, Carbenicillin, Colistin and Colistimethate sodium.

Summary

Antibiotic Assay Media are used in the performance of antibiotic assays and conforms to the specifications of USP (113) and FDA.

Principle

Papaic digest of soyabean meal and casein enzymic hydrolysate in this medium provide the essential nutrient for growth of test organisms. Sodium chloride maintains the osmotic balance. Dipotassium phosphate provide buffering action. Dextrose is the carbon and energy source and agar provide solid substratum.

Formula*

Ingredients in Grams/Litre

Casein enzymic hydrolysate	17.00
Papaic digest of soyabean meal	3.00
Sodium chloride	5.00
Dextrose	2.50
Dipotassium phosphate	2.50
Agar	12.00

Final pH (at 25°C) 7.2±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 42 gm the powder in 1000 ml distilled water containing 10 ml of Polysorbate 80..
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics upto 18-48 Hour at 36-37.5°C.

Organisms (ATCC)	Growth	Inhibition zone with
Pseudomonas aeruginosa (27853)	Luxuriant	Carbenicillin
Bordetella bronchiseptica	Luxuriant	Polymixin B, Colistimethate sodium, Colistin

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Antibiotic Assay Medium No.12 (Nystatin Assay Agar) AM50014

Use

Antibiotic Assay Medium No.12 (Nystatin Assay Agar) is used for microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae*.

Summary

This medium is prepared from the Groove and Randall formula (36.2)Antifungal antibiotics like Amphotericin B and Nystatin can be assayed using this medium.

Principle

Peptic digest of animal tissue, yeast and beef extract are essential nutrients, minerals and growth factors for the growth of test organism. Dextrose in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is by sodium chloride which maintain the cell intergrity and viability. Freshly prepared plates should be used for antibiotic assays.

Formula*

Ingredients in Grams/Litre

Peptic digest of animal tissue	10.00
Sodium chloride	10.00
Dextrose	10.00
Beef extract	2.50
Yeast extract	5.00
Agar	25.00

Final pH (at 25°C) 6.1±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 62.5 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressu re (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 18-24 hours.

Organisms (ATCC)	Growth	Inhibition zone with
Saccharomyces cerevisiae (2601)	Luxuriant	Amphotericin B, Nystatin

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Antibiotic Assay Medium No. 35 — AM500414

Use

Antibiotic Assay Medium No. 35 is used for the microbiological assay of Bleomycin using *Mycobacterium smegmatis*

Summary

This medium is formulated in accordance to CFR (110.3). This medium is employed widely as base agar for agar diffusion assay of Bleomycin using *Mycobacterium smegmatis.*

Principle

Peptic digest of animal tissue and beef extract in this medium provide the essential nutrient for growth of test organisms. Agar provides excellent solid substratum for support and over layering of seed agar, for the assay of Bleomycin. Addition of glycerol is important for provision of carbon to the test organism. To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized culture can be overlaid. Even distribution of the layer is important.

Formula*

Ingredients in grams per liter

Peptone	10.000
Beef extract	10.000
Sodium chloride	3.000
Agar	17.000

Final pH (at 25°C) 7.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40 gm the powder in 1000 ml distilled water containing 10 ml glycerol.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C. Pour into sterile petri plates as desired.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics upto 18-48 Hour at 36-37.5°C.

Organisms (ATCC)	Growth	Inhibition zone with
Mycobacterium smegmatis (607)	Luxuriant	Bleomycin

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Antifungal Assay Agar — AM10041/AM50041

Use

Antifungal Assay Agar is recommended for assaying the antifungal activity.

Summary

Antifungal Assay Agar is recommended for assaying the antifungal activity of pharmaceutical and other products using the cylinder plate or paper disc method.

Principle

Antifungal Assay Agar was formulated by Berger and Lazecka to conveniently assay the antifungal activity of of pharmaceutical and other products by both base and seed layers using the cylinder plate or paper disc methods. The principle used here is that antibiotics of varying concentrations are impregnated on the paper disc or small cylinder, which will diffuse into the medium containing the test organisms. The appearance of a zone of inhibition surrounding the disc is indicative of sensitivity. By comparing the diameter of the zones to the standard, it is possible to determine whether the test organisms are susceptible or resistant to the respective antibiotic.

Formula*

Ingredients in grams per liter

Dextrose	50.0
Tryptone	4.0
Sodium Citrate	4.5
Potassium Phosphate	0.55
Citric Acid	1.0
Pyridoxine hydrochloride	0.00025

Thiamine	0.00025
Inositol	0.025
Calcium Pantothenate	0.0025
Niacin	0.0025
Potassium Chloride	0.425
Calcium Chloride	0.125
Magnesium Sulphate	0.125
Ferric Chloride	0.0025
Manganese Sulphate	0.0025
Biotin	0.000008
Agar	15.0

Final pH (at 25° C) 5.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 75.76 grams of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Pale beige coloured, homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured clear gel.

Cultural Response

Cultural characteristics after 18-48 hours at 30° C.

Organisms (ATCC)	Growth	RGI
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Asperigillus niger (16404)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Refer to U.S.P. and other appropriate references for procedures and interpretation of results.

Precautions / Limitations

1. The assay of antibiotics is a highly skilled process, which requires very close attention to the details specified in the official publications, and these must be consulted.
2. Inspect the petriplates before inoculation. Keep the plates at 2-8° C for half an hour after placing the antibiotics discs for diffusion to take place.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Artificial Sea Water AM50042

Use

Artificial sea-water used for maintaining or cultivating organisms and plants living in the sea-water.

Summary

Artificial seawater is a complex mix of many purified salts formulated to have a composition similar to natural seawater when dissolved in distilled water. The quality of sea water is dependent on the formula, the quality of the raw materials and the uniformity of the blending. The salinity is the sum of all of the dissolved ions. Natural seawater is generally considered to have a salinity of 35 parts per thousand (ppt) or grams of salt per kilogram of water.

Principle

Artificial sea-water is based on the formulation described by Layman and Fleming (73.1). The high salt content helps to simulate sea water. These salts provide marine environment for maintenance and cultivation of sea living organisms. The mixture of inorganic salts approximating the composition of sea water, is used in place of natural sea water for the growth of marine organisms(79.2).

Formula*

Ingredients in grams per liter

Sodium chloride	24.6
Potassium chloride	0.67
Calcium chloride. $2H_2O$	1.36
Magnesium sulphate. $7H_2O$	6.29
Magnesium chloride. $6H_2O$	4.66
Sodium bicarbonate	0.18

Final pH at 25°C 8.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.76 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Filter through Whatman Filter paper.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Ashby's Glucose Agar AM50043

Use

Ashby's Glucose Agar is used for cultivation of *Azotobacter* species by using glucose as carbon source.

Summary

Subha Rao has formulated the Ashby's media(105.1). It is recommended for the isolation of *Azotobacter* species.

Principle

Glucose acts as carbon source. *Azotobacter* species is a non-symbiotic nitrogen fixer. It uses atmospheric nitrogen as nitrogen source.

Formula*

Ingredients in grams per liter

Glucose	20.0
Dipotassium phosphate	0.20
Magnesium sulphate	0.20
Sodium chloride	0.20
Potassium sulphate	0.10
Calcium carbonate	5.00
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 40.7 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Off white coloured, homogeneous free flowing powder.

Prepared Appearance

Whitish opalescent gel form in petridishes.

Cultural Response

Cultural characteristics upto 5 days at 37°C.

Organisms (ATCC)	Growth	RGI
Azotobacter vinelandii (478)	Luxuriant	More than 70%
Azotobacter nigricans (35009)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of *Azotobacter* species.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Ashby's Mannitol Agar AM50044

Use

Ashby's Mannitol Agar is used for cultivation of *Azotobacter* species by using mannitol as carbon source.

Summary

Subha Rao (105.1) has formulated the Ashby's media. It is recommended for the isolation of *Azotobacter* species.

Principle

Mannitol acts as carbon source. *Azotobacter* species is a non-symbiotic nitrogen fixer. It uses atmospheric nitrogen as nitrogen source.

Formula*

Ingredients in grams per liter

Mannitol	20.0
Dipotassium phosphate	0.20
Magnesium sulphate	0.20
Sodium chloride	0.20
Potassium sulphate	0.10
Calcium carbonate	5.00
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 40.7 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Off white coloured, homogeneous free flowing powder.

Prepared Appearance

Whitish opalescent gel form in petridishes.

Cultural Response

Cultural characteristics upto 5 days at 37°C.

Organisms (ATCC)	Growth	RGI
Azotobacter vinelandii (478)	Luxuriant	More than 70%
Azotobacter nigricans (35009)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of *Azotobacter* species.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Asparagine Broth AM50045

Use

Asparagine Broth is used for the preparation of Coccidioidin and Histoplasmin antigens for immunodiagnostic work.

Summary

A dimorphic fungus, *Histoplasma capsulatum* causes histoplasmosis, a systemic fungal disease. *H. capsulatumis* an obligate intracellular organism residing in macrophages of the reticuloendothelial system. Of current concern is the increased incidence of histoplasmosis in patients with AIDS (49.3). *Coccidiodes immitis*, the causative agent of coccidioidomycosis (Valley fever) is endemic in hot regions with dry climate and alkaline soil. Patients with AIDS are at a risk of developing coccidioidomycosis.

Principle

Asparagine Broth is a chemically defined medium used for the preparation of Coccidioidin and Histoplasmin antigens. The amino acid asparagine, favours the synthesis of antigens from Histoplasma and Coccidiodes . Salts included in the medium buffer the medium well. Dextrose and slightly acidic pH of the medium helps for the luxuriant growth of the fungi.

Formula*

Ingredients in grams per liter

Ingredients in grams per liter	
L-Asparagine	7.00
Ammonium chloride	7.00
Dipotassium phosphate	1.31
Sodium citrate	0.90
Magnesium sulphate	1.50
Ferric citrate	0.30
Dextrose	10.00

Final pH (at 25°C) 6.8±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 28.01 grams in 1000 ml distilled water containing 25 ml glycerol.
2. Mix thoroughly and then dispense in a wide bottom flask, to give a depth of 1to 1.5 inches.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Off-white to yellow homogeneous free flowing powder

Prepared Appearance

Light yellow coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics an incubation at 35-37°C for 1 week

Organisms(ATCC)	Growth
Coccidiodes immitis	Luxuriant
Histoplasma capsulatum (10230)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Asparagine Proline Broth AM1005/AM5005

Use

Asparagine Proline Broth is used for the cultivation of *Pseudomonas aeruginosa* using the membrane filter technique.

Summary

Pseudomonads are widely distributed in soil and water including sinks and drains. *Pseudomonads* can multiply in recreational waters in the presence of sufficient nutrients, as it is an opportunist, hence it can be transmitted to human beings through use of natural fresh and marine recreational waters that may be contaminated by wastewater.

Principle

Asparagine Proline Broth contains the two amino acids DL-asparagine and L-proline required by *Pseudomonas* for its growth. Phosphates and sulphates incorporated in the medium provide ions for growth as well as buffers the medium.

Formula*

Ingredients in grams per liter

DL-Asparagine	2.0
L-Proline	1.0
Magnesium Sulphate	0.5
Dipotassium Phosphate	1.0
Potassium Sulphate	10.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 14.5 gms of the powder in 1000 ml distilled water containing 25ml ethanol.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Dispense as desired.

Quality Control

Dehydrated Appearance

White coloured, homogeneous free flowing powder.

Prepared Appearance

Colourless, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18- 24 hours at 35°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	None to poor
Pseudomonas aeruginosa (27853)	Luxuriant with greenish yellow pigment

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Azide Blood Agar Base

AM1006/AM5006

Use

Azide Blood Agar Base is used for the isolation and differentiation of *streptococci* and *staphylococci* and when supplemented with blood is used for determining haemolytic reactions.

Summary

Various workers indicated that sodium azide could be used to inhibit gram-negative organisms while allowing the growth of gram-positive organisms. This medium is similar to the one used by Edwards for the isolation of mastitis *streptococci*. Sodium azide has a bacteriostatic effect on gram-negative organisms but permits growth of gram-positive organisms such as *streptococci* and some strains of *staphylococci*. *Proteus* species are slightly more resistant than other *Enterobacteriaceae* but swarming is prevented by a concentration of about 0.01% of sodium azide, thus permitting the isolation of streptococci from mixed bacterial populations (Synder and Lichstein). At the above concentration and pH, sodium azide exerts no appreciable effect on haemolysis so that the medium with added blood (5-10% sheep, rabbit or horse blood) is used for isolating, cultivating and determining haemolytic reactions of fastidious pathogens. It is recommended for the isolation of *streptococci* from cheese (23).

Principle

Peptone and beef extract provide nitrogen, carbon, vitamins and amino acids. Sodium azide is the selective agent while sodium chloride maintains the osmotic balance. Supplementation with 5-10% blood provides additional growth factors for fastidious organisms.

Formula*

Ingredients in grams per liter

Peptone Special	10.0
Sodium Chloride	5.0
Beef Extract	3.0
Sodium Azide	0.2
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 33.2 gms of the powder in 1000 ml distilled water and mix well.
2. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes
4. To prepare blood agar plates, aseptically add 5% v/v sterile defibrinated blood to the medium cooled to 45-50°C and mix well. Dispense as desired.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials and it is advisable to flush off the disposables with water.

Quality Control

Dehydrated Appearance

Beige to yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium - Yellow coloured, slightly opalescent gel.

With addition of 5% v/v sterile defibrinated blood - Cherry red opaque gel, which darkens on standing.

Cultural Response

Cultural characteristics after 18-48 hours at 35°C.

Organisms(ATCC)	Growth	Haemolysis	RGI
Enterococcus faecalis (29212)	Luxuriant	Alpha / gamma	More than 70%
Escherichia coli (25922)	None to poor	-	0%
Streptococcus pneumoniae (6303)	Luxuriant	Alpha	More than 70%
Streptococcus pyogenes(19615)	Good to luxuriant	Beta	More than 70%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. Process each specimen appropriately, and inoculate directly onto the surface of the medium.
2. Use streak plate method for isolation, stab the agar several times with the inoculating wire loop to deposit beta-haemolytic streptococci beneath the agar surface as subsurface growth displays the most reliable haemolytic reactions.
3. Use light inoculum for best results and incubate anaerobically for enhancement of haemolytic reactions.
4. Incubate plates aerobically, anaerobically or in the presence of increased carbon dioxide.

Interpretation of Results

1. Examine plates for growth and haemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of haemolysis on Blood Agar media can be described.
   a) Alpha haemolysis – reduction of haemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolouration of the medium.
   b) Beta haemolysis – lysis of the red blood cells, causing a clear zone surrounding the colony.
   c) Gamma haemolysis – wherein there is no lysis of the red blood cells and hence no change is observed in the medium.
   d) Alpha-prime haemolysis – a small zone of complete haemolysis that is surrounded by an area of partial lysis.

Precautions / Limitations

1. *Proteus* and *Escherichia* species may not always be inhibited on the Edward's formulation.
2. Use a light inoculum for best selective results.
3. Anaerobic incubation is shown to enhance haemolytic reactions.
4. Haemolytic pattern of streptococci on this medium are somewhat different than those observed on ordinary Blood Agar.
5. Azide Blood Agar Base is recommended for selective use and should be inoculated in parallel with non-selective media.
6. Sodium azide enhances haemolysis and alpha and beta haemolytic zones may be extended.
7. Haemolytic patterns may vary with the source of animal blood or base medium used.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Azide Dextrose Broth AM50061

Use

Azide Dextrose Broth is recommended for detection and cultivation of *Streptococci* in water, sewage, milk and other material.

Summary

Various workers indicated that sodium azide could be used to inhibit gram-negative organisms while allowing the growth of gram-positive organisms. Rothe, Mullmann and Seligmann formulated Azide Dextrose Broth for quantitative determination of *enterococci* in water, sewage, food and other materials, suspected of contamination with sewage (82.1).

Principle

Peptone, special and beef extract provide amino acids and other growth factors. Dextrose is a carbon and energy source. Sodium azide is the selective agent while sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Peptone, special	15.0
Beef extract	4.5
Dextrose	7.5
Sodium chloride	7.5
Sodium azide	0.20

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 34.7gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.

4. Dispense into tubes.
5. Sterilize by autoclaving at 118ºC (12 lbs pressure) for 15 minutes.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials and it is advisable to flush off the disposables with water.

Quality Control

Dehydrated Appearance

Light Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms(ATCC)	Growth
Enterococcus faecalis (29212)	Luxuriant
Escherichia coli (25922)	Inhibited

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

B_{12} Assay Agar (using *E.coli* mutant culture) AM1007

Use

B_{12} Assay Agar using *E.coli* mutant culture is used for the microbiological assay of vitamin B_{12} by the cup plate or disc plate method.

Summary

B_{12} Assay is carried out according to the procedures of the vitamin B_{12} Activity Assay as per the USP (114). *E.coli* mutant 113-3D is the test organism used.

Principle

Vitamin B_{12} Assay Agar is a vitamin B_{12} free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of the test organism *Escherichia coli* 113-3D ATCC 11105. Incorporation of vitamin B_{12} in specified increasing amounts gives a growth response curve that can be measured by the plate method.

Formula*

Ingredients in grams per liter

Dipotassium Phosphate	14.0
Monopotassium Phosphate	6.0
Ammonium Chloride	5.0
Glucose	5.0
DL-Asparagine	3.0
Ammonium Nitrate	2.0
Sodium Chloride	1.0
Magnesium Sulphate	0.2
L-Arginine Hydrochloride	0.2
Ammonium Sulphate	0.1
Calcium Chloride	0.001
Zinc Sulphate	0.00009
Ammonium Molybdate	0.00001
Borax	0.00001
Ferrous Sulphate	0.000054
Manganese Chloride	0.000046
Copper Sulphate	0.000025
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 51.5 gms of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
3. Mix well to distribute the slight precipitate evenly.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Generally satisfactory results are obtained with B_{12} levels ranging from 0 to 300 ng per ml.

Quality Control

Dehydrated Appearance

Very light to light beige, homogeneous, free flowing powder with a tendency to clump.

Prepared Appearance

Light amber coloured, clear gel, which may have a slight precipitate.

Cultural Response

Cultural characteristics after 24 hours at 37°C.

Organism (ATCC)	Growth	RGI
Escherichia coli 113-3D (11105)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Refer to U.S.P. and other official publications for assay procedure.

Interpretation of Results

1. Refer to U.S.P. and other official publications for assay procedure.

Precautions /Limitations

1. Care must be taken to avoid contamination of media or glassware in microbiological assay procedures.
2. Detergents or other chemicals present in the glassware may give erroneous results and therefore glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Sterilization and cooling conditions must be kept uniform throughout the assay.
4. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
5. All conditions of the assay must be followed precisely as outlined in the references.
6. The use of altered or deficient media may cause mutants having different nutritional requirements and will not give satisfactory response.
7. Over heating or over sterilization will give unsatisfactory results.
8. Sometimes lumps may be formed which will not effect the performance of the medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

B_{12} Assay Medium — AM10071

Use

B_{12} Assay Medium is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Summary

Three types of Vitamin assay media are generally used in the microbiological assay of vitamins.:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To quantify a vitamin under test.

B_{12} Assay Medium is used for the microbiological assay of vitamin B_{12} according to USP using *E.coli* mutant 113-3D ATCC 11105 and *Lactobacillus leichmannii* as the test organism.

Principle

B_{12} Assay Medium is a vitamin B_{12}-free dehydrated medium containing all nutrients and vitamins essential for the cultivation of *E.coli* mutant 113-3D ATCC 11105 and *Lactobacillus leichmannii*.

Formula*

Ingredients in grams per liter

Vitamin Assay Casamino Acids	15.0 g
Dextrose	40.0 g
Asparagine	0.2 g
Sodium Acetate	20.0 g
Ascorbic Acid	4.0 g
L-Cystine	0.4 g
DL-Tryptophan	0.4 g
Adenine Sulphate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Xanthine	20 mg
Riboflavin	1 mg
Thiamine Hydrochloride	1 mg
Biotin	10µg
Niacin	2 mg
p-Aminobenzoic Acid	2 mg
Calcium Pantothenate	1 mg
Pyridoxine Hydrochloride	4 mg
Pyridoxal Hydrochloride	4 mg
Pyridoxamine Hydrochloride	800 µg
Folic Acid	200 µg
Monopotassium Phosphate	1.0 g
Dipotassium Phosphate	1.0 g
Magnesium Sulphate	0.4 g
Sodium Chloride	20.0 mg
Ferrous Sulphate	20.0 mg
Manganese Sulphate	20.0 mg
Polysorbate 80	2.0 g

Final pH (at 25° C) 6.0 ± 0.1

* Formula adjusted to suit performance parameters

Directions

1. Suspend 85 grams of the powder in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 2-3 minutes to dissolve the powder completely.
3. Dispense in quantities of 5 ml in tubes, evenly dispersing the precipitate.
4. Add to the tubes standard or test samples.
5. Adjust the tube volume to 10 ml with distilled water.
6. Sterilize by autoclaving at 121° C (15 lbs pressure) for 5 minutes.

Quality Control

Dehydrated Appearance

Very light to light beige, homogeneous, with a tendency to clump.

Prepared Appearance

Very light to light amber, clear may have a slight precipitate.

Cultural Response

Cultural characteristics after 16-24 hours at 35-37° C when tubes incubated with caps loosened.

Organisms (ATCC)	Growth
E.coli mutant 113-3D (11105)	Luxuriant
Lactobacillus leichmannii (7830)	Luxuriant

Procedure

1. Refer to U.S.P. and other appropriate references for procedures and interpretation of results.

Precautions / Limitations

1. Ensure that the medium as well as the glassware used in the assay is free from contamination.
2. Heat all glassware at 250° C for at least one hour to burn off any organic residues that might be present before use.
3. Ensure that the sterilization and cooling conditions are uniform throughout the assay.
4. The test organisms used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose. The use of altered or deficient media may give rise to mutants having different nutritional requirements and hence will not give a satisfactory response.
5. Use the aseptic technique throughout the assay procedure.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

BAT (Bacillus acidoterrestris thermophilic) Medium AM100711

Use

BAT Medium is used for the detection of *Alicyclobacillus* in fruit jucies.

Summary

BAT Medium supports the growth of *Alicyclobacilli. Alicyclobacilli* are aerobe, gram-positive spore forming bacteria, whose optimum of growth is at low pH value and increased temperatures. *Alicyclobacilli* are spoilages organisms especially effecting the quality of fruit jucies(13.1).

Principle

The BAT agar supports the growth of *Alicyclobacilli.* The low pH-value in combination with the high incubation temperature inhibit the contaminating flora in growth.

Formula*

Ingredients in grams per liter

Yeast extract	2.0
Glucose	5.0
Calcium chloride	0.25
Magnesium sulfate	0.5
Ammonium sulfate	0.2
Potassium-dihydrogenphosphate	3.0
Zinc sulfate	0.00018
Copper sulfate	0.00016
Manganese sulfate	0.00015
Cobalt-chloride	0.00018
Boric acid	0.00010
Sodium molybdate	0.00030
Agar	18.00

Final pH (at 25°C) 4.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.

Note: The medium has a spontaneous pH of 5.3 ±0.2 in order to maintain the gel strength during autoclavation. Adjustment of the pH to 4.0 ±0.2 is made after the autoclavation.

4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C. Adjust the pH to 4.0 ±0.2 by adding 1.7 ml 1 NH_2SO_4. Mix well and pour into petri dishes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow colour clear gel forms in petri plates.

Cultural Response

Cultural characteristics after 16-24 hours at 35-37° C when tubes incubated with caps loosened.

Organisms (ATCC)	Growth	RGI
Alicyclobacillus acidocaldarius DSMZ 446	Good	More than 70%
Alicyclobacillus acidoterrestris DSMZ 2498	Good	More than 70%
Alicyclobacillus cycloheptanicus DSMZ 4006	Good	More than 70%
Alicyclobacillus hesperidium DSMZ 12766	Good	More than 70%
Staphylococcus aureus ATCC 25923	None	0%
Escherichia coli ATCC 25922	None	0%

Application and interpretation

1. Inoculate the medium by spreading 0.1 ml on the surface.

2. Membranefilter technique can be used with samples being filterable.
3. Incubation for 3-5 days at 45 ±1.0 °C.
4. Count all colonies growing on the BAT agar as suspicious *Alicyclobacilli.*
5. Confirm the suspicious colonies by further testing.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

B_{12} Maintenance Media (For *E.coli* Mutant) AM1008

Use

B_{12} Maintenance Media is used for the propagation, cultivation and maintenance of *Escherichia coli* 113-3D, ATCC 11105, which is the test organism in the vitamin B_{12} assay.

Summary

Escherichia coli mutant species grow poorly on non-selective culture media and require special nutrients for its growth. B_{12} Maintenance Media for *E.coli* mutant is used for carrying stock cultures to preserve the viability and sensitivity of the test organism for its intended purpose.

Principle

Tryptone is the source of carbon and nitrogen; yeast extract serves as the energy source as well as supplies B-complex vitamins. Sucrose is the fermentable carbohydrate, liver extract provides B-vitamins, potassium phosphate is the buffer and sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sucrose	12.0
Yeast Extract	5.0
Tryptone	5.0
Monopotassium Phosphate	0.5
Magnesium Sulphate	0.2
Sodium Chloride	0.1
Liver Extract	0.05
Ferrous Sulphate	0.001
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.85 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Dispense as required.

Quality Control

Dehydrated Appearance

Light yellow coloured, free flowing homogeneous powder.

Prepared Appearance

Light to medium amber, slightly opalescent gel that may have a slightly flocculent precipitate.

Cultural Response

Cultural characteristics after 16-24 hours at 35°C.

Organisms (ATCC)	Growth
Escherichia coli mutant 113-3D (11105)	Luxuriant

Storage

Store below 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bacillus Cereus Agar Base AM1009/AM5009

Use

Bacillus Cereus Agar Base with added supplements is used as a selective medium for the isolation and enumeration of *Bacillus cereus.*

Summary

Bacillus Cereus Agar Base with selective supplements is based on the highly specific diagnostic and selective PEMBA medium, developed by Holbrook and Anderson (44) for the isolation and enumeration of *B.cereus* in foods. It supports the growth of even a small number of *B.cereus* cells and spores in the presence of a large number of other food contaminants. Colonies of *B.cereus* are readily identified and confirmed by microscopic examination.

Principle

Peptone level of 0.1% and sodium pyruvate improve egg yolk precipitation and enhance sporulation. Bromothymol blue is added as a pH indicator to detect mannitol fermentation. Moulds if present in large numbers, can be suppressed by the addition of filter sterilized cycloheximide in a concentration of 40 mcg per ml of the medium. The primary diagnostic features of the medium are the colonial appearance, precipitation of hydrolyzed lecithin and the failure of *B.cereus* to utilize mannitol. The typical colonies of *B.cereus* are crenated, about 5 mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour. These features distinguish *B.cereus* from other *Bacillus* species except *B.thuringiensis.* Other egg yolk-precitipating

organisms, which can grow on the medium, include *S.aureus, S.marcescens* and *P.vulgaris,* which are distinguished from *B.cereus* by colony form and colour. These organisms also produce an egg yolk-clearing reaction in contrast to the egg yolk precipitate formed by *B.cereus*.

Formula*

Ingredients in grams per liter

Peptone	1.00
Mannitol	10.00
Sodium Pyruvate	10.00
Sodium Chloride	2.00
Magnesium Sulphate	0.10
Monopotassium Phosphate	0.25
Disodium Phosphate	2.50
Bromothymol Blue	0.12
Agar	15.00

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20.5 gms of the powder in 475 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Polymixin B Selective Supplement (AS021) and 25 ml sterile Egg Yolk Emulsion (AS010).
6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium - Green coloured, clear to slightly opalescent gel.

With addition of 5% Egg Yolk Emulsion - Yellowish green coloured, opaque gel.

Cultural Response

Cultural characteristics after 24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	Egg Yolk Reaction
Bacillus cereus (10876)	Good to luxuriant	Blue	Precipitation
Proteus vulgaris (13315)	Good to luxuriant	Green	-
Staphylococcus aureus (25923)	Good to luxuriant	Yellow	Clearing

Procedure

For Quantitative test

1. Samples should be processed in a manner suitable for the source material, liquid, solid, semi-solid or frozen.
2. Homogenize 10 gms of the food sample for 30 seconds in 90 ml of 0.1% Peptone Water.
3. Dried foods should first be rehydrated by soaking 20 gms in Tryptone Salt solution for 50 minutes at room temperature.
4. Add a further 90 ml of Peptone Water to give a final dilution of 10^{-1}.
5. Homogenize for 30 seconds.
6. Further dilutions of the homogenate should be made in 0.1% Peptone Water.
7. Inoculate 0.1 ml or 1.0 ml of the 10^{-1} dilution and higher dilutions on to the surface of the medium using spread plate or pour plate method.
8. Incubate the plates at 35°C for 24 hours.

For examining clinical specimens inoculate plates using standard procedures like streak plate method.

Interpretation of results

1. Typical colonies of *B.cereus* are crenated, about 5 mm in diameter with a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour.
2. The spores stain pale green to mid green, are paracentral or central in position and do not swell the sporangium.
3. Lipid globules and the vegetative cytoplasm are both red.
4. Only *B.cereus* are capable of possessing lipid globules in their vegetative cells when grown on selective medium and the presence of lipid globules is recommended as a rapid and confirmatory test for *B.cereus*.

For Quantitative test

1. Leave the plates for a further 24 hours at room temperature in order to detect all the *Bacillus cereus* colonies.
2. Report the results as the number of *B.cereus* colonies per gram weight of the food sample.

Precautions / Limitations

1. Identify *B.cereus* by colony form, colour, egg yolk hydrolysis and confirm with cell and spore morphology.
2. Occasional strains of *B.cereus* show weak or negative egg yolk reactions.
3. Confirm the presumptive identification of *B.cereus* by the rapid confirmatory staining procedure.
4. On this medium *B.cereus* is indistinguishable from *B.thuringiensis*. Other organisms like *S.aureus, P.vulgaris* and *S.marcescens* may also grow on this medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bacteroides Bile Esculin Agar (BBE) AM1010/AM5010

Use

Bacteroides Bile Esculin Agar is used for the selective isolation, cultivation and presumptive identification of the *Bacteroides fragilis* group.

Summary

Among the most frequently encountered anaerobes in human clinical infections are members of the "*Bacteroides fragilis*" group. These pathogens frequently occur in a mixture of microorganisms, which may overgrow the primary isolation medium. Livingston et al described this as a primary plating medium, which was found to provide selective recovery of the *B.fragilis* group and also evidence for presumptive identification (71).

Principle

Tryptone, soya peptone and hemin provide essential nutrients. Oxgall is the selective agent, which inhibits almost all gram-negative bacilli except *B.fragilis*. The medium is made more selective by the addition of Bacteroides Selective Supplement containing gentamicin which inhibits most organisms other than the esculin positive *Bacteroides* that can tolerate bile. Differentiation of the *B.fragilis* group is based on esculin hydrolysis, which produces esculetin and dextrose. Esculitin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a dark brown to black complex that appears in the medium surrounding colonies of the members of the *B. fragilis* group.

Formula*

Ingredients in grams per liter

Tryptone	15.0
Soya Peptone	5.0
Oxgall	20.0
Sodium Chloride	5.0
Esculin	1.0
Ferric Ammonium Citrate	0.5
Vitamin K_1	0.01
Hemin	0.01
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 61.52 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and aseptically add 2 vials of Bacteroides Selective Supplement (AS001).
5. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated appearance

Yellow coloured, homogeneous free flowing powder.

Prepared appearance

Slightly amber coloured, clear to slightly opalescent gel with a bluish tinge.

Cultural response

Cultural characteristics after 40-48 hours at 35°C when incubated anaerobically.

Organisms (ATCC)	Growth	Esculin Hydrolysis	RGI
Bacteroides fragilis (25285)	Good to luxuriant	+	More than 70%
Bacteroides vulgatus (8482)	Good to luxuriant	-	More than 70%
Clostridium perfringenes (11124)	Inhibited	-	More than 70%
Proteus mirabilis (12453)	None to poor	-	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. As some strains of the *B.fragilis* group may not grow due to the selective nature of the medium, it is advisable to include a non-selective Blood Agar medium simultaneously.
2. All media should be pre-reduced.
3. Incubate immediately under anaerobic conditions for at least 48 hours at 35-37°C.

Interpretation of results

1. After incubation, colonies of the *B.fragilis* group should be greater than 1 mm in diameter and appear grey, circular, entire and raised.
2. Most anaerobes other than *B.fragilis* are generally inhibited.
3. Blackening of the medium around the colonies indicates esculin hydrolysis.

Precautions / Limitations

1. *B.vulgatus* may not hydrolyze esculin.

Storage

Store below 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Baird Parker Agar Base — AM1011/AM5011

Baird Parker Agar Base IP — AM101111/AM501111

Baird Parker Agar Base USP — AM101112/AM501112

Baird Parker Agar Base (Agar Medium 0) EP — AM101113/AM501113

Baird Parker Agar Base (Agar Medium 0) BP — AM101114/AM501114

Use

Baird Parker Agar Base with added supplements (Egg Yolk Tellurite Emulsion) is used for the selective isolation and enumeration of coagulase positive *staphylococci* from clinical and non-clinical specimens.

Summary

Baird Parker Agar was developed by Baird-Parker (3, 4) from the tellurite-glycine formulation of Zebovit et al (124) for the recovery of coagulase positive *staphylococci.* It was suggested that this medium be substituted for Vogel and Johnson Agar (VJ) (2,107) because it was less inhibitory than VJ Agar, yet more selective and also possessed a diagnostic aid (egg yolk reaction) not present in VJ Agar. Subsequently, it was officially accepted by the AOAC and is also recommended by the USP and IP for use in microbial limit tests (114, 46). Baird Parker Agar is recommended by APHA for the examination of milk (39) and foods (20) and is also included in the Bacteriological Analytical Manual for testing of cosmetics (113).

Principle

Tryptone, beef extract and yeast extract provide nitrogenous compounds, carbon, sulphur and other growth factors. Sodium pyruvate protects the injured cells, helps recovery and stimulates the growth of *S.aureus* without destroying the selectivity. Glycine enhances the growth of *Staphylococcus.* Lithium chloride inhibits most of the micro flora except *Staphylococcus aureus.* The tellurite additive inhibits egg-yolk clearing strains other than *S.aureus* and imparts a black colour to the colonies. The egg yolk, apart from being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg-yolk reaction). Egg yolk makes the medium yellow, opaque. Proteolytic bacteria produce a clear zone around colonies in egg yolk containing medium. A clear zone around grey-black colonies on this medium is diagnostic for coagulase positive staphylococci. An opaque zone of lipolytic activity may be developed around the colonies on further incubation.

Identity of *Staphylococcus aureus* isolated on Baird Parker Agar must be confirmed with a coagulase reaction. Coagulase activity can be detected by adding plasma fibrinogen mixture in place of egg yolk emulsion. 375 mg bovine fibrinogen, 2.5 ml plasma, 2.5 mg trypsin inhibitor and 2.5 mg potassium tellurite is dissolved in 10 ml sterile distilled water and added to 90 ml sterile, molten medium stored at 45-50^{0}C. On this medium staphylococcal coagulase positive colonies are white to grey black surrounded by an opaque zone of coagulase activity, within 24-40 hours of incubation at 35^{0}C. Reduction in tellurite is required because of the absence of egg yolk emulsion, resulting in translucent agar and white to grey coloured colonies of *staphylococci.*

Formula*

Ingredients in grams per liter

Tryptone	10.0
Yeast Extract	1.0
Beef Extract	5.0
Sodium Pyruvate	10.0
Glycine	12.0
Lithium Chloride	5.0
Agar	20.0

Final pH (at 25^{0}C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 63 gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50^{0}C and aseptically add 50 ml concentrated Egg Yolk Emulsion (AS010) and 3 ml sterile 3.5% Potassium Tellurite Solution (AS023) or 50ml Egg Yolk Tellurite Emulsion (AS011). One vial of BP Sulpha supplement can be added if desired.
6. Mix thoroughly, but gently and pour into sterile petri plates.

Warning: Lithium chloride is harmful, bodily contact and inhalation of vapours must be avoided. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium - Light amber coloured clear to slightly opalescent gel.

With addition of Egg Yolk Tellurite Emulsion - Yellow coloured opaque gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37^{0}C.

Organisms (ATCC)	Growth	Colour of colony	Lecithinase production	RGI
Bacillus subtilis (6633)	None to poor	Dark brown	-	0%
Escherichia coli (25922)	None to poor	Large brown black	-	0%
Micrococcus luteus (10240)	Poor to good	Very small in shades of brown black	-	More than 70%
Proteus mirabilis (25933)	Good to luxuriant	Brown to black	-	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	Grey black shiny	+	More than 70%
Staphylococcus epidermidis (12228)	Poor to good	Black	-	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

For Quantitative test

1. Food samples are macerated in a suitable broth medium, dilutions are made if required and inoculated by spread plate method onto the agar surface.
2. Incubate plates aerobically for 24 hours at 35-37^{0}C.

Interpretation of Results

1. Typical colonies of *S.aureus* are black, shiny, convex and surrounded by clear zones (E-Y reaction) of approximately 2-5 mm. Coagulase negative staphylococci generally do not grow well; if growth occurs, the typical clear zones are absent.
2. If negative, reincubate for additional 24 hours.

Quantitative results

1. Count the plates with 20-200 typical *Staphylococcus aureus* like colonies, express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. Also perform coagulase test.

Precautions / Limitations

1. Colonies of some contaminating organisms may digest the coagulase halo reaction.
2. Regardless of negative reactions, consider all doubtful colonies as *S.aureus* and carry out further tests like coagulase reaction because some strains of *S.aureus* give negative egg yolk reactions (in foods, especially cheese).
3. Baird Parker Agar Base with supplements is selective for coagulase positive staphylococci, but other bacteria including *Proteus* species may grow (addition of 50mg/l Sulphamethazine is found to suppress growth and swarming of *Proteus* species).
4. The majority of contaminating flora that grows produces white to brown colonies with no clearing of the egg yolk
5. Prepare fresh medium for best results.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Baird Parker Agar Base BIS — AM101115/AM501115

Use

Baird Parker Agar Base BIS is a medium with added supplements for selective isolation and enumeration of coagulase positive *staphylococci* from food and other materials in compliance with BIS specification IS:5887 (Part III) 1976.

Summary

Baird Parker Agar was developed by Baird-Parker from the tellurite-glycine formulation of Zebovit et al., (124) for the recovery of coagulase positive *staphylococci*. It was suggested that this medium be substituted for Vogel and Johnson Agar (VJ) because it was less inhibitory than VJ Agar, yet more selective and also possessed a diagnostic aid (egg yolk reaction) not present in VJ Agar. Subsequently, it was officially accepted by the AOAC and is also recommended by the USP and IP for use in microbial limit tests. Baird Parker Agar is recommended by APHA for the examination of milk and foods and is also included in the Bacteriological Analytical Manual for testing of cosmetics.

Principle

Tryptone, meat extract and yeast extract provide nitrogenous compounds, carbon, sulphur and other growth factors. Sodium pyruvate protects the injured cells, helps recovery and stimulates the growth of S.aureus without destroying the selectivity. Glycine enhances the growth of *Staphylococcus*. Lithium chloride inhibits most of the micro flora except *Staphylococcus aureus*. The tellurite additive inhibits egg-yolk clearing strains other than S.aureus and imparts a black colour to the colonies. The egg yolk, apart from being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg-yolk reaction). Egg yolk makes the medium yellow, opaque. Proteolytic bacteria produce a clear zone around colonies in egg yolk containing medium. A clear zone around greyblack colonies on this medium is diagnostic for coagulase positive *staphylococci*. An opaque zone of lipolytic activity may be developed around the colonies on further incubation.

Identity of *Staphylococcus aureus* isolated on Baird Parker Agar must be confirmed with a coagulase reaction. Coagulase activity can be detected by adding plasma fibrinogen mixture in place of egg yolk

emulsion. 375 mg bovine fibrinogen, 2.5 ml plasma, 2.5 mg trypsin inhibitor and 2.5 mg potassium tellurite is dissolved in 10 ml sterile distilled water and added to 90 ml sterile, molten medium stored at 45- 50°C. On this medium staphylococcal coagulase positive colonies are white to grey black surrounded by an opaque zone of coagulase activity, within 24-40 hours of incubation at 35°C. Reduction in tellurite is required because of the absence of egg yolk emulsion, resulting in translucent agar and white to grey coloured colonies of *staphylococci.*

Formula*

Ingredients in grams per liter

Tryptone	10.0
Yeast extract	1.0
Meat extract	5.0
Sodium pyruvate	12.0
Glycine	12.0
Lithium chloride	5.0
Agar	20.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65 gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (AS010) and 3 ml sterile 3.5% Potassium Tellurite Solution (AS023) or 50ml Egg Yolk Tellurite Emulsion (AS011). One vial of BP Sulpha supplement can be added if desired.
6. Mix thoroughly, but gently and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium - Light amber coloured clear to slightly opalescent gel.

With addition of Egg Yolk Tellurite Emulsion - Yellow coloured opaque gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	Lecithinase production	RGI
Bacillus subtilis (6633)	None to poor	Dark brown	-	0%
Escherichia coli (25922)	None to poor	Dark brown black	-	0%
Micrococcus luteus (10240)	Poor to good	Very small shades of brown black	-	0%
Proteus mirabilis (25933)	Good to luxuriant	Brown to black	-	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	Grey black shiny	+	More than 70%
Staphylococcus epidermidis (12228)	Poor to good	Black	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Warning: Lithium chloride is harmful, bodily contact and inhalation of vapours must be avoided. On contact with skin, wash with plenty of water immediately.

Procedure

For Quantitative test

1. Food samples are macerated in a suitable broth medium, dilutions are made if required and inoculated by spread plate method onto the agar surface.
2. Incubate plates aerobically for 24 hours at 35-37°C.

Interpretation of Results

1. Typical colonies of S.aureus are black, shiny, convex and surrounded by clear zones (E-Y reaction) of approximately 2-5 mm. Coagulase negative staphylococci generally do not grow well; if growth occurs, the typical clear zones are absent. 2. If negative, reincubate for additional 24 hours.

Quantitative results

1. Count the plates with 20-200 typical Staphylococcus aureus like colonies, express as colony forming

   units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. Also perform coagulase test.

Precautions / Limitations

1. Colonies of some contaminating organisms may digest the coagulase halo reaction.
2. Regardless of negative reactions, consider all doubtful colonies as *S.aureus* and carry out further tests like coagulase reaction because some strains of *S.aureus* give negative egg yolk reactions (in foods, especially cheese).
3. Baird Parker Agar Base with supplements is selective for coagulase positive staphylococci, but other bacteria including *Proteus* species may grow (addition of 50mg/l Sulphamethazine is found to suppress growth and swarming of Proteus species).
4. The majority of contaminating flora that grows produces white to brown colonies with no clearing of the egg yolk

5. Prepare fresh medium for best results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Beef Extract Agar AM10111/AM50111

Use

Beef Extract Agar is a general-purpose nutrient medium, which supports the growth of not particularly fastidious bacteria.

Summary

Beef Extract Agar is a general-purpose nutrient medium, which is also recommended for preparation of pure cultures of *Candida*s pecies before carrying out fermentation studies and for cultivation and maintenance of a wide variety of microorganisms including *Pseudomonas aeruginosa*.

Principle

Peptone and beef extract provide nutritious sources of nitrogen and carbon. Sodium chloride acts as a source of electrolytes. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptone	10.0
Beef Extract	3.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25° C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 33 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
5. Allow the tubes to cool in a slanting position if required.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35° C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant
Candida albicans (10231)	Luxuriant
Salmonella serotype *Typhi* (6539)	Luxuriant
Pseudomonas aeruginosa (27853)	Luxuriant

Procedure

1. Use standard streaking methods to obtain isolate organisms.

Interpretation of Results

1. Luxuriant growth of non-fastidious organisms is seen.

Precautions / Limitations

1. Inspect the petriplates for contamination before inoculation.
2. Some strains may fail to grow on this medium due to variation in nutritional requirements of organisms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Beef Extract Broth AM50112

Use

Beef Extract Broth is a general-purpose nutrient medium, which supports the growth of not particularly fastidious bacteria.

Summary

Beef Extract Broth is a general-purpose nutrient medium, which is also recommended for preparation of pure cultures of *Candida* species before carrying out fermentation studies and for cultivation and maintenance of a wide variety of microorganisms including *Pseudomonas aeruginosa* (31.1).

Principle

Peptic digest of animal tissue and beef extract provide nutritious sources of nitrogen and carbon. Sodium chloride acts as a source of electrolytes.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.0
Beef extract	3.0
Sodium chloride	5.0

Final pH (at 25° C) 7.2±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Allow the tubes to cool in a slanting position if required.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35° C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant
Candida albicans (10231)	Luxuriant
Salmonella serotype Typhi (6539)	Luxuriant
Pseudomonas aeruginosa (27853)	Luxuriant

Procedure

1. Use standard methods to obtain isolate organisms.

Interpretation of Results

1. Luxuriant growth of non-fastidious organisms is seen.

Precautions / Limitations

1. Inspect the tubes for contamination before inoculation.
2. Some strains may fail to grow on this medium due to variation in nutritional requirements of organisms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bile Esculin Agar — AM1012/AM5012

Bile Esculin Agar ISO — AM1012/AM501211

Use

Bile Esculin Agar is a differential medium used for isolation and presumptive identification of group D *streptococci/ enterococci* from foods.

Summary

Swan (106) formulated Bile Esculin Agar for the isolation and identification of group D *streptococci* from foods. Originally, Bile Esculin Test was used for the identification of *enterococci*. However, since group D *streptococci* share the test with *enterococci*, it is advisable that other tests such as salt tolerance be performed while identifying *enterococci*. Meyer and Schonfeld showed that majority of *enterococci* were able to grow in esculin and split it, while other *streptococci* could not. This medium is used to differentiate *enterococci* and *Streptococcus bovis* from other *streptococci*. Rochaix (94) explored the value of esculin hydrolysis in the identification of *enterococci*. Bile Esculin Agar is included in the Bacteriological Analytical Manual for food and cosmetics testing (113) and is recommended by APHA for the examination of foods (20) and water and wastewater (36).

Principle

Oxgall inhibits gram-positive bacteria other than group D *streptococci / enterococci*. Ferric citrate is an indicator of esculin hydrolysis and resulting esculetin formation. *Enterococci/* group D *streptococci* hydrolyze the glycoside esculin to dextrose and esculetin which reacts with ferric citrate producing brownish black complex. This medium is also shown to aid differentiation of genus *Klebsiella-Enterobacter-Serratia* from other *Enterobacteriaceae* on the basis of esculin hydrolysis.

Formula*

Ingredients in grams per liter

Peptone	5.0
Beef Extract	3.0
Esculin	1.0
Oxgall	40.0
Ferric Citrate	0.5
Agar	15.0

Final pH (at 25°C) 6.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 64.5 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Mix and dispense in tubes as desired.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Allow the tubed medium to solidify in slanted position.

Quality Control

Dehydrated Appearance

Brownish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel with a bluish tinge.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C in an increased atmosphere of carbon dioxide.

Organisms (ATCC)	Growth	Esculin Hydrolysis	RGI
Enterococcus faecalis (29212)	Luxuriant	+	More than 70%
Streptococcus bovis (27960)	Luxuriant	+	More than 70%
Proteus mirabilis (25933)	Luxuriant	-	More than 70%
Staphylococcus aureus (25923)	Good	-	More than 70%
Streptococcus pyogenes (19615)	Partial to Non poor	-	0%-70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key: + = blackening of medium

\- = no change

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate the medium with two or three colonies
2. Incubate at 37°C for 18-24 hours aerobically.

Interpretation of Results

1. Blackening of the medium around the colonies indicates a positive result for esculin hydrolysis.
2. For slants, if more than half of the slant is blackened within 24-48 hours, the test is positive; if less than half of it is blackened or no blackening occurs within 24-48 hours, the test is negative.

Precautions / Limitations

1. Use a light inoculum. A heavy inoculum may cause difficulty in interpretation of results and decrease the ability of the bile to inhibit growth of other gram-positive organisms that may hydrolyze esculin.
2. A few *streptococci* may grow on the medium in the presence of bile without hydrolysis of esculin.
3. Growth without blackening of the medium is a negative result.
4. Gram-negative rods may grow on this medium and hydrolyze esculin.
5. Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* if present (isolated from human infections) may give a positive bile-esculin reaction.
6. Occasionally, strains of *Streptococcus viridans* blacken the medium or shows weakly positive reactions.
7. This medium

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.is not recommended for primary isolation of specimens.

Bile Esculin Azide Agar AM501212

Use

A medium for selective isolation and presumptive identification of fecal *Streptococci.*

Summary

Bile Esculin Agar formulated by Swan (106). Bile Esculin Azide Agar is the modified form of Bile Esculin Agar as described by Isenberg (46.1). Bile Esculin Azide Agar is a selective medium for Group D *Streptococci.*

Principle

Bile Esculin Azide Agar is a medium riched with casein enzymic hydrolysate, proteose peptone and yeast extract. Sodium azide acts as an inhibitor for Gram-negative organisms. Oxgall is used to inhibit gram positive bacteria other than *enterococci.* Hydrolysis of Esculin helps to detect Group D *Streptococci.*

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	17.0
Proteose peptone	3.0
Beef extract	5.0
Oxgall	10.0
Sodium chloride	5.0
Esculin	1.0
Ferric ammonium citrate	0.50
Sodium azide	0.15
Agar	15.0

Final pH (at 25°C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 56.65 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Medium amber coloured clear to slightly opalescent gel with a bluish tinge forms in petri plate.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	Esculin Hydrolysis	RGI
Enterococcus faecalis (29212)	Luxuriant	+	More than 70%
Streptococcus bovis (27960)	Luxuriant	+	More than 70%
Proteus mirabilis (25933)	Luxuriant	-	More than 70%
Staphylococcus aureus (25923)	Good	-	More than 70%
Streptococcus pyogenes (19615)	None-poor	-	0% or 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Key: + = blackening of medium

– = no change

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bile Salt Agar AM10121/AM50121

Use

Bile Salt Agar is used for the isolation and enumeration of enteric *bacilli.*

Summary

A variety of special differential and selective culture media are used in the isolation of enteric bacteria from clinical specimens. A selective agent Sodium Taurocholate has been incorporated in Bile Salt Agar specifically to enhance the growth of enteric *bacilli.*

Principle

Tryptone and Meat Extract provide sources of nitrogen, minerals and amino acids. Sodium Chloride maintains the osmotic equilibrium. Sodium taurocholate is a selective agent that inhibits growth of gram-positive organisms. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Tryptone	10.0
Meat Extract	5.0
Sodium Chloride	5.0
Sodium Taurocholate	5.0
Agar	18.0

Final pH (at 25° C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 43.0 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 20 minutes.
4. Cool to 60-70° C.
5. Pour into sterile petriplates. Remove air bubbles if any using a flame before solidifying.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Enterobacter aerogenes (13048)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Fair to good	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Proteus vulgaris (13315)	Luxuriant	More than 70%
Salmonellae serotype *enteritidis* (13076)	Luxuriant	More than 70%
Shigella flexneri (12022)	Luxuriant	More than 70%
Staphylococcus aereus (25923)	Fair to good	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bile Salt Agar BIS — AM10122/AM50122

Use

Bile Salt Agar is used for the isolation and enumeration of bile tolerant enteric *bacilli* in compliance with BIS specification IS:5887 (Part5) 1976 reaffirmed 1986.

Summary

A variety of special differential and selective culture media are used in the isolation of enteric bacteria from clinical specimens. A selective agent Sodium Taurocholate has been incorporated in Bile Salt Agar specifically to enhance the growth of enteric *bacilli.*

Principle

Tryptone and Meat Extract provide sources of nitrogen, minerals and amino acids. Sodium Chloride maintains the osmotic equilibrium. Sodium taurocholate is a selective agent that inhibits growth of gram-positive organisms. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptone	10.0
Meat Extract	5.0
Sodium Chloride	5.0
Sodium Taurocholate	5.0
Agar	15.0

Final pH (at 25° C) 8.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.0 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 120° C (15 lbs pressure) for 20 minutes.
4. Cool to 60-70° C.
5. Pour into sterile petriplates. Remove air bubbles if any using a flame before solidifying.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Enterobacter aerogenes (13048)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Fair to good	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Proteus vulgaris (13315)	Luxuriant	More than 70%
Salmonellae serotype *enteritidis* (13076)	Luxuriant	More than 70%
Shigella flexneri (12022)	Luxuriant	More than 70%
Staphylococcus aereus (25923)	Fair to good	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bismuth Sulphite Agar — AM1013/AM5013

Use

Bismuth Sulphite Agar is a highly selective medium used for preliminary identification of *Salmonella* species, particularly *S.typhi* from clinical and non-clinical specimens.

Summary

Bismuth Sulphite Agar is a modification of the original Wilson and Blair (121, 122) selective medium. It is recommended by various associations (20, 36,39) for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological materials, food, sewage, water supplies, etc. More positive isolates of *S.typhi* were obtained on this medium compared to Endo Agar, Eosin Methylene Blue Agar and Deoxycholate Agar. It is recommended by USP and IP for use in microbial limit testing (114, 46). For food testing, this medium is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milk and butter. It is also included in the Bacteriological Analytical Manual for food testing (113).

Principle

Beef extract and peptone provide nitrogen, growth factors and trace elements. Dextrose is the energy source. Disodium hydrogen phosphate is the buffer. Bismuth sulphite and brilliant green are complimentary in inhibiting gram-positive bacteria and intestinal gram-negative bacteria (coliform group) while allowing *Salmonella* to grow luxuriantly. This inhibitory action permits the use of a much larger inoculum than possible with other media employed for similar purposes. The use of larger inocula greatly increases the possibility of recovering the intestinal pathogens. Ferrous sulphate detects H_2S production. *S.typhi, S.enteritidis* and *S.typhimurium* typically grow as black colonies with surrounding metallic sheen resulting from H_2S production and reduction of sulphite to black ferric sulphide. *S.paratyphi A* produces light green colonies. *Shigellae* species are mostly inhibited on this medium.

Formula*

Ingredients in grams per liter

Beef Extract	5.0
Peptone	10.0
Dextrose	5.0
Disodium Hydrogen Phosphate	4.0
Ferrous Sulphate	0.3
Bismuth Sulphite Indicator	8.0
Brilliant Green	0.025
Agar	20.0

Final pH (at 25°C) 7.7 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 52.33 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. DO NOT AUTOCLAVE.
5. Disperse the precipitate evenly while dispensing (the sensitivity of the medium depends mainly upon uniform dispersion of freshly precipitated bismuth sulphite in the final gel) and use the medium the same day it is prepared.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish yellow coloured opaque gel with a flocculent precipitate.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Enterobacter aerogenes (13048)	None to poor	Brown to green*	0% or More than 70%
Enterococcus faecalis (29212)	Inhibited	-	0%
Escherichia coli (25922)	None to poor	Brown to green*	0% or More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Black with metallic sheen	More than 70%
Shigella flexneri (12022)	None to poor	Brown	0% or More than 70%
Salmonella serotype Typhi (19430)	Luxuriant	Black with metallic sheen	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Key: *depends on the inoculum density.

Procedure

1. Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media.
2. Inoculate directly on Bismuth Sulphite Agar and one or more of the following agars like Deoxycholate Citrate Agar, XLD Agar, Brilliant Green Agar, MacConkeys Agar and SS Agar.
3. Also, inoculate in an enrichment broth such as Selenite F Broth or Tetrathionate Broth Base.
4. Subculture onto Bismuth Sulphite Agar and any of the other selective media after 12-18 hours incubation. Examine the plates after 18 hours incubation and subculture suspect colonies to identification media like TSI agar or Kligler Iron Agar.

For isolating *Salmonella* species from food:

1. Samples must be selectively enriched.
2. Streak 10 microlitres of selective enrichment broth onto Bismuth Sulphite Agar.
3. Incubate plates for 24-48 hours at 35°C.

For isolation of *Salmonella* from clinical specimens:

1. Inoculate fecal specimens or rectal swabs onto a small area of the agar and streak using four-quadrant method to give discrete colonies.
2. Incubate plates at 24-48 hours at 35°C and examine for colonies resembling *Salmonella* species.

Interpretation of Results

1. The typical discrete *S.typhi* colonies are black and often surrounded by a black or brownish black zone, which may be several times the size of the colony.
2. In reflected light, preferably daylight, the zone exhibits a distinctly characteristic metallic sheen. In heavy growth areas the organism frequently appears as small light green colonies. This emphasizes the importance of inoculating plates by the four quadrant method so that some areas are sparsely populated to give discrete colonies.
3. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.
4. Generally, *Shigella* species other than *S.flexneri* and *S.sonnei* are inhibited. These two, do grow on this medium to produce brown to green raised colonies with depressed centers but show a crater like appearance.
5. *E.coli* is partially inhibited on this medium. If at all present, it produces small brown or greenish glistening colonies. The colour however, is confined to the colony itself and shows no metallic sheen.

6. *Enterobacter* colonies if present exhibit a silvery sheen, appreciably lighter in colour than that produced by *S.typhi.*
7. To isolate *S.typhi* for agglutination or fermentation studies, pick characteristic colonies from this medium and subculture on MacConkey Agar. The purified colonies from MacConkey Agar may then be inoculated in differential tubed media like TSI Agar.
8. All cultures that give reactions consistent with *Salmonella* species on this medium should be confirmed biochemically as *Salmonella* species before any serological testing is performed.

Precautions / Limitations

1. DO NOT AUTOCLAVE or overheat as it may destroy the selectivity of the medium.
2. Prepared plates should not be stored for longer then two days at 2-8^{0}C; after which time the dye oxidizes to give a green medium that can be inhibitory to some salmonellae.
3. The medium may be inhibitory to some strains of salmonellae and therefore should not be used as the sole selective medium for these organisms. *S.sendai, S.berta, S.gallinarum, S.abortus-equi* and *S.cholerae-suis* are markedly inhibited.
4. It is important to streak for well isolated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S.typhi* colonies will appear light green in these circumstances and may thus be misinterpreted as negative growth for *S.typhi.*
5. *S.typhi* and *S.arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S.arizonae* is usually inhibited on this medium.
6. Some members of the coliform group that produce H_2S may grow on this medium, giving colonies similar to *S.typhi.* However, they may be differentiated because they produce gas from lactose in differential media, for example, Triple Sugar Iron Agar. *Proteus* species may be differentiated on the basis of urea hydrolysis in Urea Broth or on Urea Agar Base.
7. Colonies on this medium may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium like MacConkey Agar.
8. All plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Bismuth Sulphite Agar Medium IP (Twin Pack) AM10131/AM50131

Use

Bismuth Sulphite Agar is a highly selective medium used for preliminary identification of *Salmonella* species, particularly *S.typhi* from clinical and non-clinical specimens in compliances with IP.

Summary

Bismuth Sulphite Agar is a modification of the original Wilson and Blair (121, 122) selective medium. It is recommended by various associations (20, 36,39) for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological materials, food, sewage, water supplies, etc. More positive isolates of *S.typhi* were obtained on this medium compared to Endo Agar, Eosin Methylene Blue Agar and Deoxycholate Agar. It is recommended by USP and IP for use in microbial limit testing (114, 46). For food testing, this medium is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milk and butter. It is also included in the Bacteriological Analytical Manual for food testing (113).

Principle

Beef extract and peptone provide nitrogen, growth factors and trace elements. Disodium hydrogen phosphate is the buffer. Bismuth sulphite and brilliant green are complimentary in inhibiting gram-positive bacteria and intestinal gram-negative bacteria (coliform group) while allowing *Salmonella* to grow luxuriantly. This inhibitory action permits the use of a much larger inoculum than possible with other media employed for similar purposes. The use of larger inocula greatly increases the possibility of recovering the intestinal pathogens. Ferric Citrate detects H_2S production. *S.typhi, S.enteritidis* and *S.typhimurium* typically grow as black colonies with surrounding metallic sheen resulting from H_2S production and reduction of sulphite to black ferric sulphide. *S.paratyphi* A produces light green colonies. *Shigellae* species are mostly inhibited on this medium.

Formula*

Ingredients in grams per liter

Part A

Beef extract	6.0
Peptone	10.0
Ferric Citrate	0.4
Brilliant green	0.01

Agar	24.0
Part B	
Ammonium bismuth citrate	3.0
Sodium sulphate	10.0
Anhydrous disodium hydrogen phosphate	5.0

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 40.41 gms of Part A of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 115°C (10 lbs pressure) for 30 minutes.
5. Suspend the 22.54 gms of Part B of powder in 100 ml distilled water.
6. Mix, heat to boiling, cool to room temperature, add 1 volume of Part B to 10 volume of Part A previously melted and cooled to a room temperature of 55°C and pour.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish yellow coloured opaque gel with a flocculent precipitate.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth	Colour of colony
Enterobacter aerogenes (13048)	None to poor	Brown to green*
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	None to poor	Brown to green*
Salmonella serotype Enteritidis (13076)	Luxuriant	Black with metallicsheen
Shigella flexneri (12022)	None to poor	Brown
Salmonella serotype Typhi (19430)	Luxuriant	Black with metallic sheen

Key: *depends on the inoculum density.

Procedure

1. Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media.
2. Inoculate directly on Bismuth Sulphite Agar and one or more of the following agars like Deoxycholate Citrate Agar, XLD Agar, Brilliant Green Agar, MacConkeys Agar and SS Agar.
3. Also, inoculate in an enrichment broth such as Selenite F Broth or Tetrathionate Broth Base.
4. Subculture onto Bismuth Sulphite Agar and any of the other selective media after 12-18 hours incubation. Examine the plates after 18 hours incubation and subculture suspect colonies to identification media like TSI agar or Kligler Iron Agar.

For isolating Salmonella species from food:

1. Samples must be selectively enriched.
2. Streak 10 microlitres of selective enrichment broth onto Bismuth Sulphite Agar.
3. Incubate plates for 24-48 hours at 35°C.

For isolation of *Salmonella* from clinical specimens:

1. Inoculate fecal specimens or rectal swabs onto a small area of the agar and streak using four- quadrant method to give discrete colonies.
2. Incubate plates at 24-48 hours at 35°C and examine for colonies resembling *Salmonella* species.

Interpretation of Results

1. The typical discrete *S.typhi* colonies are black and often surrounded by a black or brownish black zone, which may be several times the size of the colony.
2. In reflected light, preferably daylight, the zone exhibits a distinctly characteristic metallic sheen. In heavy growth areas the organism frequently appears as small light green colonies. This emphasizes the importance of inoculating plates by the four quadrant method so that some areas are sparsely populated to give discrete colonies.
3. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.
4. Generally, *Shigella* species other than *S.flexneri* and *S.sonnei* are inhibited. These two, do grow on this medium to produce brown to green raised colonies with depressed centers but show a crater like appearance.
5. *E.coli* is partially inhibited on this medium. If at all present, it produces small brown or greenish glistening colonies. The colour however, is confined to the colony itself and shows no metallic sheen.
6. *Enterobacter* colonies if present exhibit a silvery sheen, appreciably lighter in colour than that produced by *S.typhi.*
7. To isolate *S.typhi* for agglutination or fermentation studies, pick characteristic colonies from this medium and subculture on MacConkey Agar. The purified colonies from MacConkey Agar may then be inoculated in differential tubed media like TSI Agar.
8. All cultures that give reactions consistent with *Salmonella* species on this medium should be confirmed biochemically as *Salmonella* species before any serological testing is performed.

Precautions / Limitations

1. DO NOT AUTOCLAVE or overheat as it may destroy the selectivity of the medium.

2. Prepared plates should not be stored for longer than two days at 2- 8°C; after which time the dye oxidizes to give a green medium that can be inhibitory to some salmonellae.
3. The medium may be inhibitory to some strains of salmonellae and therefore should not be used as the sole selective medium for these organisms. *S.sendai, S.berta, S.gallinarum, S.abortus-equi* and *S.choleraе-suis* are markedly inhibited.
4. It is important to streak for well isolated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S.typhi* colonies will appear light green in these circumstances and may thus be misinterpreted as negative growth for *S.typhi*.
5. *S.typhi* and *S.arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S.arizonae* is usually inhibited on this medium.
6. Some members of the coliform group that produce H_2S may grow on this medium, giving colonies similar to *S.typhi*. However, they may be differentiated because they produce gas from lactose in differential media, for example, Triple Sugar Iron Agar. *Proteus* species may be differentiated on the basis of urea hydrolysis in Urea Broth or on Urea Agar Base.
7. Colonies on this medium may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium like MacConkey Agar.
8. All plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.

Use and Disposal of Dehydrated Culture Media

Inoculation of culture media with bacteria, deliberately or accidentally, leads to a very great number of organisms being produced. High concentrations of any organisms are potentially hazardous and must be disposed off safely. Therefore, after use, prepared plates, samples, sample containers or other contaminated material must be sterilized or incinerated before discarding. All autoclaved biohazards should be disposed off in accordance with state and local environmental regulations.

Only qualified personnel who have been trained in microbiological procedures should handle all infected specimens and inoculated culture media. User should ensure that any machinery or apparatus used and by chance contaminated must be safely disinfected or sterilized. The environment in which microbiological cultures are handled must also be taken into account.

Bibliography

1. Wilson and Blair, 1926. J. Pathol. Bacteriol. 29:310.
2. Wilson and Blair. 1931. J. Hyg. 31:138.
3. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25/NF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
4. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol. 2.
5. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
6. Data on file: Microxpress, a division of Tulip Diagnostics (P) Ltd.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bismuth Sulphite Agar Medium USP — AM10132/AM50132

Use

Bismuth Sulphite Agar is a highly selective medium used for preliminary identification of *Salmonella* species, in compliance with USP.

Summary

Bismuth Sulphite Agar is a modification of the original Wilson and Blair selective medium. It is recommended by various associations for the isolation and preliminary identification of *Salmonella typhi* and other *salmonellae* from pathological materials, food, sewage, water supplies, etc. More positive isolates of *S.typhi* were obtained on this medium compared to Endo Agar, Eosin Methylene Blue Agar and Deoxycholate Agar. It is recommended by USP and IP for use in microbial limit testing. For food testing, this medium is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milk and butter. It is also included in the Bacteriological Analytical Manual for food testing.

Principle

Beef extract and peptone provide nitrogen, growth factors and trace elements. Dextrose is the energy source. Disodium hydrogen phosphate is the buffer. Bismuth sulphite and brilliant green are complimentary in inhibiting gram-positive bacteria and intestinal gram-negative bacteria (coliform group) while allowing *Salmonella* to grow luxuriantly. This inhibitory action permits the use of a much larger inoculum than possible with other media employed for similar purposes. The use of larger inocula greatly increases the possibility of recovering the intestinal pathogens. Ferrous sulphate detects H_2S production. *S.typhi*, *S.enteritidis* and *S.typhimurium* typically grow as black colonies with surrounding metallic sheen resulting from H_2S production and reduction of sulphite to black ferric sulphide. *S.paratyphi* A produces light green colonies. *Shigellae* species are

mostly inhibited on this medium.

Formula*

Ingredients in grams per liter

Beef extract	5.0
Pancreatic digest of casein	5.0
Peptic digest of animal tissue	5.0
Dextrose	5.0
Sodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.025
Agar	20.0

Final pH (at 25°C) 7.6 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 52.32 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. DO NOT AUTOCLAVE.
5. Disperse the precipitate evenly while dispensing (the sensitivity of the medium depends mainly upon uniform dispersion of freshly precipitated bismuth sulphite in the final gel) and use the medium the same day it is prepared.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish yellow coloured opaque gel with a flocculent precipitate.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth	Colour of colony
Enterobacter aerogenes (13048)	None to poor	Brown to green*
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	None to poor	Brown to green*
Salmonella serotype Enteritidis (13076)	Luxuriant	Black with metallic sheen
Shigella flexneri (12022)	None to poor	Brown
Salmonella serotype Typhi (19430)	Luxuriant	Black with metallic sheen

Key: *depends on the inoculum density.

Procedure

1. Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media.
2. Inoculate directly on Bismuth Sulphite Agar and one or more of the following agars like Deoxycholate Citrate Agar, XLD Agar, Brilliant Green Agar, MacConkeys Agar and SS Agar.
3. Also, inoculate in an enrichment broth such as Selenite F Broth or Tetrathionate Broth Base.
4. Subculture onto Bismuth Sulphite Agar and any of the other selective media after 12-18 hours incubation. Examine the plates after 18 hours incubation and subculture suspect colonies to identification media like TSI agar or Kligler Iron Agar.

For isolating *Salmonella* species from food:

1. Samples must be selectively enriched.
2. Streak 10 microlitres of selective enrichment broth onto Bismuth Sulphite Agar.
3. Incubate plates for 24-48 hours at 35°C.

For isolation of *Salmonella* from clinical specimens:

1. Inoculate fecal specimens or rectal swabs onto a small area of the agar and streak using four-quadrant method to give discrete colonies.
2. Incubate plates at 24-48 hours at 35°C and examine for colonies resembling *Salmonella* species.

Interpretation of Results

1. The typical discrete *S.typhi* colonies are black and often surrounded by a black or brownish black zone, which may be several times the size of the colony.
2. In reflected light, preferably daylight, the zone exhibits a distinctly characteristic metallic sheen. In heavy growth areas the organism frequently appears as small light green colonies. This emphasizes the importance of inoculating plates by the four quadrant method so that some areas are sparsely populated to give discrete colonies.
3. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.
4. Generally, *Shigella* species other than *S.flexneri* and *S.sonnei* are inhibited. These two, do grow on this medium to produce brown to green raised colonies with depressed centers but show a crater like appearance.
5. *E.coli* is partially inhibited on this medium. If at all present, it produces small brown or greenish glistening colonies. The colour however, is confined to the colony itself and shows no metallic sheen.
6. *Enterobacter* colonies if present exhibit a silvery sheen, appreciably lighter in colour than that produced by *S.typhi.*
7. To isolate *S.typhi* for agglutination or fermentation studies, pick characteristic colonies from this medium and subculture on MacConkey

Agar. The purified colonies from MacConkey Agar may then be inoculated in differential tubed media like TSI Agar.

8. All cultures that give reactions consistent with *Salmonella* species on this medium should be confirmed biochemically as *Salmonella* species before any serological testing is performed.

Precautions / Limitations

1. DO NOT AUTOCLAVE or overheat as it may destroy the selectivity of the medium.
2. Prepared plates should not be stored for longer than two days at 2- 8^{0}C; after which time the dye oxidizes to give a green medium that can be inhibitory to some *salmonellae.*
3. The medium may be inhibitory to some strains of *salmonellae* and therefore should not be used as the sole selective medium for these organisms. *S.sendai, S.berta, S.gallinarum, S.abortus-equi* and *S.cholerae-suis* are markedly inhibited.
4. It is important to streak for well isolated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S.typhi* colonies will appear light green in these circumstances and may thus be misinterpreted as negative growth for *S.typhi.*
5. *S.typhi* and *S.arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S.arizonae* is usually inhibited on this medium.
6. Some members of the coliform group that produce H_2S may grow on this medium, giving colonies similar to *S.typhi.* However, they may be differentiated because they produce gas from lactose in differential media, for example, Triple Sugar Iron Agar. *Proteus* species may be differentiated on the basis of urea hydrolysis in Urea Broth or on Urea Agar Base.
7. Colonies on this medium may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium like MacConkey Agar.
8. All plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Blood Agar Base — AM1014/AM5014

Use

Blood Agar Base is a non-selective general-purpose medium to which blood may be added for use in isolation and cultivation of *streptococci,* and other fastidious pathogenic organisms like *Neisseria,* etc. It is also used for detection of haemolytic activity.

Summary

Without addition of blood the medium may be employed as a Nutrient Agar, or as a medium for the short-term maintenance of stock cultures. With added blood or serum, the medium is suitable for the cultivation of many fastidious organisms as well as determination of haemolytic reactions, which is an important diagnostic criteria for organisms like *streptococci, staphylococci,* etc. However, haemolytic reactions depend on the animal blood used. Group A streptococci gives best results on sheep blood. *Haemophilus haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* on horse blood. Norton found that slight acidic pH (6.8 ± 0.2) shows distinct haemolytic reaction and is ideal for cultivation of *streptococci* and pneumococci. The low pH also helps in stabilization of red blood corpuscles and helps in producing clear haemolytic zone. Blood Agar Base is specified in standard methods for food testing (20) and is included in the Bacteriological Analytical Manual for testing of cosmetics (113).

Principle

Beef heart infusion and tryptose provide nitrogen, carbon and other growth factors. Sodium chloride maintains the osmotic balance. Supplementation with blood provides additional growth factors for fastidious organisms and is the basis for determining haemolytic reactions.

Formula*

Ingredients in grams per liter

Beef Heart, Infusion from	500.0
Sodium Chloride	5.0
Tryptose	10.0
Agar	15.0

Final pH (at 25^{0}C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.
5. To prepare Blood Agar, cool the base to 45-50^{0}C and aseptically add 5% v/v sterile, defibrinated blood.

6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Basal Medium - Light amber coloured, slightly opalescent gel.

With addition of 5% v/v sterile defibrinated blood - Cherry red opaque gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth w/o blood	Growth w / blood	Haemolysis
Neisseria meningitidis (13090)	Luxuriant	Luxuriant	None
Staphylococcus aureus (25923)	Luxuriant	Luxuriant	Beta
Staphylococcus epidermidis (12228)	Luxuriant	Luxuriant	None
Streptococcus pneumoniae (6303)	Fair	Luxuriant	Alpha
Streptococcus pyogenes (19615)	Fair	Luxuriant	Beta

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. After streaking, stab the agar several times to deposit beta haemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable haemolytic reactions owing to the activity of both oxygen stable and oxygen labile streptolysins.
3. Plates may be incubated in an atmosphere containing 3-10% carbon dioxide since many pathogens require carbon dioxide on primary isolation.
4. Incubate at 35-37°C for 18-24 hours.

Interpretation of Results

Colony morphology of some organisms on Blood Agar containing 5% sheep blood:

1. Haemolytic *streptococci* may appear as opaque or translucent, greyish, small or large, matt or mucoid colonies, surrounded by a zone of haemolysis.
2. *Pneumococci* usually appear as very flat, smooth, translucent, greyish and sometimes mucoid colonies surrounded by a narrow zone of alpha (green) haemolysis.
3. *Staphylococci* appear as opaque, white to golden yellow colonies with or without zones of beta haemolysis.
4. *Listeria* may form small zones of beta haemolysis.
5. Other organisms of clinical significance may also grow on this medium.

Precautions / Limitations

1. The animal blood used (horse or sheep) and the incubation conditions (aerobic or anaerobic) affect the haemolytic reactions of organisms on this medium.
2. Colonies of *Haemophilus haemolyticus* are beta haemolytic on horse and rabbit blood agar and therefore must be distinguished from colonies of beta haemolytic streptococci. The use of sheep blood has been recommended to obviate this problem since sheep blood does not support the growth of *H. Haemolyticus*.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bordet Gengou Agar Base — AM1015/AM5015

Use

Bordet Gengou Agar Base is used for detection and isolation of *Bordetella pertussis* from clinical specimens.

Summary

Bordet Gengou Agar Base with the addition of sterile blood and glycerol is used in clinical laboratories for diagnosing whooping cough. Specimens like aspirated bronchial or nasopharyngeal secretions, perinasal swabs, etc; are used to isolate *Bordetella pertussis*, the causative agent of whooping cough. The present formulation is a modification by Kendrich (54) of the Bordet Gengou Agar originally formulated by Bordet and Gengou (7), and is prepared according to APHA requirement.

Principle

Peptone and potato infusion provide carbon and nitrogen compounds while glycerol and blood provide additional nutrients and enable the detection of haemolytic reactions, which help in the identification of *B.pertussis*. This medium supports luxuriant growth of *Bordetella* and can be used for mass cultivation of *Bordetella pertussis* for vaccine production and maintenance of stock cultures. Enrichment of the basal medium with 15% sheep blood aids in the detection of *B.pertussis* by virtue of its haemolytic reaction and with 25% human blood aids in the detection of *Mycobacterium* species from small sputum inocula and streptomycin sensitivity testing.

The medium can be made selective for *Bordetella* by the addition of Bordetella Selective Supplement, containing cephalexin at a concentration of 40 mg / litre. Amphotericin B at a concentration of 10 microgram per ml can be added as an antifungal agent.

Formula*

Ingredients in grams per liter

Potato, Infusion from	125.0

Peptone	10.0
Sodium Chloride	5.5
Agar	20.0

Final pH (at 25°C) 6.7 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40 gms of the powder in 1000 ml distilled water containing 10 ml glycerol and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and aseptically add 15-20% sterile, fresh defibrinated blood (sheep, rabbit, human or horse).
5. Mix well, (avoid incorporation of air bubbles) and pour into sterile petri plates.
6. Two vials of Bordetella Selective Supplement (AS004) may be aseptically added if required.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium - Light yellow coloured, clear to slightly opalescent gel.

With addition of sterile 15% defibrinated blood - Cherry red coloured, opaque gel.

Cultural Response

Cultural characteristics after 3-4 days at 35-37°C.

Organisms (ATCC)	Growth	Haemolysis	RGI
Bordetella bronchiseptica (4617)	Good to luxuriant	Gamma	More than 70%
Bordetella pertussis (8467)	Good to luxuriant	Beta	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. Incubate plates in an inverted position in a moist chamber (60% humidity) at 37°C for up to 7 days.
3. Examine the plates daily with or without a microscope to detect the presence of *B.pertussis* and spreading bacteria or moulds that could mask the presence of this species.
4. Plates may be discarded as negative if no growth occurs after incubation for 7 days.

Interpretation of Results

1. *B.pertussis* produce small, domed, glistening colonies that have a 'bisected pearl' appearance surrounded by a zone of haemolysis with an indefinite periphery. Some strains, however, are non-haemolytic.

Precautions / Limitations

1. Some *Haemophilus* species, if present, will grow on this medium and cross-react with *B.pertussis* antisera. It may be prudent to rule out X and V factor dependence.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

B. Q. Vaccine Medium — AM50151

Use

B.Q. Vaccine Medium (Thioglycollate Broth w/ liver extract) is recommended for the cultivation of anaerobic organisms on large scale.

Summary

Black Quarter is an infectious bacterial disease of sheep and cattle, caused by *Clostridium* species. B.Q. Vaccine is used to vaccinate cattle to prevent Black Quarter disease. B.Q. Vaccine Medium (Thioglycollate Broth w/ Liver extract) is used for mass cultivation of anaerobes for the vaccine production.

Principle

Peptic digest of animal tissue serves as a source of nitrogen and carbon. Sodium chloride maintains the osmotic balance. Liver and muscle tissue support the growth of anaerobic bacteria. Sodium thioglycollate acts as reducing agent and maintains a low oxygen tension in the medium.

Formula*

Ingredients in grams per liter

Dipotassium phosphate	4.0
Liver tissues	250.0
Muscle tissues	250.0
Peptic digest of animal tissue	10.0
Sodium chloride	5.0
Sodium thioglycollate	1.0

Final pH (at 25°C) 8.2 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend the 30 gms of powder in 1000 ml distilled water.

2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 50°C and aseptically add 0.5% sterile glucose solution. Mix thoroughly.

Quality Control

Dehydrated Appearance

Yellow coloured homogeneous free flowing powder.

Prepared Appearance

Light amber coloured clear to slightly opalescent solution

Cultural Response

Cultural characteristics after 18-48 hours at at 35-37°C under anaerobic condition.

Organisms (ATCC)	Growth
Clostridium sporogenes (11437)	Luxuriant
Clostridium perfringens (12919)	Luxuriant
Bacillus cereus (10876)	Good
S. Pyogenes (19615)	Luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brain Heart Infusion Agar — AM1016/AM5016

Use

Brain Heart Infusion Agar is a general-purpose medium used for the cultivation of wide variety of organisms including bacteria, yeasts and moulds.

Summary

Meat infusions were utilized as growth-supporting ingredients in a large number of culture media and though were cumbersome to prepare enabled the cultivation of organisms in both solid and liquid media. Nowadays, peptones have largely replaced infusions as nutritional additives in culture media, however infusions are still utilized in specific media. Brain Heart Infusion Agar can be used as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens. Brain Heart Infusion Agar slant is used for the cultivation and maintenance of pure cultures. This medium enriched with 10% sheep blood can be used to isolate systemic fungi that may grow poorly on non-enriched medium. It is also used for the cultivation of streptococci, *Neisseria* and other fastidious organisms. Brain Heart Infusion Agar is recommended by APHA for the examination of foods (20) and is included in the Bacteriological Analytical Manual for testing of cosmetics (113).

Principle

Peptone and infusion from calf brain and beef heart provide sources of nitrogen, carbon, sulphur and other growth factors. Dextrose is the fermentable carbohydrate and disodium phosphate is the buffer. Sodium chloride maintains the osmotic balance. Addition of defibrinated sheep blood provides additional essential growth factors for more fastidious organisms. Addition of antimicrobials like 50 mg per liter of chloramphenicol or 40 mg per liter of streptomycin or mixture of 50 mg of gentamicin and 50 mg chloramphenicol along with 5-10% defibrinated blood is often recommended for inhibition of bacteria and isolation of pathogenic systemic fungi. A mixture of captan (0.005gm per liter) and chloramphenicol can also be used for selective isolation of pathogenic fungi (incubation at 25-30°C for 1-2 weeks). For the selective isolation of fungi, without blood, 0.5 microgram per ml of cycloheximide and 0.05 microgram per ml of chloramphenicol may be used. However, some fungi may be inhibited on this medium with gentamicin, chloramphenicol and 10% sheep blood.

Formula*

Ingredients in grams per liter	
Beef Heart, Infusion from	250.0
Calf Brain, Infusion from	200.0
Proteose Peptone	10.0
Sodium Chloride	5.0
Dextrose	2.0
Disodium Phosphate	2.5
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 52.0 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Shake well to distribute the precipitate uniformly throughout the medium and pour into sterile petri plates.
5. If required, add antimicrobials to make the medium selective.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium - Light to medium amber, clear to slightly opalescent gel.

With addition of 5-10% sterile defibrinated blood - Cherry red coloured opaque gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35- 37°C.

Organisms (ATCC)	Growth	RGI
Candida albicans (26790)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Shigella flexneri (12022)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	More than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. Since many pathogens require CO_2 on primary isolation, plates of plain Brain Heart Infusion Agar may be incubated in an atmosphere containing approximately 5-10% CO_2, at 35-37°C for 24-48 hours.
3. For isolation of fungi from contaminated specimens, a selective medium may be inoculated simultaneously and the plates incubated at 25-30°C in an inverted position with increased humidity.
4. To isolate fungi causing systemic mycoses, two sets of the medium should be inoculated, one set incubated at 25-30°C and the other set at 35-37°C.

Interpretation of Results

1. After proper incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.
2. In cultures for fungi, examine plates for fungal colonies exhibiting typical colour and morphology.
3. All cultures must be examined weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Precautions / Limitations

1. Organisms like *H.capsulatum, C.immitis* and other pathogenic fungi can produce free infective spores and therefore extreme care must be taken to avoid dissemination of infective particles while culturing.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brain Heart Infusion Broth AM1017/AM5017

Use

Brain Heart Infusion Broth is a highly nutritious general-purpose liquid medium used for the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria from a variety of clinical and non-clinical specimens.

Summary

Rosenow (96) developed the original medium by adding brain tissue to meat infusion or beef extract dextrose broth. Brain Heart Infusion Broth is used for cultivation of bacteria, yeasts and moulds and propagation of fastidious pathogenic cocci (*streptococci, meningococci, pneumococci*) associated with blood culture work and allied pathological investigations. Brain Heart Infusion Broth, 0.5 ml per tube is used for the cultivation of bacteria employed in the preparation of inoculum for micro dilution minimal inhibitory concentration (MIC) and identification test panels. The medium can be used for the preparation of inoculum in antimicrobial susceptibility test procedures and for the cultivation of anaerobes with the addition of 0.1% agar. Prereduced medium can be used for the cultivation of obligate anaerobes. Brain Heart Infusion Broth is included in the Bacteriological Analytical Manual for food and cosmetics testing (113) and is recommended by APHA for the examination of foods (20) and milk (39).

Brain Heart Infusion Broth containing 6.5% sodium chloride is used to differentiate *enterococci* from group D *streptococci* by the 6.5% salt tolerance test. With the addition of 10% defibrinated sheep blood, it is useful for the isolation and cultivation of *Histoplasma capsulatum*. Fildes enrichment (peptic digest of sheep blood) can be incorporated for the cultivation of fastidious organisms like *H.influenzae*. This medium is especially useful as a growth and suspension medium for *staphylococci*, which is to be tested for coagulase production and supplemented with yeast extract, hemin and menadione, it was found to be better in producing heavy growth of five species of *Bacteroides* than three standard anaerobic broths. This medium without dextrose is used as a basal medium to which carbohydrates are added to study fermentation reactions.

Principle

Calf brain, beef heart infusion and peptone provide carbon, nitrogen and other growth factors. Dextrose is the fermentable carbohydrate source and disodium phosphate is the buffer. The addition of 0.1% agar helps in the cultivation of microaerophiles and anaerobes because it yields conditions of reduced oxygen tension. In the formulation containing 6.5% sodium chloride, the salt acts as a differential and / or selective agent by interfering with membrane permeability and osmotic and electro kinetic equilibrium in salt intolerant organisms.

Formula*

Ingredients in grams per liter

Beef Heart, Infusion from	250.0
Calf Brain, Infusion from	200.0
Proteose Peptone	10.0
Sodium Chloride	5.0
Dextrose	2.0
Disodium Phosphate	2.5

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.0 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes or bottles as desired.
4. Sterilize at 121°C.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Enterococcus faecalis (29212)	Luxuriant
Neisseria meningitidis (13090)	Luxuriant
Streptococcus pneumoniae (6303)	Luxuriant
Streptococcus pyogenes (19615)	Luxuriant

Procedure

1. For testing liquid specimens, the bottled or tubed media should be inoculated with 1-2 drops of the specimen using a sterile pipette.
2. Swab specimens may be inserted into Broth after inoculation of plated media.
3. For anaerobic incubation, the medium must be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours before use. This may be achieved through use of anaerobic systems. Alternatively, the medium may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Interpretation of Results

1. Growth in tubes is indicated by turbidity.
2. Examine cultures by gram stain method and subculture onto appropriate media like Soyabean Casein Digest Agar with 5% sheep blood or EMB Agar.
3. Incubate the subcultures anaerobically if anaerobes are suspected.
4. *Enterococci* grow in the medium containing 6.5% sodium chloride within 24-48 hours while non-enterococcal group D streptococci fail to grow.

Precautions / Limitations

1. Tubes of Brain Heart Infusion Broth not used on the same day, should be placed in boiling water bath for a few minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.
2. Brain Heart Infusion Broth with addition of 1.5% agar should not be used for detection of haemolytic activity of *streptococci,* since the presence of dextrose in it may cause atypical haemolytic reactions when used in blood containing media.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green Agar, Modified AM1018/AM5018

Use

Brilliant Green Agar, Modified is used for selective isolation of *Salmonella* other than *S.typhi* from clinical and non-clinical samples.

Summary

Kristensen et al (62) first described Brilliant Green Agar as a primary plating medium for isolation of *Salmonella,* which was further modified by Kauffmann. Brilliant Green Agar, Modified is recommended for the isolation of *Salmonella,* other than *S.typhi* from faeces, water, meat and meat products, food and dairy products and poultry and poultry products. This medium is more selective than Deoxycholate Citrate Agar and other brilliant green media. The advantages claimed for the medium are that it inhibits the growth of *E.coli; Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* species, which may resemble *Salmonella. S.cholerasuis* grows well on this medium compared to Deoxycholate Citrate Agar. Brilliant Green Agar, Modified is recommended by APHA for food testing (20), USP (114) and IP (46).

Principle

Proteose peptone provides carbon, nitrogen and other growth factors while yeast extract provides B complex vitamins. Lactose and sucrose are the carbohydrate sources. Sodium chloride maintains the osmotic balance. Brilliant green inhibits majority of gram-positive and gram-negative bacteria, allowing *Salmonella* to grow. *S.typhi, E.coli, Staphylococcus aureus, Shigella, Pseudomonas* and *Proteus* species are mostly inhibited. In the presence of phenol red, lactose and sucrose, non-fermenting *Salmonella* will form white to pinkish red colonies while fermenters will form yellow colonies.

Formula*

Ingredients in grams per liter

Proteose Peptone	10.0
Yeast Extract	3.0
Sucrose	10.0
Lactose	10.0
Sodium Chloride	5.0
Brilliant Green	0.0125
Phenol Red	0.08
Agar	20.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 58.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. To increase the selectivity, aseptically add 2 vials of Sulpha Supplement (ASO_{27}) and mix well before pouring into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish brown coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	Colony Colour	RGI
Escherichia coli (25922)	None to poor	Yellowish green	0%
Salmonella serotype Enteritidis (13076)	Luxuriant	Pinkish white	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Pinkish white	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Salmonella serotype Typhi (6539)	None to poor Red		0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Interpretation of Results

1. *Salmonella* species produce pinkish-white to red colonies surrounded by brilliant red zones in the medium.
2. Lactose fermenting or sucrose fermenting organisms produce yellow to yellow-green colonies surrounded by yellow-green zones in the medium. *Proteus, Citrobacter* and *Pseudomonas* species, if present may mimic enteric pathogens by producing small red colonies.

Precautions / Limitations

1. Brilliant Green Agar, Modified being highly selective, it is recommended that this medium be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite Broth or Tetrathionate Broth Base is plated on this medium along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar, DCA and XLD Agar.
2. The recovery of many *Salmonella* species is greatly reduced if the specimens (stool samples) remain unpreserved for more than 3 hours before processing.
3. In cases of delay, inoculate the specimen onto an appropriate transport media to maintain viability of the organisms.
4. Organisms other than *Salmonella* species, like *Morganella morgani* and some *Enterobacteriaceae* may grow on this medium. Lactose fermenting *S.arizona* may be present in foods.
5. The medium is not recommended for isolation of *S.typhi, S.paratyphi* and *Shigella* species.
6. Protect the medium from light to avoid discolouration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green Agar, Modified IP (Agar Medium L) AM10181/AM50181

Use

Brilliant Green Agar, Modified is used for selective isolation of *Salmonella* other than *S. typhi* from clinical and non-clinical samples in compliance with IP.

Summary

Kristensen et al., first described Brilliant Green Agar as a primary plating medium for isolation of *Salmonella*, which was further modified by Kauffmann. Brilliant Green Agar, Modified is recommended for the isolation of Salmonella, other than *S. typhi* from faeces, water, meat and meat products, food and dairy products and poultry and poultry products. This medium is more selective than Deoxycholate Citrate Agar and other brilliant green media. The advantages claimed for the medium are that it inhibits the growth of *E. coli, Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* species, which may

resemble Salmonella. *S. cholerasuis* grows well on this medium compared to Deoxycholate Citrate Agar. Brilliant Green Agar, Modified is recommended by APHA for food testing , USP and IP .

Principle

Peptone provides carbon, nitrogen and other growth factors while yeast extract provides B complex vitamins. Lactose and sucrose are the carbohydrate sources. Sodium chloride maintains the osmotic balance. Brilliant green inhibits majority of gram-positive and gram-negative bacteria, allowing Salmonella to grow. *S. typhi, E. coli, Staphylococcus aureus, Shigella, Pseudomonas* and *Proteus* species are mostly inhibited. In the presence of phenol red, lactose and sucrose, non-fermenting *Salmonella* will form white to pinkish red colonies while fermenters will form yellow colonies.

Formula*

Ingredients in grams per liter

Peptones (meat and casein)	10.0
Yeast extract	3.0
Sucrose	10.0
Lactose monohydrate	10.0
Sodium chloride	5.0
Brilliant green	0.0125
Phenol red	0.08
Agar	12.0

Final pH (at 25ºC) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 50.09 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
4. Sterilize by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.
5. To increase the selectivity, aseptically add 2 vials of Sulpha Supplement (AS027) and mix well before pouring into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish brown coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms(ATCC)	Growth	Colour of colony	RGI
Escherichia coli (25922)	None to poor	Yellowish green	0% or 70%
Salmonella serotype *Enteritidis* (13076)	Luxuriant	Pinkish white	more than 70%
Salmonella serotype *Typhimurium* (14028)	Luxuriant	Pinkish white	more than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Salmonella serotype *Typhi* (6539)	None to poor	Red	0% or 70%

Precautions / Limitations

1. Brilliant Green Agar, Modified being highly selective, it is recommended that this medium be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite Broth or Tetrathionate Broth Base is plated on this medium along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar, DCA and XLD Agar.
2. The recovery of many Salmonella species is greatly reduced if the specimens (stool samples) remain unpreserved for more than 3 hours before processing.
3. In cases of delay, inoculate the specimen onto an appropriate transport media to maintain viability of the organisms.
4. Organisms other than Salmonella species, like *Morganella morgani* and some Enterobacteriaceae may grow on this medium. Lactose fermenting *S. arizona* may be present in foods.
5. The medium is not recommended for isolation of *S. typhi, S. paratyphi* and *Shigella* species.
6. Protect the medium from light to avoid discolouration.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green Agar, Modified USP AM10182/AM50182

Brilliant Green Agar, Modified (Agar Medium L) BP AM10184/AM50184

Use

Brilliant Green Agar, Modified is used for selective isolation of *Salmonella* other than *S.typhi* from clinical and non-clinical samples.

Summary

Kristensen et al., first described Brilliant Green Agar as a primary plating medium for isolation of *Salmonella,* which was further modified by Kauffmann. Brilliant

Green Agar, Modified is recommended for the isolation of Salmonella, other than *S.typhi* from faeces, water, meat and meat products, food and dairy products and poultry and poultry products. This medium is more selective than Deoxycholate Citrate Agar and other brilliant green media. The advantages claimed for the medium are that it inhibits the growth of *E.coli,* Pseudomonas aeruginosa and partially inhibits the growth of *Proteus* species, which may resemble *Salmonella. S.cholerasuis* grows well on this medium compared to Deoxycholate Citrate Agar. Brilliant Green Agar, Modified is recommended by APHA for food testing , USP and BP .

Principle

Proteose peptone provides carbon, nitrogen and other growth factors while yeast extract provides B complex vitamins. Lactose and sucrose are the carbohydrate sources. Sodium chloride maintains the osmotic balance. Brilliant green inhibits majority of gram-positive and gramnegative bacteria, allowing *Salmonella* to grow. *S.typhi, E.coli,* Staphylococcus *aureus, Shigella, Pseudomonas* and *Proteus* species are mostly inhibited. In the presence of phenol red, lactose and sucrose, non-fermenting *Salmonella* will form white to pinkish red colonies while fermenters will form yellow colonies.

Formula*

Ingredients in grams per liter	USP	BP
Peptic digest of animal tissue	5.0	-
Pancreatic digest of casein	5.0	-
Peptones (meat and casein)	-	10.0
Yeast extract	-	3.0
Sucrose	10.0	10.0
Lactose	10.0	-
Lactose monohydrate	-	10.0
Sodium chloride	5.0	5.0
Brilliant green	0.0125	0.0125
Phenol red	0.08	0.08
Agar	20.0	20.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 58.09 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. To increase the selectivity, aseptically add 2 vials of Sulpha Supplement (AS027)and mix well before pouring into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish brown coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	Colour of colony	RGI
Escherichia coli (25922)	None to poor	Yellowish green	0% or More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Pinkish white	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Pinkish white	0% or More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Salmonella serotype Typhi (6539)	None to poor	Red	0% or More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Precautions / Limitations

1. Brilliant Green Agar, Modified being highly selective, it is recommended that this medium be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite Broth or Tetrathionate Broth Base is plated on this medium along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar, DCA and XLD Agar.
2. The recovery of many *Salmonella* species is greatly reduced if the specimens (stool samples) remain unpreserved for more than 3 hours before processing.
3. In cases of delay, inoculate the specimen onto an appropriate transport media to maintain viability of the organisms.
4. Organisms other than *Salmonella* species, like *Morganella morgani* and some Enterobacteriaceae may grow on this medium. Lactose fermenting *S.arizona* may be present in foods.
5. The medium is not recommended for isolation of *S.typhi, S.paratyphi* and *Shigella* pecies.
6. Protect the medium from light to avoid discolouration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green, Phenol Red, Lactose Monohydrate, Sucrose Agar (Agar Medium L) EP

AM10183/AM50183

Use

Brilliant Green Agar, Modified is used for selective isolation of *Salmonella* other than *S. typhi* from clinical and non-clinical samples.

Summary

Kristensen et al., first described Brilliant Green Agar as a primary plating medium for isolation of Salmonella, which was further modified by Kauffmann. Brilliant Green Agar, Modified is recommended for the isolation of *Salmonella*, other than *S. typhi* from faeces, water, meat and meat products, food and dairy products and poultry and poultry products. This medium is more selective than Deoxycholate Citrate Agar and other brilliant green media. The advantages claimed for the medium are that it inhibits the growth of *E. coli, Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* species, which may resemble *Salmonella*. *S. cholerasuis* grows well on this medium compared to Deoxycholate Citrate Agar. Brilliant Green Agar, Modified is recommended by APHA for food testing , USP , BP (9.1) and EP (26.1) .

Principle

Peptone provides carbon, nitrogen and other growth factors while yeast extract provides B complex vitamins. Lactose and sucrose are the carbohydrate sources. Sodium chloride maintains the osmotic balance. Brilliant green inhibits majority of gram-positive and gram-negative bacteria, allowing Salmonella to grow. S. typhi, *E. coli, Staphylococcus aureus, Shigella, Pseudomonas* and *Proteus* species are mostly inhibited. In the presence of phenol red, lactose and sucrose, non-fermenting *Salmonella* will form white to pinkish red colonies while fermenters will form yellow colonies.

Formula*

Ingredients in grams per liter

Peptones (meat and casein)	10.0
Yeast extract	3.0
Sucrose	10.0
Lactose monohydrate	10.0
Sodium chloride	5.0
Brilliant green	0.0125
Phenol red	0.08
Agar	20.0

Final pH (at 25ºC) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 58.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
4. Sterilize by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.
5. To increase the selectivity, aseptically add 2 vials of Sulpha Supplement (AS027) and mix well before pouring into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish brown coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms(ATCC)	Growth	Colour of colony	RGI
Escherichia coli (25922)	None to poor	Yellowish green	0% or More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Pinkish white	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Pinkish white	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Salmonella serotype Typhi (6539)	None to poor	Red	0% or More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Precautions / Limitations

1. Brilliant Green Agar, Modified being highly selective, it is recommended that this medium be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite Broth or Tetrathionate Broth Base is plated on this medium along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar, DCA and XLD Agar.
2. The recovery of many *Salmonella* species is greatly reduced if the specimens (stool samples) remain unpreserved for more than 3 hours before processing.
3. In cases of delay, inoculate the specimen onto an appropriate transport media to maintain viability of the organisms.
4. Organisms other than Salmonella species, like *Morganella morgani* and some *Enterobacteriaceae* may grow on this medium. Lactose fermenting *S. arizona* may be present in foods.
5. The medium is not recommended for isolation of *S. typhi, S. paratyphi* and *Shigella* species.

6. Protect the medium from light to avoid discolouration.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green Bile Agar AM1019/AM5019

Use

Brilliant Green Bile Agar is used for isolating, differentiating and enumerating coliform bacteria.

Summary

Noble and Tonney (85) described Brilliant Green Bile Agar for determining the relative density of coliform bacteria in water and sewage and its usefulness in selectively isolating *Salmonella* species from other coliforms. APHA has approved this medium for the estimation of number of coliforms in test samples of various materials (20).

Principle

Peptone provides nitrogen, carbon, vitamins and other growth factors. Lactose is the fermentable carbohydrate. Basic fuchsin and erioglaucine are the pH indicators while monopotassium phosphate is the buffer. Oxgall and brilliant green combination is highly selective for coliforms, inhibiting most of the gram-positive and gram-negative bacteria.

When pH is neutral, colour of the medium is blue, while acid production from lactose turns the medium pink. Differentiation of coliforms is based on fermentation of lactose. Coliform bacteria typically ferment lactose producing acid, and in the presence of basic fuchsin produce deep red colonies surrounded by a pink halo against blue background of the medium, while *Salmonella* which do not ferment lactose, produce colourless to faint pink colonies.

Formula*

Ingredients in grams per liter

Peptone	8.25
Lactose	1.9
Sodium Sulphite	0.205
Ferric Chloride	0.0295
Basic Fuchsin	0.0776
Erioglaucine	0.0649
Monopotassium Phosphate	0.0153
Oxgall	0.00295
Brilliant Green	0.0000295
Agar	10.15

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20.7 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. For plating 10 ml quantities of water samples, prepare the medium in double strength.

Warning: Basic fuchsin is a potent carcinogen and must be handled with care so as to avoid inhalation or contact with skin.

Quality Control

Dehydrated Appearance

Light purple coloured, homogeneous free flowing powder.

Prepared Appearance

Bluish purple coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	Colour of colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink	More than 70%
Escherichia coli (25922)	Luxuriant	Deep red with bile precipitate	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless to light pink	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Precautions

1. The medium must be prepared just prior to use and when necessary to store the medium, it should be kept in the dark.
2. The medium is sensitive to light, particularly direct sunlight, which may cause a decrease in the productivity of the medium and a change in colour from deep blue to purple or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Brilliant Green Bile Broth 2%

AM1020/AM5020

Use

Brilliant Green Bile Broth 2% is used for the detection of coliform organisms in water and wastewater, foods, milk and dairy products, as well as in other materials of sanitary importance.

Summary

Brilliant Green Bile Broth 2% is formulated as per American Public Health Association specifications for use in presumptive identification and confirmation of coliforms (20, 36, 39). It is included in the Bacteriological Analytical Manual for food testing (113).

Principle

Peptone provides the essential nutrients. Lactose is the fermentable carbohydrate. Oxgall and brilliant green inhibit most of the gram-positive organisms including lactose fermenting clostridia and selected gram-negative organisms. Coliforms, which are resistant to the action of inhibitors and which ferment lactose, can replicate in this medium. Fermentation is detected by gas production and is seen as bubbles in the inverted Durham's tubes. Production of gas in the inverted Durham's tube indicates presence of faecal coliforms, since non-faecal coliforms growing in this medium do not produce gas.

It is important that the inhibitory agents in the medium are balanced with the nutrient and mineral components, so that clostridial and *bacillus* spores if present will not give false positive reactions in the medium e.g. gas formation.

Formula*

Ingredients in grams per liter

Oxgall	20.0
Lactose	10.0
Peptone	10.0
Brilliant Green	0.0133

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.01 gms of the powder in 1000 ml distilled water and mix well.
2. Warm slightly to dissolve the powder completely.
3. Dispense into tubes containing inverted Durham's tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. DO NOT AUTOCLAVE DOUBLE STRENGTH BROTH.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Emerald green coloured, clear solution, without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Gas
Bacillus cereus (10876)	Inhibited	-
Enterobacter aerogenes (13048)	Luxuriant	+
Enterococcus faecalis (19433)	None to poor	-
Escherichia coli (25922)	Luxuriant	+
Staphylococcus aureus (25923)	Inhibited	-

Procedure

1. Food macerates are decimally diluted and added to the broth in the proportion 1:10.
2. Double strength broth can be used for large volume samples. Incubation can be carried out at 44°C for 48 hours to detect *E.coli;* at 32°C for 25-48 hours to detect *coli-aerogenes* and at 4°C for 10 days to detect psychrotrophic coliform organisms.
3. In water plants, control tests where less than 1 ml to 10 ml volumes is used, it is important not to over dilute the medium. Thus 1 ml or less volumes of water can be added to 10 ml of Brilliant Green Bile Broth. For 10 ml volumes of water, double strength Brilliant Green Bile Broth should be used in equal volumes.

Interpretation of Results

1. The medium becomes turbid and yellowish-green in colour when bacterial growth occurs, and when accompanied by copious gas formation, it is presumptive of the presence of *coli-aerogenes* organisms.
2. Turbidity and gas production in the Brilliant Green Bile Broth 2% incubated at 44°C is indicative of a positive test for *E.coli.* To confirm the presence of *E.coli* carry out indole production test at 44°C in Tryptone Water.

Precautions / Limitations

1. Do not autoclave double strength broth.
2. Gram-positive sporing organisms may produce gas to give a false positive reaction if the bile / brilliant green inhibition is compromised by the food material.
3. Turbidity alone is not a positive test for coliforms.
4. It may be necessary to invert the tube prior to inoculation if bubbles are trapped in the Durham's tube.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Bromothymol Blue Lactose Agar — AM1021/AM5021

Use

Bromothymol Blue Lactose Agar is used for the detection and isolation of pathogenic staphylococci.

Summary

Bromothymol Blue Lactose Agar was developed by Chapman et al (15) for the detection and isolation of pathogenic staphylococci.

Principle

Beef extract and proteose peptone provide sources of carbon and nitrogen. Lactose is the carbohydrate source and bromothymol blue is the acid-base indicator. Pathogenic saphylococci are differentiated by their ability to grow at a high pH and in the presence of bromothymol blue indicator.

Formula*

Ingredients in grams per liter

Beef Extract	3.0
Proteose Peptone	5.0
Lactose	10.0
Bromothymol Blue	0.17
Agar	15.0

Final pH (at 25^{0}C) 8.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 33.17 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish blue coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35^{0}C.

Organisms (ATCC)	Growth	Colour of colony
Escherichia coli (25922)	Luxuriant	Yellow
Salmonella serotype Enteritidis (13076)	Luxuriant	Blue / colourless
Salmonella serotype Typhi (6539)	Luxuriant	Blue / colourless
Staphylococcus aureus (6538)	Luxuriant	Golden yellow
Staphylococcus epidermidis (12228)	Luxuriant	Blue / colourless

Procedure

1. Plates should be inoculated preferably by spread plate method and incubated for about 36 hours at 35^{0}C.

Interpretation of Results

1. Typical colonies of staphylococci appear deep / golden yellow about 90%, blue grey about 10%.

Precautions / Limitations

1. Coliforms and other organisms may also grow on this medium but are differentiated by their appearance.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Peptone Water — AM10211/AM50211

Use

Buffered peptone water used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered peptone water (BPW) is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organization for Standardization (ISO) method, for detection of Salmonella in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to Salmonellae. It is known that freezing and drying may injure *Salmonellae* so that they are unable to multiply in selective media (23.1). Pre enrichment of sublethally injured *Salmonellae* in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample (6.1).

Principle

Proteose peptone serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Proteose peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Monopotassium phosphate	1.5

Final pH (at 25°C) 7.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 20 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Interpretation of Results

Growth in the medium is indicated by the presence of turbidity compared to an uninoculated control.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Peptone Water BIS — AM10212/AM50212

Buffered Peptone Water ISO — AM10213/AM50213

Use

Buffered Peptone Water used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered Peptone Water (BPW) is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organisation for Standardisation (ISO) method, for detection of *Salmonella* in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to Salmonellae. It is known that freezing and drying may injure *Salmonellae* so that they are unable to multiply in selective media. Pre enrichment of sublethally injured *Salmonellae* in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample.

Principle

Enzymatic digest of casein serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Enzymatic digest of casein	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate anhydrous	3.5
Potassium dihydrogen phosphate (KH_2PO_4)	1.5

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 20.0 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 16-20 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Sodium Chloride-Peptone Solution pH7.0 (Harmonized) AMH50214

Use

Buffered Sodium Chloride-Peptone Solution pH 7.0 is used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered peptone water (BPW) is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organization for Standardization (ISO) method, for detection of *Salmonella* in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to *Salmonellae*. It is known that freezing and drying may injure *Salmonellae* so that they are unable to multiply in selective media. Pre enrichment of sublethally injured Salmonellae in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample.

Principle

Proteose peptone serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Peptone (meat and casein)	1.00
Sodium chloride	4.30
Disodium hydrogen phosphate dihydrate	7.23*
Potassium dihydrogen phosphate	3.6

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

* (equivalent to 0.067 M phosphate)

Directions

1. Suspend the 16.1 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

   Note: To this solution surface active agents or inactivators of antimicrobial agents may be added before autoclave, such as: Polysorbate 80 or Polysorbate 20 in1-10 gms per liter.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Peptone Water with NaCl AM50214

Use

Buffered peptone water used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered peptone water (BPW) is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organization for Standardization (ISO) method, for detection of *Salmonella* in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to *Salmonellae*. It is known that freezing and drying may injure *Salmonellae* so that they are unable to multiply in selective media. Pre enrichment of sublethally injured *Salmonellae* in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample.

Principle

Proteose peptone serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Peptone (meat and casein)	1.00
Sodium chloride	4.30
Disodium hydrogen phosphate	7.23
Potassium dihydrogen phosphate	3.56

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 16.09 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

   Note: To this solution surface active agents or inactivators of antimicrobial agents may be added before autoclave, such as: Polysorbate 80 or Polysorbate 20 in1-10 gms per liter.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Interpretation of Results

Growth in the medium is indicated by the presence of turbidity compared to an uninoculated control

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Peptone Water with NaCl IP — AM50215

Buffered Peptone Water with NaCl USP — AM502151

Buffered Peptone Water with NaCl EP — AM50216

Use

Buffered Peptone Water with NaCl used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered Peptone Water with NaCl is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organization for Standardization (ISO) method, for detection of *Salmonella* in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to Salmonellae. It is known that freezing and drying may injure *Salmonellae* so that they are unable to multiply in selective media. Pre enrichment of sublethally injured *Salmonellae* in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample.

Principle

Proteose peptone serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Peptone (meat and casein)	1.0
Sodium chloride	4.3
Disodium hydrogen phosphate	7.2
Potassium dihydrogen phosphate	3.6

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 16.1 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.

4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

   Note: To this solution surface active agents or inactivators of antimicrobial agents may be added before autoclave, such as: Polysorbate 80 or Polysorbate 20 in1-10 gms per liter.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Buffered Peptone Water with NaCl BP — AM50217

Use

Buffered peptone water used for pre-enrichment of injured *Salmonella* species. It increases recovery of *Salmonella* species prior to selective enrichment from food.

Summary

Buffered peptone water (BPW) is one of the most widely used pre enrichment broths for *Salmonella* in a wide range of foods. Moreover, it is often the medium of choice in many published reference methods, including the current International Organization for Standardization (ISO) method, for detection of *Salmonella* in foods. Edel and Kampelmaacher in their comparative study observed that food preservation methods cause subleathal injury to Salmonellae. It is known that freezing and drying may injure Salmonellae so that they are unable to multiply in selective media. Pre enrichment of sublethally injured *Salmonellae* in buffered peptone water showed superior results in comparison with direct selection methods. In addition to providing conditions for the recovery and growth of cells prior to selective enrichment, BPW buffers the pH of the growth system against pH changes brought about by the growth and metabolism of microorganisms during enrichment and those imposed by the food sample.

Principle

Proteose peptone serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Phosphates buffer the medium.

Formula*

Ingredients in grams per liter

Peptone (meat and casein)	1.00
Sodium chloride	4.30
Disodium hydrogen orthophosphate	7.23
Potassium dihydrogen orthophosphate	3.6

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 16.09 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

   Note: To this solution surface active agents or inactivators of antimicrobial agents may be added before autoclave, such as: Polysorbate 80 or Polysorbate 20 in1-10 gms per liter.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Salmonella serotype Enteritidis (13076)	Luxuriant
Salmonella serotype Typhi (19430)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant

Procedure

Test specimens as per recommended guidelines.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Campylobacter Agar Base AM50218

Use

Campylobacter agar base is used for selective isolation of *Campylobacter* species from clinical and non-clinical samples.

Summary

Campylobacter agar base is based on the formulation described by Bolton and Robertson (29.1). Campylobacter agar base is designed to isolate *Campylobacter* species from fecal specimens, food and environmental specimens. It is used as a highly nutritious basal medium, which after appropriate additions, is used for selective isolation of *Campylobacter* species from fecal specimens, food and environmental specimens. The antimicrobial agents described by Skirrow and Blaser *et al.*, markedly reduce growth of normal enteric bacteria (79.1).

Principle

Proteose peptone serves as a nitrogen source for fastidious organism like *Campylobacter.* Liver digest and yeast extract provide essential carbon, nitrogen, vitamin and amino acid sources. Sodium chloride provides the osmotic balance. The selective isolation of *Campylobacter* depends on the incorporation of the proper antimicrobial agent in the medium. Skirrow's antimicrobial supplement contains Vancomycin, Polymixin B and Trimethoprim where as antimicrobial supplement of Blaser-Wang contains Vancomycin, Polymixin B, Trimethoprim, Cephalothin and Amphotericin B. inhibits Gram-positive bacteria, Polymyxin B inhibits most Gram negative *bacilli* except *Proteus trimethoprim* is inhibitory for *Proteus* species. Cephalothin inhibits Gram-positive organisms and also inhibits enteric bacteria. Amphotericin B is an antifungal agent.

Formula*

Ingredients in grams per liter

Proteose peptone	15.00
Liver digest	2.50
Yeast extract	5.00
Sodium chloride	5.00
Agar	12.00

Final pH (at 25°C) 7.4± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend the 19.75 gms of powder in 500 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the medium to ~50°C. aseptically add 5-7% sterile lysed horse blood or 10% sheep blood. Mix thoroughly.
6. Add 1 vial of Campylobacter supplement (AS0061) to prepare Blaser's medium or add 1 vial of Campylobacter supplement (AS0071) to prepare Skirrow's medium.
7. After addition, the medium must be gently but thoroughly mixed to ensure that the antibiotics are uniformly distributed throughout the medium.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basel medium yield yellow coloured, cleared to slightly opalescent gel. Addition of 5-7% v/v lysed blood forms reddish brown coloured opalescent gel in petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 35ºC in microaerophilic atmosphere.

Organisms (ATCC)	Growth*	Growth**	RGI
Campylobacter fetus subsp. jejuni (29428)	Good - Luxuriant	Good - Luxuriant	More than 70%
Candida albicans (10231)	None to poor	Moderate	0% or More than 70%
Escherichia coli (25922)	None to poor	None to poor	0% or More than 70%
Streptococcus faecalis (29212)	None to poor	None to poor	0% or More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

* after addition of Campylobacter supplement I (Blaser- Wang), AS0061

** after addition of Campylobacter supplement II (Skirrow), AS0071

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Inoculate and incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 24-48 hours at 42°C.

Precautions / Limitations

Since Campylobacter jejuni thermophilic, it is important to incubate the plates at 42°C; otherwise, growth will be delayed. Also, the higher temperature improves selectivity by inhibiting the normal flora.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Candida Medium AM50219

Use

Candida Medium is used for cultivating *Candida* species.

Summary

Candida Medium used for the selective cultivation and differentiation of *Candida* species. Originally this medium was developed by Nickerson (84.1). It is also use for processing and incubation of specimens like tissues, skin scrapping, nails and hair (42.1 & 24.1). Differentiation of *Candida* species is based on the growth patterns and pigmentation of isolated colonies.

Principle

Mycological peptone provide essential nitrogenous nutrients while dextrose act as carbon source and phosphate maintain buffering action of medium. This medium contains sodium sulphite which is reduced by Candida species to form sulphide. Bismuth in the medium combine with the sulphide to produce brown to black pigmented colonies and zones of dark precipitate in the medium surrounding the colonies of some species. Bismuth sulphite also acts as an inhibitor of bacterial growth. Selectivity of medium is increased by incorporation of penicillin and streptomycin in the medium which helps to suppress the growth of many bacteria.

Formula*

Ingredients in grams per liter

Mycological peptone	2.50
Dextrose	5.00
Disodium hydrogen phosphate	5.00
Sodium sulphite	5.00
Bismuth sulphite indicator	3.00
Agar	15.00

Final pH (at 25°C) 7.6 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 35.5 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 50-52°C and ascetically add 0.3 units of Penicillin and 25µg Streptomycin per ml of sterile medium.
5. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 30°C.

Organisms (ATCC)	Growth	RGI
Candida albicans (10231)	Good to luxuriant	More than 70%
Candida tropicalis (1369)	Good to luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before incubating.
2. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plate in an inverted position.
5. Once incubated, the medium should be protected from light and incubated aerobically at 25-30°C with increase humidity for 48 hrs.

Interpretation of Results

Refer to appropriate reference and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Carbohydrate Consumption Broth AM50220

Use

Carbohydrate Consumption Broth is recommended for cultivation and differentiation of *Listeria* species.

Summary

Carbohydrate Consumption Broth is used for cultivation and differentiation of *Listeria* species (1.1). It has slightly different concentration of Bromo cresol purple than recommended by FDA(2.1) and ISO(46.2) committee. Differentiation is based on fermentation of glucose, dulcitol, raffinose, rhamnose and salicin.

Principle

Peptone and beef extract provide carbon and nitrogen compounds including essential amino acids, vitamins and trace elements for bacterial metabolism.

Bromo cresol purple is the pH indicator which turns yellow in acidic condition.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Sodium chloride	5.00
Beef extract	1.00
Bromocresol purple	0.10

Final pH (at 25°C) 6.8 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the16.1 gms of powder in 990 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Aseptically add 10 ml separately sterilized carbohydrate solution to give a final concentration of 0.5%.
5. Mix well and pour into sterile test tubes.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Without carbohydrate		With 1% Dextrose	
		Acid	Gas	Acid	Gas
Listeria monocytogenes (19118)	Luxuriant	—	----	+	---
Escherichia coli (25922)	Luxuriant	—	----	+	+
Staphylococcus aureus (25923)	Luxuriant	—	----	+	---

Interpretation of Results

Refer to appropriate reference and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cetrimide Agar (Harmonized Media) AMH5022

Use

Cetrimide Agar is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

Summary

Cetrimide Agar is based on the formulation described by King *et al.*, and is widely recommended for use in the examination of cosmetics, pharmaceuticals and clinical specimens for the presence of *P.aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism. Strains of *P.aeruginosa* are identified from specimens by the production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology and the characteristic grapelike odour of aminoacetophenone. *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide Agar Base is therefore, a valuable culture medium in the identification of this organism. It is also included in the Bacteriological Analytical Manual for cosmetics testing and recommended by the USP, BP and IP in Microbial Limit Tests.

Principle

Cetrimide (Cetyltrimethylammonium bromide) is a quaternary ammonium compound, cationic detergent, which is inhibitory to a wide variety of bacteria including *Pseudomonas* species other than *P.aeruginosa*. It causes nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa*. The magnesium chloride and potassium sulphate in the medium stimulates the production of pyocyanin. Pancreatic digest of gelatin provides nitrogenous compounds.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	20.0
Magnesium chloride	1.4
Dipotassium sulphate	10.3
Cetrimide	0.3
Agar	13.6

Final pH (at 25°C) 7.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.6gms of the powder in 1000 ml distilled water containing 10 ml glycerol.
2. Mix thoroughly.
3. Heat with frequent agitation and boil to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. If desired, add rehydrated contents of 1 vial of Nalidixic Selective Supplement (AS020) aseptically to sterile molten medium.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel, with slight precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Inhibited	0%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Pseudomonas maltophila (13637)	Inhibited	0%
Staphylococcus aureus (25923)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate at 35-37°C for 24-48 hours.

Precautions / Limitations

1. Certain strains of *P.aeruginosa* may not produce pyocyanin while other species of Pseudomonas do not produce pyocyanin but fluoresce under UV light.
2. Most non-*Pseudomonas* species are inhibited, and some species of Pseudomonas may also be inhibited.
3. The type of peptone used in the base may affect pigment production.
4. No single medium can be depended upon to exhibit all pigment producing *P.aeruginosa* strains.
5. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this colouration is easily distinguished from fluorescein production since this yellowing does not fluoresce.
6. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. Serratia strains may exhibit a pink pigmentation.
7. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days.
8. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cetrimide Agar Base — AM1022/AM5022

Use

Cetrimide Agar Base is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

Summary

Cetrimide Agar Base is based on the formulation described by King et al (55) and is widely recommended for use in the examination of cosmetics, pharmaceuticals and clinical specimens for the presence of *P.aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism. Strains of *P.aeruginosa* are identified from specimens by the production of pyocyanin, a blue, water-soluble, non-fluorescent, phenazine pigment in addition to their colonial morphology and the characteristic grapelike odour of aminoacetophenone. *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide Agar Base is therefore, a valuable culture medium in the identification of this organism. It is also included in the Bacteriological Analytical Manual for cosmetics testing (113) and recommended by the USP and IP in Microbial Limit Tests (114, 46).

Principle

Cetrimide (Cetyltrimethylammonium bromide) is a quaternary ammonium compound, cationic detergent, which is inhibitory to a wide variety of bacteria including *Pseudomonas* species other than *P.aeruginosa*. It causes nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa*. The magnesium chloride and potassium sulphate in the medium stimulates the production of pyocyanin. Pancreatic digest of gelatin provides nitrogenous compounds.

Formula*

Ingredients in grams per liter

Pancreatic Digest of Gelatin	20.0
Potassium Sulphate	10.0
Magnesium Chloride	1.4
Cetrimide	0.3
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 46.7 gms of the powder in 1000 ml distilled water containing 10 ml glycerol.
2. Mix thoroughly.
3. Heat with frequent agitation and boil to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

5. If desired, add rehydrated contents of 1 vial of Nalidixic Selective Supplement (AS020) aseptically to sterile molten medium.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel, with slight precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Inhibited	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Pseudomonas maltophila (13637)	Inhibited	0%
Staphylococcus aureus (25923)	Inhibited	0%

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate at 35-37°C for 24-48 hours.

Interpretation of Results

1. Colonies that are surrounded by a blue-green pigment and fluoresce under ultraviolet light (wavelength 254 nm) may be presumptively identified as *Pseudomonas aeruginosa.*

Precautions / Limitations

1. Certain strains of *P.aeruginosa* may not produce pyocyanin while other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light.
2. Most non-*Pseudomonas* species are inhibited, and some species of *Pseudomonas* may also be inhibited.
2. The type of peptone used in the base may affect pigment production.
3. No single medium can be depended upon to exhibit all pigment producing *P.aeruginosa* strains.
4. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this colouration is easily distinguished from fluorescein production since this yellowing does not fluoresce.
5. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.
6. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days.
7. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cetrimide Agar IP — AM10221/AM50221
Cetrimide Agar USP — AM10222/AM50222
Cetrimide Agar EP — AM10223/AM50223
Cetrimide Agar BP — AM10224/AM50224

Use

Cetrimide Agar Base is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

Summary

Cetrimide Agar Base is based on the formulation described by King et al., and is widely recommended for use in the examination of cosmetics, pharmaceuticals and clinical specimens for the presence of *P.aeruginosa,* as well as for evaluating the efficacy of disinfectants against this organism. Strains of *P.aeruginosa* are identified from specimens by the production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology and the characteristic grapelike odour of aminoacetophenone. *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide Agar Base is therefore, a valuable culture medium in the identification of this organism. It is also included in the Bacteriological Analytical Manual for cosmetics testing and recommended by the USP, BP and IP in Microbial Limit Tests.

Principle

Cetrimide (Cetyltrimethylammonium bromide) is a quaternary ammonium compound, cationic detergent, which is inhibitory to a wide variety of bacteria including *Pseudomonas* species other than *P.aeruginosa.* It causes nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa.* The magnesium chloride and potassium sulphate in the medium stimulates the production of pyocyanin. Pancreatic digest of gelatin provides nitrogenous compounds.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	20.0
Magnesium chloride	1.4

Dipotassium sulphate	10.0
Cetrimide	0.3
Agar	13.6

Final pH (at 25°C) 7.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.3 gms of the powder in 1000 ml distilled water containing 10 ml glycerol.
2. Mix thoroughly.
3. Heat with frequent agitation and boil to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. If desired, add rehydrated contents of 1 vial of Nalidixic Selective Supplement (AS020) aseptically to sterile molten medium.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel, with slight precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Inhibited	0%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Pseudomonas maltophila (13637)	Inhibited	0%
Staphylococcus aureus (25923)	Inhibited	0%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate at 35-37°C for 24-48 hours.

Precautions / Limitations

1. Certain strains of *P.aeruginosa* may not produce pyocyanin while other species of Pseudomonas do not produce pyocyanin but fluoresce under UV light.
2. Most non-Pseudomonas species are inhibited, and some species of *Pseudomonas* may also be inhibited.
3. The type of peptone used in the base may affect pigment production.
4. No single medium can be depended upon to exhibit all pigment producing *P.aeruginosa* strains.
5. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this colouration is easily distinguished from fluorescein production since this yellowing does not fluoresce.
6. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. Serratia strains may exhibit a pink pigmentation.
7. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days.
8. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cetrimide Broth AM1023/AM5023

Use

Cetrimide Broth is used for cultivation of *Pseudomonas aeruginosa.*

Summary

Cetrimide Broth is the modification of the formula described by King, Ward and Raney (55). It is a selective medium for the cultivation of *Pseudomonas aeruginosa* due to the presence of cetrimide.

Principle

Cetrimide (Cetyltrimethylammonium bromide) is a quaternary ammonium compound, cationic detergent, which is inhibitory to a wide variety of bacteria including *Pseudomonas* species other than *P.aeruginosa.* It causes release of phosphorous and nitrogen from bacterial cells other than *Pseudomonas aeruginosa.* Peptone and beef extract provide necessary nutrients for *Pseudomonas aeruginosa.* Sodium chloride maintains osmotic balance.

Formula*

Ingredients in grams per liter

Beef Extract	10.0
Peptone	10.0
Cetrimide	0.3
Sodium Chloride	5.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25.3 gms of the powder in 1000 ml distilled water.
2. Boil to dissolve the powder completely.
3. Dispense in tubes as per requirements.

4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to very slightly opalescent solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Inhibited
Pseudomonas aeruginosa (27853)	Luxuriant
Staphylococcus aureus (25923)	Inhibited

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chapman Stone Agar AM50231

Use

Chapman Stone Agar is recommended for the selective isolation of *Staphylococci* causing food poisoning.

Summary

Chapman (14.1) developed Staphylococcus Agar No. 110 and subsequently modified it by reducing sodium chloride concentration and incorporating ammonium sulphate in the formulation (16.1).

Principle

Casein enzymic hydrolysate, yeast extract provide nitrogen, carbon, sulphur, vitamin B and trace elements. Sodium chloride acts as a selective agent which inhibits most of the bacterial species. Mannitol is the fermentable carbohydrate and its fermentation can be detected by adding a few drops of bromo cresol purple resulting in production of yellow colour. Gelatin hydrolysis id observed as clear zones around colonies. Due to the presence of ammonium sulphate in the medium itself there is no need to flood the plate with ammonium sulphate solution for detection of gelatin liquefaction by the isolates which is knows as stone's method (103.1). After incubation of 48 hours at 35°C, cream to golden yellow colonies surrounded by clear zone are presumptively identified as *Staphylococcus aureus*. White or non-pigmented colonies, with or without a clear zone, are presumptively identified as *Staphylococcus epidermidis*.

Formula*

Ingredients in grams per liter

Casein enzyme hydrolysate	10.00
Yeast extract	2.50
Gelatin	30.00
D- mannitol	10.00
Sodium chloride	55.00
Ammonium sulphate	75.00
Dipotassium Phosphate	5.0
Agar	15.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20.25gms of the powder in 100 ml distilled water.
2. Boil to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 10 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, coarse, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-48 hours at 30°C.

Organisms (ATCC)	Growth	Gelatinase production	Mannitol fermentation	RGI
Staphylococcus aureus (25923)	Luxuriant	+	+	More than 70%
Staphylococcus epidermidis(12228)	Luxuriant	+	–	More than 70%
Escherichia coli (25922)	Inhibited	–	–	0%

* Key: Gelatinase + = clearing or halo.

Procedure

1. Use standard procedures to obtain isolated colonies from

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chloramphenicol Yeast Glucose Agar AM1024/AM5024

Use

Chloramphenicol Yeast Glucose Agar is a selective medium used for isolation and enumeration of yeasts and moulds in milk and milk products.

Accumix

Summary

The antibiotic method for enumerating yeasts and moulds in dairy products has become the method of choice, thereby replacing the traditional acidified method. The use of antibiotics for suppressing bacteria results in better recovery of injured fungal cells, which are sensitive to an acid environment and in less interference from precipitated food particles during the counting. Chloramphenicol Yeast Glucose Agar is a nutrient medium that inhibits the growth of organisms other than yeasts and moulds due to the presence of chloramphenicol. Chloramphenicol Yeast Glucose Agar is recommended by APHA for the examination of dairy products (39). The ISO committee recommends this medium for the enumeration of yeasts and moulds.

Principle

Yeast extract provides nitrogen and vitamin B complex. Dextrose is the carbohydrate source. Chloramphenicol, a thermostable antibiotic, suppresses accompanying bacterial flora, which improves the shelf life of the prepared medium; therefore the prepared medium can be used over a period of at least 4 months.

Formula*

Ingredients in grams per liter

Dextrose	20.0
Yeast Extract	5.0
Chloramphenicol	0.1
Agar	14.9

Final pH (at 25°C) 6.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40 gms of the powder in 1000 ml distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Warning: Chloramphenicol is a potent carcinogen and must be handled with care so as to avoid inhalation or contact with skin.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 2-5 days at 22-25°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Good to luxuriant	More than 70%
Candida albicans (10231)	Good to luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	More than 0%
Saccharomyces cerevisiae (9763)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Inhibited	More than 0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Prepare initial sample dilutions using 10 gms or 10 ml of sample in 90 ml of diluent, as listed below:

SAMPLE	DILUENT	PREPARATION
Milk, Liquid milk product	¼-strength Ringer's solution	Mix.
Dried milk, Whey powder, Buttermilk powder, Lactose	¼-strength Ringer's solution	Shake at 47°C.
Casein	2% Dipotassium phosphate solution	Shake at 47°C.
Cheese	2% Sodium citrate solution	Shake at 47°C.
Butter, Edible ice	¼-strength Ringer's solution	Shake at 47°C.
Custard, Dessert Fermented milk, Yogurt	¼-strength Ringer's solution	Shake.

2. Add 10 ml from the initial dilution prepared above to 90 ml of ¼-strength Ringer's solution. One milliliter (1ml) of this dilution corresponds to 0.01 gm/ml of sample.
3. Prepare further dilutions by adding 10 ml of the above 0.01 gm/ml dilution to 90 ml of diluent.
4. Pipette 1ml of each dilution into two petri plates.
5. Pour 10 ml of sterile molten agar (cooled to 45°C) into each sterile petri plate. Mix thoroughly.
6. Incubate at 25°C for 4 days.

Interpretation of Results

1. Select plates containing 30-300 colonies and count the colonies.
2. Distinguish yeasts from moulds by colony morphology.
3. Express results as yeasts and moulds "per gram" or "per milliliter".

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Christensen Citrate Agar AM1025/AM5025

Use

Christensen Citrate Agar is used for the differentiation of enteric pathogens and coliforms on the basis of citrate utilization.

Summary

Christensen Citrate Agar is a modification of Christensen Iron Agar (18). Christensen reported that all members of genera *Escherichia, Enterobacter, Citrobacter, Salmonella* and Alkalescens-Dispar group were capable of utilizing citrate as a source of energy while on the other hand *Shigella* failed to do so. Christensen Citrate Agar is recommended by APHA for the examination of foods (20).

Principle

Yeast extract provide nitrogen and vitamin B complex. L-cysteine hydrochloride is a reducing agent. Dextrose is the carbohydrate source. Sodium citrate is the energy source for citrate utilizing organisms. Sodium chloride maintains the osmotic balance. Monopotassium phosphate acts as the buffer. Organisms that metabolize citrate as the sole source of carbon cleave citrate to oxaloacetate and acetate via the citritase enzyme. Another enzyme, oxaloacetate decarboxylase, then converts oxaloacetate to pyruvate and CO_2. This CO_2 combines with sodium and water to form an alkaline sodium carbonate compound. As a result the pH of the medium rises and the indicator, phenol red changes from orange red to cerise. Presence of cerise colour indicates a positive finding for citrate utilization.

Formula*

Ingredients in grams per liter

Sodium Citrate	3.0
Yeast Extract	0.5
Monopotassium Phosphate	1.0
L-Cysteine Hydrochloride	0.1
Phenol Red	0.012
Dextrose	0.2
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.81 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense into test tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the tubes in a slanted position.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Orange red coloured, very slightly opalescent gel.

Cultural Response

Cultural characteristic after 24-48 hours at 37°C

Organisms (ATCC)	Growth	Colour of the slant
Enterobacter aerogenes (13048)	Luxuriant	Cerise
Escherichia coli (25922)	Luxuriant	No change
Klebsiella pneumoniae (13883)	Luxuriant	Orange to pink
Salmonella serotype Enteritidis (13076)	Luxuriant	Cerise

For growth RGI should be more than 70%

RGI- Relative Growth Index

Interpretation of Results

1. Presence of cerise colour indicates a positive finding for citrate utilization.

Precautions / Limitations

1. Care should be taken while inoculating, as, too heavy an inoculum may give a false positive result.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chromogenic Coliform Agar AM10251/AM50251

Use

Chromogenic Coliform Agar is recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

Summary

Escherichia coli is a bacterium whose natural habitat is the enteric tract of humans and warm-blooded animals. Transmission most often occurs through ingestion of raw or uncooked beef, from person to person and recreational water sources. Thus, the presence of *E.coli* in foods is an indicator of direct and indirect fecal contamination and possible presence of enteric pathogens in water, shellfish, dairy products and other foods (74, 75, 90).

Hence, Chromogenic Coliform Agar is recommended for simultaneous detection of *Escherichia coli* and total coliforms in water and food samples. The chromogenic

mixture in this medium consists of chromogenic substrates, which release insoluble coloured compounds when hydrolysed by a specific enzyme, which permits differentiation of pathogenic microorganisms.

Principle

Peptone special and sodium pyruvate provide essential growth nutrients. Dipotassium hydrogen phosphate and Potassium dihydrogen phosphate act as buffers. Sodium lauryl sulphate inhibits the growth of gram-positive organisms. The chromogenic mixture in this medium contains two substrates namely Salmon-GAL and X-glucuronide.

The characteristic enzyme for coliforms, ß-D-galactosidase cleaves the Salmon-GAL substrate and causes a salmon to red colour of the coliform colonies.

The substrate X-glucuronide is used for the identification of ß-D-glucuronidase, which is characteristic for *E. coli*. *E. coli* cleaves both Salmon-GAL and X-glucuronide, so that positive colonies take on a dark-blue to violet colour. These are easily distinguished from other coliform colonies, which have a salmon to red colour. As part of an additional confirmation of *E. coli*, the inclusion of tryptophan improves the indole reaction, thereby increasing detection reliability when it is used in combination with the Salmon-GAL and X-glucuronide reaction.

Formula*

Ingredients in grams per liter

Peptone special	3.0
Sodium chloride	5.0
Sodium pyruvate	1.0
Tryptophan	1.0
Sodium lauryl sulphate	0.1
Dipotassium hydrogen phosphate	3.0
Potassium dihydrogen phosphate	1.7
Chromogenic mixture	0.2
Agar	12.0

Final pH (at 25° C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 27 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. When a high number of gram-positive accompanying bacteria are expected, add 5 mg/L Novobiocin.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Colourless, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37° C.

Organisms (ATCC)	Colony Colour	Salmon GAL	X-Glucuro-nide	Indole
Escherichia coli (25922)	Dark blue/violet	+	+	+
Enterobacter cloacae (13047)	Salmon to red	+	-	-
Citrobacter freundii (8090)	Salmon to red	+	-	-
Klebsiella pneumoniae (13883)	Light pink	+	-	-
S. serotype Enteridis (13076)	Colourless	-	-	-
Shigella flexneri (12022)	Colourless	-	-	-
Enterococcus faecalis (29212)	Inhibited	-	-	-

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate the medium by the pour plate method or by spreading the sample material on the surface of the plates. In addition the membrane-filter-technique can also be used.
2. Incubate for 24 hours at 35 - 37°C.

Interpretation of Results

1. *E. coli:* dark-blue to violet colonies (Salmon-GAL and X-glucuronide reaction).
2. Total coliforms: salmon to red colonies (Salmon-GAL reaction) and dark-blue to violet colonies (*E. coli*).
3. Other gram-negative microorganisms: colourless colonies, except for some organisms, which possess ß-D-glucuronidase activity. These colonies appear light blue to turquoise.
4. In order to confirm *E. coli*, coat the dark-blue to violet colonies with a drop of Kovac's indole reagent. If the reagent turns to a cherry-red colour after some seconds, a positive indole formation confirms the presence of *E. coli*.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chromogenic *E. coli* Agar — AM10252/AM50252

Use

Chromogenic *E.coli* Agar is recommended for the detection and enumeration of *E. coli* in foods without further confirmation on membrane filter or by indole reagent.

Summary

Escherichia coli is a common Gram-negative microorganism, which may be present in food and water samples. The detection of *E.coli* and its differentiation from other coliforms is important in medical and environmental analyses (75,90). Chromogneic *E.coli* Agar is a differential agar for presumptive identification of *E.coli* from other coliforms by presence of an enzyme glucoronidase produced by *E.coli* strains. The chromogenic agent in this medium consists of chromogenic substrates, which release insoluble coloured compounds when hydrolysed by a specific enzyme, which permits differentiation of pathogenic microorganisms.

Principle

Tryptone and Peptone special provide essential growth nutrients. Bile salts mixture inhibits the growth of gram-positive organisms. Sodium chloride maintains the osmotic equilibrium. Disodium hydrogen phosphate and Sodium dihydrogen phosphate act as buffers. The chromogenic agent X-glucuronide is used for identification of *E. coli*. *E.coli* cleaves X-glucuronide, giving a colouration to the colonies, which are easily distinguished from other coliform colonies.

Formula*

Ingredients in grams per liter

Tryptone	14.0
Peptone, special	5.0
Sodium chloride	2.4
Bile Salts mixture	1.5
X-Glucuronide	0.075
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Agar	12.0

Final pH (at 25° C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36.58 grams of the powder in 1000 ml distilled water.
2. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
3. Cool to 50° C and pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 44° C.

Organisms (ATCC)	Growth	Colony Colour	RGI
Escherichia coli (25922)	Luxuriant	Blue	More than 70%
S. serotype Enteridis (13076)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	--	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Dry the surface of the medium in the prepared plates.
2. Prepare the food sample by diluting 1 in 5 or 1 in 10 (as appropriate) with 0.1% (w/v) sterile Peptone Water and homogenize in a stomacher or laboratory blender.
3. Pipette 0.5 ml or 1.0 ml (as appropriate) of the homogenate on to the petriplate and spread over the surface with a sterile glass spreader.
4. Incubate plates for 18 - 24 hours at 35 - 37° C.

Interpretation of Results

1. Clear differentiation of blue coloured E.coli colonies observed.

Precautions / Limitations

1. Avoid contact with eyes, wear appropriate mask while handling the product.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chromogenic Enterococci Broth — AM10253/AM50253

Use

Chromogenic Enterococci Broth is recommended for identification and differentiation of *Enterococci* from water samples.

Summary

The presence of *Enterococci*, which account for most of the faecal *streptococci*, serves as an indicator for faecal contamination. It is more specific than the presence of coliforms, which may originate from non-faecal sources (74). Chromogenic *Enterococci* Broth is recommended for identification and differentiation of *Enterococci* from other coliforms. The chromogenic mixture in this medium consists of chromogenic substrates, which release insoluble coloured

compounds when hydrolysed by a specific enzyme, which permits differentiation of pathogenic microorganisms.

Principle

Peptone special provides essential nutrients and nitrogenous compounds to the medium. Sodium azide inhibits the growth of gram-negative organisms. Sodium chloride maintains the osmotic equilibrium. Disodium hydrogen phosphate acts as a buffer. The chromogenic substrate is cleaved by the enzyme -D-glucosidase produced by *Enterococci* resulting in colour change of the medium from light yellow to blue green.

Formula*

Ingredients in grams per liter

Peptone, special	10.0
Sodium chloride	5.0
Sodium Azide	0.3
Chromogenic Mixture	0.04
Disodium hydrogen phosphate	1.0

Final pH (at 25° C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 16.59 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
4. Dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37° C.

Organisms (ATCC)	Growth	Colour of the Broth
Enterococcus faecalis (29212)	Luxuriant	Blue
Escherichia coli (25922)	None to poor	Light yellow
Staphylococcus aureus (25923)	None to poor	Light yellow
Pseudomonas aeruginosa (27853)	None to poor	Light yellow

Procedure

1. Inoculate the medium by the pour-plate method or by spreading the sample material on the surface of the medium. In addition, the membrane-filter technique may also be used.
2. Incubate at 24 ± 4 hours at 35-37°C. If there is no color change or any visible growth after 24±4 hours, continue the incubation up to 44±4 hours.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials and it is advisable to flush off the disposables with water.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chromogenic Improved Salmonella Agar AM10255/AM50255

Use

Chromogenic Improved Salmonella Agar for identification and differentiation of *Salmonella* from water sample.

Summary

Salmonella Medium was developed by *A. Rambach. Salmonella* is ubiquitous in animal populations and is generally isolated from the intestines of animals and humans. It is one of the most prevalent organisms associated with food borne illness, which is often linked to animal origin. Illness caused by *Salmonella* have been associated with poultry, chocolate, dairy and vegetable products.

Salmonella medium is intended for the identification and differentiation of *Salmonella* species based on the formation of an insoluble pigment. The addition of chromogenic substrate in the medium facilitrates detection of *Salmonella* species from other normal flora.

Principle

Specially selected peptone supply the nutrients. Gram-positive organisms are generally inhibited as a result of the selective medium base used.

Formula

Ingredients Gms/Liter

Peptone special	8.0
Yeast extract	2.0
Sodium deoxycholate	1.0
Chromogenic substance	3.25
Agar	12.0

Final pH (at 25°C) 7.3 ±0.2

Directions

1. Suspend 26.25 gms of the powder in 1000 ml-distiled water and mix thoroughly.

2. Boil with frequent agitation to dissolve the powder completely.
3. DO NOT AUTOCLAVE.
4. Cool to 50°C and pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Red coloured, coarse, free flowing powder.

Prepared Appearance:

Reddish pink coloured slightly opalescent gel forms in periplates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour	RGI of colony
Escherichia coli (25922)	Luxuriant	Blue	More than 70%
Salmonella Serotype Typhimurium (14028)	Luxuriant	Light pink	More than 70%
S. Serotype Enteritidis (13076)	Luxuriant	Pink	More than 70%
Proteus vulgaris (13315)	Good	Light brown	More than 70%
Staphylococcus aureus (25923)	Inhibited	–	0%
Bacillus subtillus (6633)	Inhibited	–	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Chromogenic UTI Agar AM10254/AM50254

Use

Chromogenic UTI Agar is a differential medium recommended for the presumptive identification of microorganisms mainly causing urinary tract infections.

Summary

Escherichia coli, Enterococci, Klebsiella, and *Proteus* are frequently encountered microorganisms in urinary tract infections. For many years, blood, CLED and MacConkey Agars have been used for the detection of urinary pathogens as well as for differentiation of a few of them (123). However, chromogenic media have proved more specific for direct differentiation of microorganisms. Chromogenic UTI Agar has been formulated on these lines. It facilitates and expedites the identification of some gram-negative and gram-positive bacteria based on reactions of the chromogenic substrates with specific enymes produced by these pathogens. The chromogenic mixture in this medium consists of chromogenic substrates, which release insoluble coloured compounds when hydrolysed by a specific enzyme, which permits differentiation of pathogenic microorganisms.

Principle

Peptone special provides essential growth nutrients. The chromogenic mixture consists of artificial substrates, which release insoluble coloured compounds when hydrolysed by a specific enzyme, permitting differentiation of Enterococci, *E. coli* and coliforms.

Formula*

Ingredients in grams per liter

Peptone, special	15.0
Chromogenic mixture	2.45
Agar	15.0

Final pH (at 25° C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 32.45 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
4. Cool to 50° C and pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24 hours at 35-37° C.

Organisms (ATCC)	Growth	Colony Colour	RGI
Escherichia coli (25922)	Luxuriant	Pink-red	More than 70%
Enterococcus faecalis (29212)	Luxuriant	Blue, small	More than 70%
Staphylococcus aureus (25923)	Luxuriant	Golden yellow	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Colourless	More than 70%
Proteus mirabilis (10975)	Luxuriant	Light brown	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant mucoid	Blue to purple,	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Inoculate the urine specimen on to the medium.
2. Incubate plates aerobically at 35-37^{0}C for not less than 20-24 hours.
3. Observe for growth.

Precautions / Limitations

1. Do not incubate the plates in an atmosphere supplemented with carbon dioxide.
2. Avoid exposure to light during incubation as light may destroy the chromogens. However, once the colony colour develops, exposure to light is permissible.
3. Colonies that show their natural colour and do not react with chromogenic substrates must be further differentiated with appropriate biochemical or serological tests.
4. In rare cases, Listeria species might be present in urine. Hence, it is necessary to perform a gram stain of all microorganisms isolated. Isolates of *Aeromonas hydrophila* may produce rose colonies and can be differentiated from *E. coli* by performing the oxidase test.
5. This medium will not support growth of fastidious microorganisms such as *Neisseria* species.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

C.L.E.D. Agar with Andrade Indicator AM1026/AM5026

Use

C.L.E.D. Agar with Andrade indicator is used for the isolation, enumeration and presumptive identification of microorganisms from urine, giving good colonial differentiation.

Summary

Sandys (101) observed that restricting the electrolytes on a solid medium might prevent the swarming of *Proteus*. Previous chemical methods used to inhibit swarming of *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulphonamides to the culture medium. This electrolyte-deficient medium was modified for use in urine culture by substituting lactose and sucrose instead of mannitol and increasing the concentrations of bromothymol blue indicator and agar. The medium was further modified by the incorporation of cystine in order to enhance the growth of cystine-dependent "dwarf colony " coliforms and by the deletion of sucrose. This new medium, Cystine-Lactose-Electrolyte-Deficient (C.L.E.D.) Agar is ideal for dip-inoculation techniques and for urinary bacteriology in general.

Principle

C.L.E.D. Agar with Andrade indicator is similar to C.L.E.D. Agar with Bromothymol blue except in this medium Andrade indicator (Acid Fuchsin in 1N Sodium Hydroxide) is incorporated. The essential nutrients are supplied by peptone, tryptone and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Addition of Andrade indicator enhances the appearance of colony and aids in the identification of microorganisms. At different pH values, the colour of the medium varies from the standard medium.

pH	Colour of the medium
7.4	deep blue
7.0	bluish grey
6.8	pale grey
6.6	pinkish grey
6.4	bright red with whitish tinge
6.0	bright red

Formula*

Ingredients in grams per liter

Lactose	10.0
Tryptone	4.0
Peptone	4.0
Beef Extract	3.0
L-Cystine	0.128
Andrade Indicator	0.10
Bromothymol Blue	0.02
Agar	15.0

Final pH (at 25^{0}C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36.25 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Greenish blue coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Greyish green, mucoid	More than 70%
Enterococcus faecalis (29212)	Luxuriant	Orange, yellow or green	More than 70%
Escherichia coli (25922)	Luxuriant	Bright pink with halo	More than 70%
Proteus mirabilis (25933)	Luxuriant	Blue-green	More than 70%
Staphylococcus aureus (25923)	Luxuriant	Golden yellow	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	Greyish green	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Inoculate the medium as soon as possible after the specimen is received in the laboratory.
2. It is recommended that quantitative methods be used for culturing urine specimens.
3. Incubate at 35-37^{0}C for 24 hours.

Interpretation of Results

1. Count the number of colonies on the plate or dipstick. Multiply by the dilution factor to convert the count to CFU per ml of the sample.
2. Contaminant bacteria usually appear in low numbers and vary in colony morphology.
3. Urinary pathogens will usually yield high counts having uniform colonial morphology and should not be sub cultured directly to routine media for identification and susceptibility testing.

Precautions / Limitations

1. The medium should not be incubated for more than 24 hours since, if lactose fermenters predominate, the whole medium may turn pink, masking the presence of non-lactose fermenters.
2. Factors that may cause urine counts from infected patients to be low include: apid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.
3. *Shigella* species may not grow on this medium.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

C.L.E.D. Agar with Bromothymol Blue AM1027/AM5027

Use

C.L.E.D. Agar with Bromothymol Blue is used for the isolation, enumeration and presumptive identification of microorganisms from urine.

Summary

Sandys (101) observed that restricting the electrolytes on a solid medium might prevent the swarming of *Proteus*. Previous chemical methods used to inhibit swarming of *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulphonamides to the culture medium. This electrolyte medium was modified for use in urine culture by substituting lactose and sucrose instead of mannitol and increasing the concentrations of bromothymol blue indicator and agar. The medium was further modified by the incorporation of cystine in order to enhance the growth of cystine-dependent "dwarf colony " coliforms and by the deletion of sucrose. This new medium, Cystine-Lactose-Electrolyte-Deficient (C.L.E.D.) Agar is ideal for dip-inoculation techniques and for urinary bacteriology in general.

Principle

The essential growth nutrients are supplied by peptone, tryptone and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Bromothymol blue is used as the pH indicator to differentiate lactose fermenters from non-lactose fermenters. Organisms that ferment lactose will lower the pH and change the colour of the medium from green to yellow. Electrolyte sources are reduced in order to restrict the swarming of *Proteus* species.

Formula*

Ingredients in grams per liter

Tryptone	4.0
Peptone	4.0
Lactose	10.0
Beef Extract	3.0
L-Cystine	0.128
Bromothymol Blue	0.02
Agar	15.0

Final pH (at 25^{0}C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36.15 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.

3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogenous, free flowing powder.

Prepared Appearance

Green coloured, very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35- 37°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Enterococcus faecalis (29212)	Luxuriant	Slight yellowish or greenish	More than 70%
Escherichia coli (25922)	Luxuriant	Yellow opaque, center slightly deep yellow	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant	Yellow to whitish blue	More than 70%
Proteus vulgaris (13315)	Luxuriant	Blue	More than 70%
Staphylococcus aureus (25923)	Luxuriant	Golden yellow	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

1. Inoculate the medium as soon as possible after the specimen is received in the laboratory.
2. It is recommended that quantitative methods be used for culturing urine specimens.
3. Incubate at 35-37°C for 18-24 hours.

Interpretation of Results

1. Count the number of colonies on the plate or dipstick. Multiply by dilution factor to convert the count to CFU per ml of the sample.
2. Contaminant bacteria usually appear in low numbers, which vary in colony morphology. Urinary pathogens will usually yield high counts having uniform colonial morphology and should not be subcultured directly to routine media for identification and susceptibility testing.
3. Typical colony morphology on C.L.E.D. Agar is as follows:

E.coli	Yellow colonies, opaque, center slightly deeper yellow.
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid.
P.aeruginosa	Green colonies with typical matted surface and rough periphery.
Enterococci	Small yellow colonies, about 0.5 mm in diameter.
S.aureus	Deep yellow colonies, uniform in colour.
Coagulase negative staphylococci	Pale yellow colonies, more opaque than *E.faecalis.*

Precautions / Limitations

1. The medium should not be incubated for more than 24 hours since, if lactose fermenters predominate, the whole medium will turn pink, thus masking the presence of non-lactose fermenters.
2. Factors that may cause urine counts from infected patients to be low include: rapid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.
3. *Shigella* species may not grow on this medium.?

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Coagulase Mannitol Agar Base AM1028/AM5028

Use

Coagulase Mannitol Agar Base with added plasma is used for isolation and differentiation of *staphylococci* from clinical specimens or for classifying pure cultures.

Summary

Coagulase-positive and coagulase-negative *Staphylococcus* species have major medical significance. Coagulase producing *staphylococci* (*S.aureus*) may be differentiated and presumptively identified on this medium based on production of coagulase and mannitol utilization. Chapman (16) introduced the first selective medium for isolating and differentiating *staphylococcal* species. Several years later, Zebovit et al (124) and Marwin (79) introduced tellurite-glycine media designed to selectively isolate coagulase positive staphylococcal species. The present formulation is based on Esber and Faulconer (26) formulation.

Principle

Coagulase production is dependent on the presence of mannitol and a protein factor in the brain heart infusion and blood serum (plasma). During utilization of mannitol, the pH of the medium drops, causing the bromocresol purple indicator

to change from purple to yellow, producing yellow zones around these colonies. An opaque area of coagulated plasma forms around the colonies of organisms that also produce coagulase. In contrast some coagulase-negative species that do not utilize mannitol, such as *Staphylococcus epidermidis*, do not change the colour of the medium and it remains clear. Other coagulase-negative species may utilize mannitol and produce a yellow zone around the colony, but an opaque zone will not be produced.

Formula*

Ingredients in grams per liter

Tryptone	10.5
Mannitol	10.0
Brain Heart Infusion	5.0
Sodium Chloride	3.5
Soya Peptone	3.5
Bromocresol Purple	0.02
Agar	14.5

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 47.02 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 118°C -121°C (12-15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C.
6. Add 7-15% v/v sterile, pre-tested, rabbit plasma to the basal medium.
7. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Light grey coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Mannitol Fermentation	Coagulase production	RGI
Staphylococcus aureus (25923)	Luxuriant	+ (yellow)	+ (opaque zone)	More than 70%
Staphylococcus epidermidis (12228)	Luxuriant	- (purple)	-	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Inoculate and incubate at 35-37°C, and examine for growth after 18-24 hours.
2. Avoid prolonged incubation because it may cause the opaque zones surrounding coagulase-positive organisms to become clear.

Interpretation of Results

1. Coagulase-positive organisms will produce opaque zones; coagulase-negative organisms will produce no opacity.
2. Organisms that utilize mannitol produce yellow zones. *S.aureus* may be presumptively identified as those colonies with opaque, yellow zones around them.

Precautions

1. Some old or mutant strains of *S.aureus* may be weak coagulase producers or exhibit negative coagulase reaction and should be subcultured and retested if in doubt.
2. *E.coli* also uses mannitol and may be weakly coagulase-positive. Colonial morphology and a gram stain should readily allow for differentiation from *S.aureus.*

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Columbia Blood Agar Base AM1029/AM5029

Use

Columbia Blood Agar Base is used as an efficient base for the preparation of Blood Agar, Chocolate Agar and for various selective and identification media in isolating and cultivating fastidious microorganisms.

Summary

Columbia Blood Agar Base was developed by Ellner et al (24) and is used for isolating, cultivating and determining the haemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, it can be used as a general-purpose medium. It is also used for the selective cultivation of *Brucella* and *Campylobacter* species by the addition of the respective selective supplement. Columbia Blood Agar Base is recommended by APHA for the examination of foods (20).

Principle

Peptone special provides essential nutrients. Corn starch serves as the energy source and also neutralizes toxic metabolites. Columbia Blood Agar Base is used as a base for media containing blood and for selective media formulations in

which different combinations of antimicrobial agents are added as additives. It also promotes typical colonial morphology; better pigment production and more sharply defined hemolytic reaction. Sheep blood permits the detection of hemolysis and also provides heme (X factor), which is required for the growth of many bacteria. However, it is devoid of V factor (Nicotinamide Adenine Dinucleotide) and hence *Haemophilus influenzae*, which needs both X and V factors, will not grow on this medium.

Formula*

Ingredients in grams per liter

Peptone Special	23.0
Sodium Chloride	5.0
Corn Starch	1.0
Agar	15.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 44 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C before the addition of heat sensitive compounds.
   - For Blood Agar: Add 5% sterile defibrinated sheep blood to sterile cool base.
   - For Chocolate Agar: Add 10% sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation. The medium may be made selective by the addition of different antimicrobials to sterile base.
   - For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement, Modified (AS006) to 500 ml of sterile molten base containing 5-10% v/v inactivated Horse Serum (AS015) and 1% w/v sterile dextrose.
   - For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (AS025) or Strepto Supplement (AS026) to 500 ml of sterile molten base along with 25ml sterile defibrinated horse blood.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium - Light amber coloured, clear to slightly opalescent gel.

With addition of 5% defibrinated blood - Cherry red coloured, opaque gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth with 5% Blood	Haemolysis	RGI
Staphylococcus aureus (25923)	Luxuriant	Beta or gamma	More than 70%
Staphylococcus epidermidis (12228)	Luxuriant	Gamma	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	Alpha	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	Beta	More than 70%
Neisseria meningitidis (13090)	Luxuriant	None	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate plates at 35-37°C for 18-48 hours.
3. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Interpretation of Results

1. After incubation most plates will show an area of confluent growth.
2. Because the streaking procedure is in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen.
3. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Precautions / Limitations

1. *Brucella* cultures are highly infective and must be handled under properly protected conditions.
2. *Campylobacter* species are best grown at 42°C (except *C.fetus* subspecies *fetus*) in a microaerophilic atmosphere.
3. Staph/Strepto supplemented plates should be incubated aerobically at 35°C for 18 hours. Incubation in carbon dioxide-enriched air will cause inhibition of staphylococcal growth.
4. Strepto supplemented plates should be incubated aerobically or anaerobically at 35°C for 18 hours.
5. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
6. Columbia Blood Agar Base has a relatively high carbohydrate content and therefore beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha-hemolysis

7. Hemolytic reactions of some strains of group *D streptococci* are affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit Blood Agar and alpha-hemolytic on sheep Blood Agar.
8. Prepared plates of supplemented media should be used within 18 hours of preparation for maximum selectivity.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Columbia Agar (Harmonized) AMH5029

Columbia Agar Base EP AM50291

Columbia Agar Base BP AM50292

Columbia Agar Base USP (Agar Medium Q) AM50293

Use

Columbia Agar Base is used for detection of Clostridium perfringens from pharmaceutical products.

Summary

Columbia Blood Agar Base was developed by Ellner et al., and used for isolating, cultivating and determining the haemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, it can be used as a general-purpose medium.

Principle

Pancreatic digest of casein, meat peptic digest, heart pancreatic digest and yeast extract provide essential nutrients. Maize starch serves as the energy source and also neutralizes toxic metabolites. Sodium chloride maintains the osmotic pressure.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	10.0
Meat peptic digest	5.0
Heart pancreatic digest	3.0
Yeast extract	5.0
Maize starch	1.0
Sodium chloride	5.0
Agar	15.0

Final pH (at 25°C) 7.3± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 44 gms of the powder in 1000ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C before the addition of heat sensitive compounds.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium- Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35ºC.

Organisms (ATCC)	Growth	RGI
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Staphylococcus epidermidis (12228)	Luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%
Neisseria meningitidis (13090)	Luxuriant	More than 70%
Clostridium sporogenes (11437)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate plates at -37ºC for 18-48 hours.
3. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Interpretation of Results

1. After incubation most plates will show an area of confluent growth.
2. Because the streaking procedure is in effect, a "dilution"technique, diminishing numbers are deposited on the streaked areas. Consequently, one or more of these areas should be exhibit-isolated colonies of the organisms contained in the specimen.
3. Further growth of each organism may be semi quantitatively scored on the basis of growth in each of the streaked areas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cooked Meat Medium

AM1030/AM5030

Use

Cooked Meat Medium is used for the cultivation and maintenance of aerobes and anaerobes, especially *Clostridium* species.

Summary

Cooked Meat Medium is a modification of Robertson's (93) original formula and it is also called Chopped Meat Medium (69). Cooked Meat Medium is used for the cultivation and maintenance of clostridia and for determining the proteolytic activity of anaerobes. It also supports the growth of many spore forming and non-spore forming strict anaerobes. This medium is recommended by FDA's Bacteriological Analytical Manual for enumeration and identification of *Clostridium perfringens* from foods and cosmetics (113) and by APHA for the examination of foods (20).

Principle

Proteose peptone provides carbon and nitrogen. Sodium chloride maintains the osmotic balance. The muscle protein in the heart tissue is a source of amino acids and other nutrients, it also provides reducing substances, particularly glutathione, which allows the growth of strict anaerobes. The sulphydryl groups, which exert the reducing effect, are more available in denatured protein; hence meat particles are cooked for use in this medium. Growth is indicated by turbidity or bubble formation by some organisms. Blackening and disintegration of meat particles indicate proteolysis.

Formula*

Ingredients in grams per liter

Beef Heart, Infusion from	454.0
Proteose Peptone	20.0
Sodium Chloride	5.0
Dextrose	2.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 12.5 gms of the powder in 100 ml distilled water.
2. Mix thoroughly.
3. Allow to stand for 15 minutes until all the particles are thoroughly wetted.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality control

Dehydrated Appearance

Brown coloured granules.

Prepared Appearance

Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth	RGI
Clostridium sporogenes (11437)	Luxuriant	More than 70%
Enterococcus faecalis (29215)	Luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	More than 70%
Clostridium perfringenes (12924)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Preferably use freshly reconstituted, sterile medium, which is inoculated as soon as it has cooled to approximately 35°C.
2. Inoculate at the bottom of the tube in the meat particles.
3. For aerobic organisms, incubate up to 7 days at 35°C with loosened caps, examine daily for turbidity, gas or changes in the meat particles.
4. For anaerobic organisms, incubate up to 21 days at 35°C, make films and subculture at regular intervals.
5. For maintenance of stock culture, hold at room temperature after initial incubation at 35°C. Subculture every 4-6 months.

Interpretation of Results

1. Saccharolytic organisms usually produce acid and gas.
2. Proteolytic organisms generally cause blackening and dissolution of the meat particles.

Precautions / Limitations

1. Tubes not used on the day of preparation should be placed in a boiling water bath or steamed for about 15 minutes to remove dissolved oxygen, cooled without agitation, then inoculated.
2. Meat particles in the medium may cause turbidity, which could be interpreted as positive growth.
3. Meat particles blacken only in the presence of alkali, which is a result of the ammonia produced by proteolytic organisms.
4. The reaction observed in the medium is useful for characterization, not speciation, of the organism.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Cooked Meat Medium BIS

AM103011/AM503011

Use

A medium for cultivation and maintenance of aerobes, especially *Clostridium* species in compliance with BIS specification IS:5887(Part 2) 1976.

Summary

Cooked Meat Medium was originally developed by Robertson for the cultivation of certain anaerobes isolated from wounds. The present formulation is recommended by BIS for the detection and enumeration of bacteria responsible for food poisoning especially *Clostridium welchii* (11.1). This medium with addition of further 10% Sodium chloride is used as a salt medium for isolation of *Staphylococci* (11.2). It is used for cultivation and maintenance of Clostridia and for determining proteolytic activity of anaerobes (77.1). FDA has recommended a slight modification of this medium for enumeration and identification of *Clostridium perfringens* from foods.

Principle

Cooked Meat Medium contains beef heart, the muscle protein which provides amino acids and other nutrients. It also contains glutathione a reducing substance which permits the growth of obligate anaerobes. The sulphydryl groups which impart reducing effect are more available in denatured protein and hence the cooked meat is added in the medium. The growth in this medium is indicated by the turbidity or bubble formation by some organisms. Blackening and disintegration of the meat particles indicate proteolysis. For best results, medium should be used on the day it is prepared, otherwise it should be boiled or steamed for a few minutes and allowed to cool without agitation and then inoculated.

Formula*

Ingredients in grams per liter

Beef Heart, Infusion from(500gm)	107.90
Peptic digest of animal tissue	5.0
Sodium Chloride	2.5

Final pH (at 25°C) 7.8 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 115.40 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Allow to stand for 15 minutes until all the particles are thoroughly wetted.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality control

Dehydrated Appearance

Brown coloured granules.

Prepared Appearance

Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth
Clostridium botulinum (25763)	Luxuriant
Clostridium perfringens (12924)	Luxuriant
Clostridium sporogenes (11437)	Luxuriant
Streptococcus pneumoniae (6303)	Luxuriant
Enterococcus faecalis (29212)	Luxuriant

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Preferably use freshly reconstituted, sterile medium, which is inoculated as soon as it has cooled to approximately 35°C.
2. Inoculate at the bottom of the tube in the meat particles.
3. For aerobic organisms, incubate up to 7 days at 35°C with loosened caps, examine daily for turbidity, gas or changes in the meat particles.
4. For anaerobic organisms, incubate up to 21 days at 35°C, make films and subculture at regular intervals.
5. For maintenance of stock culture, hold at room temperature after initial incubation at 35°C. Subculture every 4-6 months.

Interpretation of Results

1. Saccharolytic organisms usually produce acid and gas.
2. Proteolytic organisms generally cause blackening and dissolution of the meat particles.

Precautions / Limitations

1. Tubes not used on the day of preparation should be placed in a boiling water bath or steamed for about 15 minutes to remove dissolved oxygen, cooled without agitation, then inoculated.
2. Meat particles in the medium may cause turbidity, which could be interpreted as positive growth.
3. Meat particles blacken only in the presence of alkali, which is a result of the ammonia produced by proteolytic organisms.
4. The reaction observed in the medium is useful for characterization, not specialization, of the organism.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Corn Meal Agar

AM10301/AM50301

Use

Corn Meal Agar is a general-purpose medium for the cultivation of fungi.

Summary

To facilitate a more rapid differentiation of strains of *Candida albicans* from other yeasts and from other species of *Candida*, there arose a need for culture conditions, which would result in the rapid formation of mycelia, chlamydospores or both. Pollack and Benham reported the usefulness of Corn Meal Agar for studying the morphology of *Candida* (89). In 1960, Walker and Huppert modified the basic formulation of Corn Meal Agar by adding Polysorbate 80, which stimulated rapid and abundant chlamydospore formation (117). This modified formulation is recommended for the production and visualization of chlamydospores. Besides, the addition of dextrose enhances fungal growth and pigment production.

Principle

Corn Meal Agar is a relatively simple medium, consisting of an infusion of corn meal and agar. This infusion product contains sufficient nutrients to support the growth of fungal species. The polysorbate 80 is a mixture of oleic esters which when added to the basal medium, stimulates the production of chlamydospores. Dextrose when added to Corn Meal Agar provides an energy source that enhances fungal growth and chromogenesis.

Formula*

Ingredients in grams per liter

Corn meal, infusion from	50.0
Agar	15.0

Final pH (at 25° C) 6.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 17 grams of the powder in 1000 ml distilled water. If desired, add 1% Polysorbate 80 or 1% Dextrose and mix thoroughly.
2. Heat with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, coarse, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel.

Cultural Response

Cultural characteristics after 4 days when incubated at 25 ± 2° C when incubated for 4 days.

Organisms (ATCC)	Growth	Chlamydospores	RGI
Asperigillus niger (16404)	Luxurinat	_	More than 70%
Candida albicans (10231)	Luxuriant	+	More than 70%
Candida kefyr (8553)	Good	_	More than 70%
Saccharomyces uvarum (9080)	Luxuriant	_	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	_	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. To prepare plated media, place the medium in a boiling water bath until the medium becomes liquefied.
2. Pour the molten medium into a sterile petriplate and allow to solidify.
3. With the help of an inoculating needle, streak the medium with growth from a pure culture and incubate at 25 ± 2° C.
4. Examine at regular intervals for up to 28 days for growth and pigmentation.
5. If Dextrose is added to the medium, it is necessary to incubate for up to 4 weeks to allow sufficient time for pigmentation to develop.
6. If Polysorbate 80 is added to the medium, the production of chlamydospores should be tested using the Dalmau Plate Method.

Interpretation of Results

1. Observe cultures for growth and morphology.
2. If Polysorbate 80 is added to a medium, most strains of *C. albicans* and *C. stellatoidea* form chlamydospores within 24-48 hours.
3. If Dextrose is added to the medium, observe for chromogenesis macroscopically.

Precautions / Limitations

1. Some *Candida* species lose their ability of chlamydospores formation by repeated subculturing.
2. When streaking, with a sterile inoculating needle, lightly touch the yeast colony and make two separate streaks. Do not dig into the agar. Flame a cover slip and after it cools, place it over the central area of the stab marks to provide slightly reduced oxygen tension.
3. Glucose supplemented Corn Meal Agar should not be used for chlamydospores production by Candida species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Czapek Dox Agar

AM10302/AM50302

Use

Czapek Dox Agar is used for general cultivation of fungi, yeasts and soil bacteria.

Summary

Czapek Dox agar has been developed according to the formula of Thom and Church. This medium has a defined chemical composition. Czapek Dox Agar is a semi synthetic medium containing sodium nitrate as the sole source of nitrogen.

Principle

Sucrose serves, as a sole source of carbon and sodium nitrate is the source of nitrogen. Dipotassium phosphate is the buffering agent. Magnesium sulphate, Potassium chloride and Ferrous sulphate are source of essential cations. medium is also a highly satisfactory substrate for chlamydospore production by *Candida albicans*. The pH is slightly alkaline. Czapek Dox Agar supports abundant growth of almost all saprophytic *Aspergilli* with characteristic mycelia and conidia formation.

Formula*

Ingredients in grams per liter

Sucrose	30.0
Sodium nitrate	2.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.50
Potassium chloride	0.50
Ferrous sulphate	0.01
Agar	15.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 49gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Creamish white, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent solution or gel with slight precipitate forms in petriplates.

Cultural Response

Cultural characteristics after 48-72 hours at 30ºC.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Prepare the medium. After plating inoculate the medium.
2. Incubate the medium at 30ºC.

Interpretation of Results

Refer to appropriate references and procedures for result.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Decarboxylase Agar Base

AM503023

Use

Decarboxylase Agar Base is used to differentiate bacteria on the basis of their ability to decarboxylate the amino acid added to the medium.

Summary

Decarboxylase agar base is formulated as described by Moeller to differentiate bacteria on the basis of their ability to decarboxylate the amino acids.

Principle

The medium is useful for the identification of the *Enterobacteriaceae* and other gram-negative bacilli. Production of ornithine decarboxylase is especially useful for differentiating *Enterobacter* and *Klebsiella* species as the former produces this enzyme and are motile while later are nonmotile and do not synthesize this enzyme. Peptic digest of animal tissue and yeast extract supply nitrogenous nutrients for the bacterial growth. Dextrose is the fermentable carbohydrate. Bromo cresol purple is the pH indicator which change colour from purple to yellow in acidic condition. Decarboxylase activity is stimulated by acidic pH and hence the amino acids are decarboxylated or degraded to form corresponding amine. Production of these amines increase the pH of the medium changing the colour of the indicator and inturn the medium from yellow to purple violet.

Each isolate must be inoculated into a tube of the basal medium without amino acid. If this tube becomes alkaline then the best is invalid. Exposure of the medium to air may cause alkalinization so the inoculated tubes if covered with a layer of sterile mineral oil will give best result.

Formula*

Peptic digest of animal tissue	5.0

Yeast extract	3.0
Dextrose	1.00
Bromo Cresol Purple	0.02
Agar	15.0

Final pH (at 25°C)6.5±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.02 gms in 1000 ml distilled water.
2. Boil to dissolve the medium completely.
3. Add 5 gms of desired L-Amino acid (L-Lysine, L-Arginine, L-Ornithine) in hydrochloride form per liter of the medium.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Dispense into sterile test tubes and cool in a slanted position.
6. When L-Orinithine hydrochloride is used, readjustment of pH is necessary.

Quality Control

Dehydrated Appearance

Creamish yellow, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear gel forms in tubes as slants.

Cultural Response

Cultural characteristics after upto 4 days at 35-37°C.

Organisms (ATCC)	Lysine	Arginine	Ornithine
Citrobacter freundii (8090)	-	±	±
Enterobacter aerogenes (13048)	+	-	+
Escherichia coli (25922)	±	±	±
Klebsiella pneumoniae (13883)	+	-	-
Proteus mirabilis (25933)	-	-	+
Salmonella serotype Paratyphi A	-	(+)or+	
Salmonella serotype Typhi (6539)	+	(+) or -	-
Shigella flexneri (12022)	-	- or (+)	-
Shigella sonnei (25931)	-	±	+
Shigella dysenteriae (13313)	-	- or (+)	-
Serratia marcescens (8100)	+	-	+

Key: - = negative reaction, yellow colour
+ = positive reaction, purple colour
(+) = delayed positive reaction

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Deoxycholate Agar AM10303/AM50303

Use

A medium for direct differential count of coliforms in dairy products and for isolation of enteric pathogens from rectal swabs, faeces and other pathological specimens.

Summary

Deoxycholate Agar is a modification of Leifson's original formula. It demonstrates improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using sodium deoxycholate and sodium citrate in specified amounts as inhibitors of gram-positive bacteria. It is used for isolation of enteric pathogens from rectal swabs, faeces or other specimens for the routine examination of clinical samples, it is recommended that MacConkey Agar (AM 1059/5059), Bismuth Sulphite Agar (AM 1013/5013) etc. should be used in parallel with this media.

It is also used for enumeration of coliforms in dairy products & water.

Principle

Peptic Digest of Animal Tissue supply nutrients, nitrogen compounds and amino acids. Lactose is the fermentable carbohydrate. Sodium citrate and sodium deoxycholate inhibit gram-positive bacteria. Neutral red is a pH indicator. Agar is the solidifying agent. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colourless colonies. The majority of normal intestine bacteria ferment lactose except *Salmonella* and *Shigella.*

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.0
Lactose	10.0
Sodium chloride	5.00
Dipotassium phosphate	2.00
Sodium deoxycholate	1.00
Sodium citrate	1.00
Ferric citrate	1.00
Neutral red	0.03
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 45.03 gms of powder in 1000 ml distilled water.

2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Avoid excessive heating, as it is detrimental to the medium. DO NOT AUTOCLAVE OR REHEAT.
4. Pour into adequate containers homogenizing the medium well enough to distribute the calcium carbonate.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, Clear to very slightly opalescent gel .

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colonies	RGI
S. serotype Typhimurium (14028)	Good	Colourless	More than 70%
S. serotype Enteritidis (13076)	Good	Colourless	More than 70%
Escherichia coli (25922)	Good	Pink with bile precipitate.	More than 70%
Staphylococcus aureus (25923)	Inhibited	–	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. Inoculate directly on the surface of the medium.
2. Incubate plates at 35-37°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters observed.

Interpretation of Results

1. Non lactose fermenters produce transparent, Colourless to pink or tan coloured colonies with or without bile precipitation.
2. Lactose fermenters produce red colonies with or without bile precipitation.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Deoxycholate Citrate Agar AM1031/AM5031

Use

Deoxycholate Citrate Agar is a selective medium used for the isolation of enteric pathogens particularly *Salmonella* and *Shigella* species.

Summary

Deoxycholate Citrate Agar is a modification of Deoxycholate Agar formulated by Leifson (67), which demonstrates improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium deoxycholate in specified amounts as inhibitors of gram-positive bacteria. In comparison, Deoxycholate Citrate Agar has increased concentrations of sodium citrate and sodium deoxycholate for reliably isolating many *Salmonella* and *Shigella* species while inhibiting coliforms and many *Proteus* species. This medium is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods. The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other micro flora. Deoxycholate Citrate Agar is recommended by APHA for the examination of foods (20) and in the IP for use in Microbial Limit test (46).

Principle

Heart infusion is a source of carbon and nitrogen and is preferred because the inhibition of coliforms produced is greater than when an extract or simple peptone is used. Proteose peptone provides carbon, nitrogen, vitamins and minerals. Lactose is the fermentable carbohydrate. Sodium citrate and sodium deoxycholate inhibit gram-positive bacteria, coliforms and *Proteus* species. Ferric ammonium citrate aids in the detection of H_2S-producing bacteria. Neutral red is a pH indicator.

Bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colourless colonies. Bacteria producing H_2S will have black centers. The majority of normal intestinal bacteria ferment lactose and do not produce H_2S (red colonies without black centers). *Salmonella* and *Shigella* species do not ferment lactose but *Salmonella* may produce H_2S (colourless colonies with or without black centers). Lactose fermenting colonies may have a zone of precipitation around them caused by the precipitation of deoxycholate in the presence of acid.

Formula*

Ingredients in grams per liter

Sodium Citrate	20.0
Proteose Peptone	10.0
Heart Infusion Solids	10.0
Lactose	10.0
Sodium Deoxycholate	5.0

Ferric Ammonium Citrate	2.0
Neutral Red	0.02
Agar	13.5

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 70.52 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Avoid excessive heating, as it is detrimental to the medium.
4. DO NOT AUTOCLAVE.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	H_2S	RGI
Enterococcus faecalis (29212)	Inhibited	-	-	More than 70%
Escherichia coli (25922)	Poor	Pink with bile precipitate	-	0%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	+	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Colourless	+	More than 70%
Shigella flexneri (12022)	Good	Colourless	-	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate directly onto the surface of the medium.
2. Incubate plates at 35-37°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters are observed.

Interpretation of Results

1. Non-lactose fermenters produce transparent, colourless to light pink or tan coloured colonies with or without black centers.
2. Lactose fermenters produce red colonies with or without bile precipitation.

Precautions / Limitations

1. Deoxycholate Citrate Agar is heat sensitive. Avoid excessive or prolonged heating during reconstitution. Do not autoclave or remelt.
2. The medium is best used freshly prepared.
3. Stock cultures of *Shigella* species may predominantly be in the R-phase when subcultured away from Deoxycholate Citrate Agar. Such cultures are difficult to use for control purposes without first heavily streaking the culture on Deoxycholate Citrate Agar plates and picking off the few S-phase colonies i.e. the macro-colonies on the agar surface, for further subculture.
4. When performing biochemical tests on colonies picked from the surface of DCA plates, purity of subcultures should be carried out because the colony may be contaminated with *E.coli* present as micro colonies.
5. Coliform strains may be encountered that will grow on this medium, making it difficult to detect pathogens.
6. Heavy inocula should be distributed over the entire surface of the medium to prevent complete masking of pathogens by coliform organisms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Deoxycholate Citrate Agar IP — AM10311/AM50311

Use

Deoxycholate Citrate Agar is a selective medium used for the isolation of enteric pathogens particularly *Salmonella and Shigella* species in compliance with IP.

Summary

Deoxycholate Citarte Agar is a modification of Deoxycholate agar formulated by Leifson's (67), which demonstrates improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using sodium deoxycholate and sodium citrate in specified amounts as inhibitors of gram-positive bacteria. In comparison, deoxycholate Citrate Agar has incresed concentrations of sodium citrate and sodium deoxycholate for reliably isolating many *Salmonella* and *Shigella* species while inhibiting coliforms and many *proteus* species. This medium is used for the isolation maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods. The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other micro flora. Deoxycholate Citrate Agar is recommended by APHA for the examination of foods and in the IP for use in Microbial Limit Test.

Principle

Peptone supply nutrients, nitrogen compounds and amino acids. Lactose is the fermentable carbohydrate. Sodium citrate and sodium deoxycholate inhibit

gram-positive bacteria, coliforms and Proteus species. Ferric ammonium citrate aid in the detection of H_2S producing bacteria. Neutral red is a pH indicator.

Bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colourless colonies. Bacteria producing H_2S will have black centers. The majority of normal intestine bacteria ferment lactose and do not produce H_2S (red colonies without black centers). Lactose fermenting colonies may have a zone of precipitation around them caused by the precipitation of deoxycholate in the presence of acid.

Formula*

Ingredients in grams per liter

Trisodium citrate	8.5
Beef extract	5.0
Peptone	5.0
Lactose	10.0
Sodium deoxycholate	5.00
Ferric citrate	1.00
Neutral red	0.02
Agar	13.5

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 69.52 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Avoid excessive heating, as it is detrimental to the medium. DO NOT AUTOCLAVE OR REHEAT.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, Clear to very slightly opalescent gel .

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colonies	H2S	RGI
Enerococcus faecalis (29212)	Inhibited	–	–	0%
Escherichia coli (25922)	Poor	Pink with bile precipitate	–	0%
S. serotype Enteritidis (13076)	Luxuriant	Colourless	+	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Colourless	+	More than 70%
Shigella flexneri (12022)	Good	Colourless	–	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate directly on the surface of the medium.
2. Incubate plates at 35-37°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters observed.

Interpretation of Results

1. Non lactose fermenters produce transparent, Colourless to pink or tan coloured colonies with or without bile precipitation.
2. Lactose fermenters produce red colonies with or without bile precipitation

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Deoxycholate Citrate Agar EP — AM10312/AM50312
Deoxycholate Citrate Agar BP — AM50313

Use

Deoxycholate Citrate Agar is a selective medium used for the isolation of enteric pathogens particularly *Salmonella* and *Shigella*s pecies.

Summary

Deoxycholate Citarte Agar is a modification of Deoxycholate Agar formulated by Leifson's (67), which demonstrates improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using sodium deoxycholate and sodium citrate in specified amounts as inhibitors of gram-positive bacteria. In comparison, deoxycholate Citrate Agar has increased concentrations of sodium citrate and sodium deoxycholate for reliably isolating many *Salmonella* and *Shigella* species while inhibiting coliforms and many *Proteus* species. This medium is used for the isolation maximum recovery of intestinal pathogens belonging to *Salmonell*a and *Shigella* groups from foods. The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other micro flora. Deoxycholate Citrate Agar is recommended by APHA for the examination of foods and in the BP for use in Microbial Limit Test.

Principle

Heart Infusion is a source of carbon and nitrogen and is preferred because the inhibition of coliforms produced is greater than when an extract or simple peptone is used. Proteose peptone provides carbon, nitrogen vitamins and minerals. Lactose is the fermentable carbohydrate. Sodium citrate and sodium deoxycholate inhibit gram-positive bacteria, coliforms and Proteus species. Ferric ammonium citrate aid in the detection of H_2S producing bacteria. Neutral red is a pH indicator.

Bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colourless colonies. Bacteria producing H_2S will have black centers. The majority of normal intestine bacteria ferment lactose and do not produce H_2S (red colonies without black centers). Lactose fermenting colonies may have a zone of precipitation around them caused by the precipitation of deoxycholate in the presence of acid.

Formula*

Ingredients in grams per liter

Sodium citrate	20.0
Beef extract	10.0
Meat peptone	10.0
Lactose monohydrate	10.0
Sodium deoxycholate	5.00
Iron (III) citrate	1.00
Neutral red	0.02
Agar	13.50

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 69.52 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Avoid excessive heating, as it is detrimental to the medium. DO NOT AUTOCLAVE OR REHEAT.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, Clear to very slightly opalescent gel .

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colonies	H_2S	RGI
Enerococcus faecalis (29212)	Inhibited	–	–	0%
Escherichia coli (25922)	Poor	Pink with bile precipitate	–	0%
S. serotype Enteritidis (13076)	Luxuriant	Colourless	+	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Colourless	+	More than 70%
Shigella flexneri (12022)	Good	Colourless	–	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate directly on the surface of the medium.
2. Incubate plates at 35-37°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters observed.

Interpretation of Results

1. Non lactose fermenters produce transparent, Colourless to pink or tan coloured colonies with or without bile precipitation.
2. Lactose fermenters produce red colonies with or without bile precipitation.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Dextrose Agar AM1032/AM5032

Dextrose Broth AM1033/AM5033

Use

Dextrose Agar with or without blood is used for cultivation of a wide variety of microorganisms. Dextrose Broth is used for the cultivation of fastidious microorganisms and for detecting gas production from enteric bacilli.

Summary

Dextrose Agar with 5% defibrinated blood is recommended for the isolation of many fastidious bacteria, including *Haemophilus* and *Neisseria*. This medium has a high concentration of dextrose, which makes it suitable for the production of early, abundant organism growth and shortens the lag period of older cultures. Dextrose Agar facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment. The low agar concentration provides suitable conditions for both aerobic growth in the clear upper zones and

for microaerophilic growth in the lower, flocculent agar zones.

Dextrose Broth is a highly nutritious broth for the isolation of fastidious microorganisms and for specimens containing a low inoculum. This medium is used for antibiotic sensitivity testing using tube dilution method as it is found to be superior when compared to Soyabean Peptone Medium, particularly for sensitivity testing of Neomycin and Chlortetracycline. Dextrose Agar and Dextrose Broth are both specified in the Compendium of Methods for the microbiological examination of foods (20).

Principle

Dextrose serves as the carbon source, the high concentration of which in Dextrose Agar is a distinguishing characteristic of this medium from other formulations used as Blood Agar bases. Beef extract and tryptose provide nitrogen, sulphur, carbon, amino acids, minerals and vitamins. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter	Dextrose Agar	Dextrose Broth
Tryptose	10.0	10.0
Dextrose	10.0	5.0
Sodium Chloride	5.0	5.0
Beef Extract	3.0	3.0
Agar	15.0	-
Final pH (at 25°C)	7.3 ± 0.2	7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.
   Dextrose Agar - 43 gms
   Dextrose Broth - 23 gms
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. OPTIONAL: To prepare Blood Agar add 5% v/v sterile defibrinated sheep blood to sterile Dextrose Agar cooled to 45-50°C. Mix well and dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Dextrose Agar - Light yellow coloured, clear to slightly opalescent gel.
Dextrose Broth - Light yellow coloured clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth on Dextrose Agar and in Dextrose Broth	Growth with blood on Dextrose Agar	RGI
Bordetella pertussis (8467)	Good to luxuriant	Luxuriant	More than 70%
Clostridium perfringenes (12919)	Fair to good	Luxuriant	More than 70%
Neisseria meningitidis (13090)	Good to luxuriant	Luxuriant	More than 70%
Escherichia coli (25922)	Good to luxuriant	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Good to luxuriant	Luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Precautions / Limitations

1. Dextrose Agar is not suitable for observation of haemolysis when supplemented with 5% sheep, rabbit or horse blood, because of the high concentration of dextrose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Dextrose Peptone Agar — AM1034/AM5034
Dextrose Peptone Broth — AM1035/AM5035

Use

Dextrose Peptone Agar and Dextrose Peptone Broth are general-purpose media used for the cultivation of a wide variety of microorganisms.

Summary

Dextrose Peptone Media are formulated as suggested by Williams (120). Dextrose Peptone Agar and Dextrose Peptone Broth are used for the cultivation of microorganisms that are fastidious, or present in small numbers. Dextrose Peptone Agar is an excellent basal medium for the preparation of Glucose Blood Agar, which supports good growth of anaerobic microorganisms. Dextrose Peptone Agar is also used for the enumeration of thermophilic bacteria responsible for flat sour spoilage of canned foods and both are recommended for routine cultivation purpose by AOAC.

Principle

Dextrose serves as the carbon and energy source. Peptone provides amino acids, peptides etc. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter	Dextrose Peptone Agar	Dextrose Peptone Broth
Dextrose	10.0	10.0
Peptone	20.0	20.0
Sodium Chloride	5.0	5.0
Agar	15.0	-

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Dextrose Peptone Agar - 50 gms

   Dextrose Peptone Broth - 35 gms
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Dextrose Peptone Agar - Light yellow coloured, clear to slightly opalescent gel.

Dextrose Peptone Broth - Light yellow coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Dextrose Tryptone Agar AM1036/AM5036

Dextrose Tryptone Broth AM1037/AM5037

Use

Dextrose Tryptone Agar is used for the cultivation and enumeration of mesophilic and thermophilic aerobic microorganisms associated with food spoilage while Dextrose Tryptone Broth is used for enrichment of mesophilic and thermophilic microorganisms associated with food spoilage.

Summary

Williams (120) formulated Dextrose Tryptone Agar, a suitable medium for the cultivation and enumeration of thermophilic bacteria. Dextrose Tryptone Broth is used for enriching "flat sour" organisms from food products while Dextrose Tryptone Agar is used for isolating "flat sour" organisms from food products. "Flat sour" spoilage of canned foods is caused *by Bacillus coagulans* (*Bacillus thermodurans*). Bacterial growth results in a 0.3 - 0.5 drop in pH, while the ends of the can remain flat. *B.coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favourable for multiplication of the bacterium can result in spoilage of the food product.

Dextrose Tryptone Agar and Dextrose Tryptone Broth are also used for isolating and enriching other food spoilage bacteria respectively, mesophilic aerobic spore formers in the genera *Bacillus* and *Sporolactobacillus* and thermophilic flat sour spore formers such as *B.stearothermophilus*. This media are used for enumerating and enriching mesophilic and thermophilic organisms respectively in cereals and cereal products, dehydrated fruits and vegetables and spices. Dextrose Tryptone Agar and Dextrose Tryptone Broth are recommended by APHA for the examination of foods (20).

Principle

Dextrose serves as the carbohydrate source. Tryptone provides carbon and nitrogen for growth. Bromocresol purple is the pH indicator.

Formula*

Ingredients in grams per liter	Dextrose Tryptone Agar	Dextrose Tryptone Broth
Tryptone	10.0	10.0
Dextrose	5.0	5.0
Agar	15.0	-
Bromocresol Purple	0.04	0.04
Final pH (at 25°C)	6.7 ± 0.2	6.7 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water and mix well.

   Dextrose Tryptone Agar - 30 gms

   Dextrose Tryptone Broth - 15 gms

2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Dextrose Tryptone Agar - Purple coloured, slightly opalescent gel.

Dextrose Tryptone Broth - Purple coloured, slightly opalescent solution.

Cultural Response

Cultural characteristics after 48 hours at 55°C.

Organisms (ATCC)	Growth on Dextrose Tryptone Agar & in Dextrose Tryptone Broth	Colour of colony on Tryptone Agar	RGI
Bacillus stearothermophilus (7953)	Good to luxuriant	Yellow	More than 70%
Bacillus coagulans (8038)	Good to luxuriant	Yellow	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Interpretation of Results

Dextrose Tryptone Agar

1. Acid producing organisms form yellow coloured colonies surrounded by a yellow zone.

Dextrose Tryptone Broth

1. A change in the colour of the medium from purple to yellow indicates dextrose fermentation.

Precautions / Limitations

1. Dextrose Tryptone Agar must be incubated at 55°C under humid conditions e.g. wrapped dishes or in a high humid environment.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing) AM50371

Use

Dey Engley Neutralizing Agar is recommended for disinfectant testing where neutralization of the antiseptics and disinfectant is important for determining its bactericidal activity.

Summary

Dey and Engley described a procedure of neutralizer evaluation and also formulated a medium, known as Dey-Engley Neutralizing medium (25.1). This medium neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. Sodium thioglycollate, sodium thiosulfate, sodium bisulfite, soya lecithin and polysorbate 80 act as neutralizing components.

Principle

Casein enzymic hydrolysate serves as a rich source of nitrogen and amino acid. Yeast extract provides a source of trace elements and vitamins. Dextrose is a source of energy. Five neutralizers are incorporated into the medium to inactivate different types of biocides. Sodium thiosulfate neutralizes iodine and chlorine; sodium thioglycollate neutralizes mercurials; sodium bisulphate neutralizes aldehydes; lecithin neutralizes quaternary ammonium compounds; and Polysorbate 80 neutralizes substituted phenolics. Bromocresol purple acts as an indicator, which indicates the utilization of dextrose.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	5.0
Yeast extract	2.50
Dextrose	10.0
Sodium thiosulphate	6.0
Sodium thioglycollate	1.0
Sodium bisulphate	2.50
Lecithin	7.0
Polysorbate 80	5.0
Bromo cresol purple	0.02
Agar	15.0

Final pH (at 25°C) 7.6± 0.2

Directions

1. Suspend the 54 gms of powder in 1000 ml of distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Bluish grey coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 40-48 hours at 35-370C.

Organisms (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Luxuriant	More than 70%

Escherichia coli (25922)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Selected surface are sampled by firmly pressing the agar medium against the test area. Slightly curved surfaces may be sampled with a rolling motion. Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.
2. Incubate exposed plates at 35-37°C for 48 hours.

Interpretation of Results

Growth is indicated by a colour change from purple to yellow, and visible colonies are found on incubated plate.

Storage

Store at 2- 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Dey-Engley Neutralizing Broth (D/E Broth Disinfectant Testing) AM50372

Use

Dey Engley Neutralizing Broth is recommended for disinfectant testing where neutralization of the antiseptics and disinfectant is important for determining its bactericidal activity.

Summary

Dey and Engley described a procedure of neutralizer evaluation and also formulated a medium, known as Dey-Engley Neutralizing medium (25.1). This medium neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. Sodium thioglycollate, sodium thiosulfate, sodium bisulfite, soya lecithin and polysorbate 80 act as neutralizing components.

Principle

Casein enzymic hydrolysate serves as a rich source of nitrogen and amino acid. Yeast extract provides a source of trace elements and vitamins. Dextrose is a source of energy. Five neutralizers are incorporated into the medium to inactivate different types of biocides. Sodium thiosulfate neutralizes iodine and chlorine; sodium thioglycollate neutralizes mercurials; sodium bisulphate neutralizes aldehydes; lecithin neutralizes quaternary ammonium compounds; and Polysorbate 80 neutralizes substituted phenolics. Bromocresol purple acts as an indicator, which indicates the utilization of dextrose.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	5.0
Yeast extract	2.50
Dextrose	10.0
Sodium thiosulphate	6.0
Sodium thioglycollate	1.0
Sodium bisulphate	2.50
Lecithin	7.0
Polysorbate 80	5.0
Bromo cresol purple	0.02

Final pH (at 25°C) 7.6± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 39 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Dispense in tubes or adequate containers and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Bluish grey coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C.

Organisms (ATCC)	**Growth**
Bacillus subtilis (6633)	Luxuriant
Escherichia coli (25922)	Luxuriant
Pseudomonas aeruginosa (27853)	Luxuriant
Salmonella serotype Typhimurium (14028)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant

Procedure

1. Add 1 ml of disinfectant solution to one tube of Dey-Engley Neutralizing Broth.
2. Add desired amount of culture.
3. Incubate tubes at 35°C for 40-48 hours.

4. Do further testing to determine bacteriostatic or bactericidal activity of the solution by inoculating samples from broth onto Dey-Engley Neutralizing Agar (AM 50371).

Interpretation of Results

Growth is indicated by a colour change from purple to yellow, or pellicle formation.

Growth on the plates from negative broth tubes indicates a bacteriostatic substance.

No growth the plates from negative broth tubes indicates a bactericidal substance. All positive broth tubes should be positive on the plates.

Storage

Store at 2- 8^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Dichloron 18% Glycerol (DG18) Agar AM50373

Use

Dichloran Glycerol Medium Base with Chloramphenicol is recommended for selective isolation of xerophilic moulds from food samples.

Summary

Hocking and Pitt (45.5) formulated Medium Dichloran Glycerol and is recommended for isolation and enumeration of xerophilic moulds from dried and semidried foods. The glycerol at 18% (w/w) lowers the water activity from 0.999 to 0.95 (6.2) without causing any problem. This restrictive characteristic makes the medium especially suitable for foods.

Principle

Peptic digest of animal tissue provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.00
Dextrose	10.00
Monopotassium phosphate	1.00
Magnesium sulphate	0.50
Dichloran	0.002
Chloramphenicol	0.10
Agar	15.00

Final pH (at 25°C) 5.6±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15.8 grams in 500 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Add 110 grams of glycerol.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after an incubation at 25°C for upto 6 days.

Organisms (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Inhibited	0%
Candida albicans (10231)	Good-luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Mucor racemosus (42647)	Good-luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Good-luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

(9763)

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Dichloran Rose Bengal chloramphenicol (DRBC) Agar AM50374

Use

Dichloran Rose Bengal chloramphenicol (DRBC) Agar used as a selective medium for the enumeration of yeast and moulds in food industries

Summary

Dichloran Rose Bengal chloramphenicol (DRBC) Agar is based on the Dichloran Rose Bengal Chlortetracycline agar formula described by King, Hocking and Pitt

(55.4). DRBC Agar conforms with APHA guidelines for the mycological examination of foods, containing chloramphenicol rather than chlortetracycline as originally proposed (6.2.1). DRBC Agar is a selective medium that supports good growth of yeasts and molds.

Principle

Peptone provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium sulfate is a source of divalent cations and sulfate. The antifungal agent, dichloran, is added to the medium to reduce colony diameters of spreading fungi. Rose bengal suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Agar is the solidifying agents.

Formula*

Ingredients in grams per liter

Bacteriological peptone	5.0
Glucose	10.0
KH2PO4	1.0
MgSO4.7H2O	0.5
Rose Bengal (5% aqueous soln. w/v)	0.05
Dichloran (0.2% in ethanol w/v)	0.002
Chloramphenicol	0.1
Agar	15.0

Final pH (at 25°C)5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 31.6 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool at 50°C and pour into plates.

Quality Control

Dehydrated Appearance

Pink colour, homogeneous, free flowing powder.

Prepared Appearance

Bright pink coloured, slightly opalescent.

Cultural Response

Cultural characteristics up to 5 days at 25+2°C.

Organisms(ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Differential Reinforced Clostridial Broth AM1038/AM5038

Use

Differential Reinforced Clostridial Broth is used for the cultivation of clostridia from water.

Summary

Differential Reinforced Clostridial Broth is based on the formulation described by Barnes and Ingram (5) and Gibbs and Freame (34). It is used for cultivation of sulphite reducing clostridia from food and enumeration in water by multiple tube method. Differentiation is based on the ability to reduce sulphite to sulphide to form black coloured iron sulphide.

Principle

Peptone, beef extract, yeast extract, starch and L-cysteine hydrochloride provide nutrients and co-factors. Dextrose serves as the energy source. Partial selectivity of the medium is achieved by the addition of sodium acetate. L-cysteine hydrochloride also acts as a reducing agent.

Formula*

Ingredients in grams per liter

Peptone	10.0
Yeast Extract	1.5
Sodium Acetate	5.0
Beef Extract	10.0
Glucose	1.0
L-Cysteine Hydrochloride	0.5
Starch	1.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29 gms of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

4. Just before use add 0.5 ml filter sterilized solution prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate to 25 ml of single strength medium or 0.4 ml and 2 ml to 10ml and 50 ml of double strength medium respectively.
5. Mix well.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics within 1 week at 30°C.

Organisms (ATCC)	Growth	H_2S production
Clostridium perfringens (13124)	Good to luxuriant	+
Clostridium sporogenes (11437)	Good to luxuriant	+

Key:

+ = Blackening of the medium

Interpretation of Results

1. Blackening of the medium presumptively indicates the presence of clostridia.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Differential Reinforced Clostridial Broth Base ISO AM503811

Use

Differential Reinforced Clostridial Broth is used for the cultivation of clostridia from water, in compliance with ISO specification ISO 6461-1: 1986.

Summary

Differential Reinforced Clostridial Broth is based on the formulation described by Barnes and Ingram and Gibbs and Freame. It is used for cultivation of sulphite reducing clostridia from food and enumeration in water by multiple tube method. Differentiation is based on the ability to reduce sulphite to sulphide to form black coloured iron sulphide.

Principle

Tryptose, meat extract, yeast extract, starch and L-cysteine hydrochloride provide nutrients and co-factors. Dextrose serves as the energy source. Partial selectivity of the medium is achieved by the addition of sodium acetate. L-cysteine hydrochloride also acts as a reducing agent.

Formula*

Ingredients in grams per liter

Tryptose	10.0
Yeast extract	1.5
Sodium acetate	5.0
Meat extract	10.0
Glucose	1.0
L-Cysteine hydrochloride	0.5
Starch	1.0

Final pH (at 25ºC) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29 gms of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Just before use add 0.5 ml filter sterilized solution prepared by mixing equal volumes of 4% w/v solution of sodium sulphite an 7% w/v ferric citrate to 25 ml of single strength medium or 0.4 ml and 2 ml to 10ml and 50 ml of double strength medium respectively.
5. Mix well.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics within 1 week at 30ºC.

Organisms (ATCC)	Growth	H_2S production
Clostridium perfringens (13124)	Good to luxuriant	+
Clostridium sporogenes (11437)	Good to luxuriant	+

Key: + = Blackening of the medium

Interpretation of results

1. Blackening of the medium presumptively indicates the presence of clostridia.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

DNase Test Agar Base

AM10381

Use

DNase Test Agar Base is a differential medium recommended for the detection of deoxyribonuclease activity to aid in the identification of bacteria and fungi isolated from clinical specimens especially pathogenic *staphylococci.*

Summary

Weckman and Catlin in their study observed that DNase activity might prove to be a taxonomic characteristic useful in the detection of strains of the micrococcus-*staphylococcus* group (118). Subsequently, DiSalvo reported a correlation between coagulase production and DNase activity. This test proves useful in differentiating *Serratia* from *Enterobacter*, *Staphylococcus aureus* from coagulase-negative *staphylococci* and *Moraxella catarrhalis* from *Neisseria* species (125).

Principle

Tryptone and Papaic Digest of Soyabean Meal provide amino acids and other complex nitrogenous substances required to support bacterial growth. Sodium chloride maintains the osmotic equilibrium. DNA is the substrate for DNase activity. DNase is an extracellular enzyme that breaks the DNA down into subunits composed of nucleotides.

The depolymerization of the DNA may be detected by flooding the surface of the medium with 1 N HCl and observing for clear zones around the medium surrounding growth. In the absence of DNase activity, the reagent reacts with the intact nucleic acid, resulting in the formation of a cloudy precipitate.

Formula*

Ingredients in grams per liter

Tryptone	15.0
Papaic Digest of Soyabean Meal	5.0
Deoxyribonucleic Acid (DNA)	2.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25° C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 42 grams of the powder in 1000 ml distilled water.
2. Heat with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 12 to 15 lbs pressure (118° C to 121° C) for 15 minutes.
4. Cool to 45° C and pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hous at 35-37° C.

Organisms (ATCC)	Growth	RGI
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Staphyloccus epidermidis (12228)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%
Serratia marcescens (8100)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Inoculate each plate / tube with the sample for testing by drawing a single streak line.
2. Incubate at 35 ± 2° C for 24-48 hours. Plates should be incubated in an inverted position and tubes with caps loosened.
3. On completion of the incubation period, flood the plates / tubes with 1 N HCl reagent to precipitate the DNA in the medium.
4. Observe for reaction.

Interpretation of Results

1. A clear area surrouding growth on the medium after the addition of 1 N HCl indicates a positive reaction i.e.; DNase activity.
2. No clearing and a cloudy precipitate around colonies throughout the medium indicate a negative reaction. This occurs due to the formation of precipitated salts in the medium.
3. Gram positive, catalase positive cocci that produce DNase can be provisionally classified as *S. aureus* and confirmed by tube coagulase or thermostable DNase tests.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Double Sugar Agar, Russell

AM50382

Use

Double Sugar Agar, Russel is used for the differentiation of gram-negative enteric bacilli on the basis of their ability to ferment dextrose and lactose with or without gas formation.

Summary

In 1911, Russell described a new double sugar tube medium for the isolation of

typhoid bacilli from urine and feces (97). Six years later, Kligler developed a simple lead acetate medium for the differentiation of the typhoid-paratyphoid group. Subsequently, Kligler evaluated culture media used in the isolation and differentiation of typhoid, dysentery and allied bacilli and endorsed Russell's medium. Bailey and Lacey substituted phenol red for the Andrade indicator previously used as a pH indicator.

Princple

This medium is based upon the original formula of Russel except the litmus is new substituted by phenol red and used for differentiating gram-negative enteric bacilli especially the colon-typhoid-salmonellae dysentery groups based on the fermentation of dextrose and lactose. After the incubation the acid production in aerobic condition (on the slant) and under anaerobic condition (in the butt) can be detected by the change in colour of the indicator. Phenol red is pH indicator in the medium. Gaseous fermentation is indicated by the splitting of the agar or by the bubble formation in the butt. Organism like Salmonella typhi capable of fermenting dextrose but not lactose, will show an initial acid slant in short incubation period. As the dextrose is consumed the reaction under aerobic condition reverts and becomes alkaline. Under anaerobic condition in the butt, the same organisms fail to revert the reaction and remain acidic.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	2.50
Casein enzymic hydrolysate	7.50
Beef extract	3.00
Lactose	10.0
Dextrose	1.00
Sodium chloride	5.00
Phenol red	0.025
Agar	15.00

Final pH (at 25ºC) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 44 grams of the powder in 1000 ml distilled water. Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes or as desired.
4. Sterilize by autoclaving at 118^{0}C (12-15 lbs pressure) for 15 minutes.
5. Allow the tubes to solidify in slanting position to form generous butt.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to very slightly opalescent gel forms in tubes as slants..

Cultural Response

Cultural characteristics after 18-40 hours at 35-37ºC.

Organisms (ATCC)	Growth	Slant	Butt	Gas	RGI
Escherichia coli (25922)	Luxuriant	A	A	+	More than 70%
Proteus vulgaris (13315)	Luxuriant	K	A	+	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	K	K	---	More than 70%
Salmonella serotype *Typhimurium* (14028)	Luxuriant	K	A	+	More than 70%
Shigella dysenteriae (13313)	Luxuriant	K	A	---	More than 70%

Key:

A = acidic reaction, yellowing of the medium

K= alkaline reaction, red colour of the medium

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

E. C. Broth AM1039/AM5039

E. C. Broth ISO AM503911

Use

EC Broth is used for detection of coliform bacteria at 35^{0}C and *Escherichia coli* at an elevated temperature of 44.5 or 45.5^{0}C.

Summary

EC Broth was developed by Hajna and Perry (42) and is used for the examination of water, milk, shellfish and other material for evidence of faecal pollution. Tennant et al (110) reported the use of this medium for the estimation of *E.coli* densities in seawater and shellfish. Fishbein and Surkiewicz (32) used EC Broth for the recovery of *E.coli* from frozen foods and nut meats. This medium is recommended for use in Most Probable Number (MPN) procedure for examination of dairy products (39) water and wastewater (36) and foods (20). The procedure employing EC Broth provides information regarding the source of the coliform group (faecal or non-faecal) when used as a confirmatory test. It is included in the Bacteriological Analytical Manual for food testing (114).

Principle

Tryptone provides nutrients for growth while lactose is the fermentable

carbohydrate. Bile salts mixture inhibits gram-positive organisms especially bacilli and faecal streptococci. The medium contains a strong potassium phosphate buffering system to control the pH during fermentation of lactose. Sodium chloride maintains osmotic balance.

Formula*

Ingredients in grams per liter

Tryptone	20.0
Lactose	5.0
Sodium Chloride	5.0
Monopotassium Phosphate	1.5
Bile Salts Mixture	1.5
Dipotassium Phosphate	4.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37 gms of the powder in 1000ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense into tubes containing inverted Durham's tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Adjust the concentration of the medium as per the sample size.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24 hours at 44.5°C ± 0.2°C.

Organisms (ATCC)	Growth	Gas
Bacillus subtilis (6633)	Inhibited	-
Enterobacter aerogenes (13048)	Inhibited	-
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	Luxuriant	+
Klebsiella pneumoniae (13883)	Luxuriant	+
Pseudomonas aeruginosa (27853)	Fair to good	-

Interpretation of Results

1. Lactose fermenting organisms produce gas, which is detected by the appearance of bubbles in the inverted Durham's tube within 24 hours, which is a presumptive evidence of the presence of coliform bacteria.
2. The development of turbidity and gas production within 48 hours at 35°C or at 45.5°C indicates the presence of coliforms.

Precautions / Limitations

1. This medium should not be used for the direct isolation of coliforms since prior enrichment in a presumptive test medium for optimal recovery of faecal coliforms is required.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

E. E. Broth, Mossel — AM103913/AM503913

Use

E E Broth, Mossel is used for selective enrichment and detection of *Enterobacteriaceae* in bacteriological examination of food and feedstuffs.

Summary

Mossel, Visser and Cornelissen developed a culture media for the selective enrichment of *Enterobacteriaceae* that enable the *Enterobacteriaceae* to multiply freely and inhibit accompanying other organisms (81.1). Media contains dextrose to facilitate growth of most *Enterobacteriaceae* including *Salmonella* and other non-lactose fermenting organisms.

EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar (AM51073). When specific organisms, rather than *Enterobacteriaceae* in general, are required subcultures must be made onto lactose differential media e.g. Deoxycholate Citrate Agar (AM1031/5031), Brilliant Green Agar, Modified (AM1018/5018), or MacConkey Agar (AM1059/5059) for the detection of non-lactose fermenting or delayed lactose fermenting organisms.

Principle

Peptic Digest of Animal Tissue supply nutrients, nitrogen compounds and amino acids. Ox bile supports the growth of enteric bacteria and inhibits other bacteria, which do not normally live in the intestine. Brilliant-green specifically inhibits the Gram-positive accompanying flora.Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Disodium phosphate and Mono-potassium phosphate are buffering agents.

Formula*

Ingredients in grams per liter

Peptic Digest of Animal Tissue	10.0
Dextrose	5.0
Ox-bile Purified	20.0
Disodium phosphate	6.45
Mono-potassium phosphate	2.0
Brilliant Green	0.0135

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 43.50 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. Do not boil. DO NOT AUTOCLAVE OR REHEAT.
4. Pour into adequate containers.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Emerald green coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid
Shigella boydii (12030)	Luxuriant	-
S.*serotype Enteritidis* (13076)	Luxuriant	±
Escherichia coli (25922)	Luxuriant	+
Enterobacter aerogenes (13048)	Luxuriant	+
Proteus mirabilis (25933)	Luxuriant	+
Staphylococcus aureus (25923)	Inhibited	-

* Key: + = Positive, yellow colouration

– = Negative, no colour change green

Procedure

1. Inoculate the E E Broth, Mossel with food or other test specimen.
2. Mix well the inoculated medium.
3. Incubate at 35-37°C for 20-24 hours.

Interpretation of Results

Acid production causes the color of EE Broth Mossel to become yellow. A negative reaction results in no colour change and the medium remains green.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

ECD Agar AM503912

Use

ECD Agar is used for selective isolation of coliforms, specially *Escherichia coli* in water and food by membrane filter technique.

Summary

The medium complies with the German-DIN-Norm 10110 for the examination of meat, with the regulation according to §35 LMBG (06.00/36) for the examination of food and with ISO standard 6391 (1996) for the enumeration of *E.coli* in meat and meat products.

Principle

Casein peptone and yeast extract provides essential growth nutrients. Bile salts inhibit gram-positive bacteria specially bacilli and faecal streptococci. Potassium phosphates control the pH.

Formula*

Ingredients in grams per liter

Casein peptone (tryptic)	20.00
Yeast extract	5.00
Bile salt	1.50
Sodium chloride	5.00
Disodium hydrogen phosphate	5.00
Potassium dihydrogen phosphate	1.50
Agar	15.00

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 53 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Bright beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellowing brown coloured, clear gel.

Cultural Response

Cultural characteristics up to 18-24 hour at 44°C.

Organisms (ATCC)	Growth	Indole formation	RGI
Escherichia coli (8739)	Luxuriant	+	More than 70%
Escherichia coli (25922)	Luxuriant	+	More than 70%
Enterobacter aerogenes (13048)	Good	-	More than 70%
Klebsiella pneumoniae (13883)	Good		More than 70%
Pseudomonas aeruginosa (27853)	Good		More than 70%

Proteus mirabilis (14153)	Good	More than 70%
Citrobacter freundii (8090)	Good	More than 70%
Clostridium perfringens (10543)	None-poor	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

ECD MUG Agar AM5039121

Use

ECD MUG Agar is recommended for demonstrating the presence of *Escherichia coli* by fluorescence in UV and positive indole test while inhibiting accompanying intestinal flora.

Summary

Feng and Hartman (30.1) developed a rapid assay for *E. coli* by incorporating 4-methylumbilliferyl-ß-gluconide (MUG) in to Lauryl Trytose Broth. E.C Medium with MUG is prepared according to the formula specified by the U.S Environmental Protection Agency and Standard methods for water and food testing (17.1).

Principle

Casein enzymic hydrolysate provides the nitrogen, vitamins and amino acids in EC medium with MUG. Lactose is the carbon source in this medium. Bile salts mixture is the selective agent against gram-positive bacteria, particularly *bacilli* and *fecal streptococci*. Dipotassium phosphate and mono potassium phosphate are buffering agents. Sodium chloride maintains the osmotic balance of the medium. *E.coli* produces the enzyme glucoronidase that hydrolysis MUG to yield a fluorogenic product that is detectable under long wave (366 nm) UV light. Tryptophan serves as the substrate for indole reaction.

Formula*

Ingredients in grams per liter

Casein peptone	20.00
Lactose	5.00
Sodium chloride	5.00
Bile salts mixture	1.50
Dipotassium hydrogen phosphate	4.00
Potassium hydrogen phosphate	1.50
Tryptophan	1.00
4-Methylumbelliferyl ß-D-Glucuronide (MUG)	0.07
Agar	15.00

Final pH (at 25°C) 7.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 53.0 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.
4. Mix well and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organisms	Growth	RGI	Indole production	Fuorescence (under 366nm)
Enterobacter aerogenes (13048)	Good-luxuriant	More than 70%	Negative reaction	Negative
Escherichia coli (25922)	Good-luxuriant	More than 70%	Positive reaction,	Red positive zone around the colony
Staphylococcus aureus (25923)	Inhibited	0%		

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Enterobacteria Enrichment, Mossel (Harmonized) AMH503913

EE Broth, Mossel EP AM503914

EE Broth, Mossel BP AM503915

EE Broth, Mossel IP AM5039151

EE Broth, Mossel USP AM5039152

Use

E E Broth, Mossel is used for selective enrichment and detection of *Enterobacteriaceae* in bacteriological examination of food and feedstuffs.

Summary

Mossel, Visser and Cornelissen developed a culture media for the selective enrichment of *Enterobacteriaceae* that enable the *Enterobacteriaceae* to multiply freely and inhibit accompanying other organisms (81.1). Media contains dextrose to facilitate growth of most *Enterobacteriaceae* including Salmonella and other non-lactose fermenting organisms. EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar (AM51073). When specific organisms, rather than *Enterobacteriaceae* in general, are required subcultures must be made onto lactose differential media e.g. Deoxycholate Citrate Agar (AM1031/5031), Brilliant Green Agar, Modified (AM1018/5018), or MacConkey Agar (AM1059/5059) for the detection of non-lactose fermenting or delayed lactose fermenting organisms.

Principle

Pancreatic digest of gelatin provides nutrients, nitrogen compounds and amino acids. Ox bile supports the growth of enteric bacteria and inhibits other bacteria, which do not normally live in the intestine. Brilliant-green specifically inhibits the Gram-positive accompanying flora. Disodium phosphate and Mono-potassium phosphate are buffering agents.

Formula*

Ingredients in grams per liter	AM5039151	AM503914, AM503915, AM5039152 & AMH503913
Pancreatic digest of gelatin	10.0	10.0
Glucose monohydrate	-	5.0
Dextrose monohydrate	5.0	-
Dehydrated Ox-bile	20.0	20.0
Disodium hydrogen phosphate, dihydrate	8.0	8.0
Potassium dihydrogen phosphate	2.0	2.0
Brilliant Green	0.015	0.015

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 45.015 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat in free flowing steam or boiling water for 30 minutes. Avoid overheating of the medium. DO NOT AUTOCLAVE OR REHEAT.
4. Pour into adequate containers.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Emerald green coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid
Shigella boydii (12030)	Luxuriant	-
S.serotype Enteritidis (13076)	Luxuriant	±
Escherichia coli (25922)	Luxuriant	+
Enterobacter aerogenes (13048)	Luxuriant	+
Proteus mirabilis (25933)	Luxuriant	+
Staphylococcus aureus (25923)	Inhibited	-

Key: + = positive, yellow colouration.

- = negative, no colour change, green.

Procedure

1. Inoculate the E E Broth, Mossel with food or other test specimen.
2. Mix well the inoculated medium.
3. Incubate at 35-37°C for 20-24 hours.

Interpretation of Results

Acid production causes the color of EE Broth Mossel to become yellow. A negative reaction results in no color change and the medium remains green.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

E. E. Broth Mossel with Oxgall

AM503916

Use

E E Broth, Mossel with Oxgall is used for selective enrichment and detection of *Enterobacteriaceae* in bacteriological examination of foods.

Summary

Mossel, Visser and Cornelissen developed a culture media for the selective enrichment of *Enterobacteriaceae* that enable the *Enterobacteriaceae* to multiply freely and inhibit accompanying other organisms. Media contains dextrose to facilitate growth of most *Enterobacteriaceae* including Salmonella and other non-lactose fermenting organisms. EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar (AM51073). When specific organisms, rather than *Enterobacteriaceae* in general, are required subcultures must be made onto lactose differential media e.g. Deoxycholate Citrate Agar (AM1031/5031), Brilliant Green Agar, Modified (AM1018/5018), or MacConkey Agar (AM1059/5059) for the detection of non-lactose fermenting or delayed lactose fermenting organisms.

Principle

Pancreatic digest of gelatin provides nutrients, nitrogen compounds and amino acids. Brilliant-green or oxgall specifically inhibits the Gram-positive accompanying flora. Disodium phosphate and Mono-potassium phosphate are buffering agents.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	12.0
Proteose peptone No. 3	8.0
Dextrose	5.0
Oxgall	10.0
Disodium phosphate	8.0
Monopotassium phosphate	2.0
Brilliant Green	0.0135

Final pH (at 25°C) 7.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 45.01 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat at 100°C for 30 minutes and cool immediately. Do not boil. DO NOT AUTOCLAVE OR REHEAT.
4. Pour into adequate containers.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Emerald green coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid
Shigella boydii (12030)	Luxuriant	-
S.serotype Enteritidis (13076)	Luxuriant	±
Escherichia coli (25922)	Luxuriant	+
Enterobacter aerogenes (13048)	Luxuriant	+
Proteus mirabilis (25933)	Luxuriant	+
Staphylococcus aureus (25923)	Inhibited	-

Key: + = positive, yellow colouration.

- = negative, no colour change, green.

Procedure

1. Inoculate the E E Broth, Mossel with Oxgall, with food or other test specimen.
2. Mix well the inoculated medium.
3. Incubate at 35-37°C for 20-24 hours.

Interpretation of Results

Acid production causes the color of EE Broth Mossel with Oxgall to become yellow. A negative reaction results in no color change and the medium remains green.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Eijkman Lactose Broth

AM503917

Use

Eijkman Lactose Broth is used for the detection and differentiation of *Escherichia coli* from other coliform organisms on the basis of their ability to grow and liberate gas from Lactose.

Summary

Eijkman (23.2) described a method for separating the strains of *Escherichia coli* from the faeces of warm blooded and cold blooded animals. This method had limitation due to the inability to obtain growth after subculturing from positive tubes incubated at 46°C, as acidity and high temperature results in death of the culture within 24-48 hours. Perry and Hajna (87.1) modified Eijkman original method by decreasing carbohydrate content and adding a phosphate buffer enabling to subculture *Escherichia coli* after incubation at 46°C for 96 hours or

longer where pH was 5.6 unlike 4.5 of Eijkman medium.

Principle

Tryptose provides nitrogen, carbon and other growth factors. Perry modified Eijkman medium using lactose for isolation of *Escherichia coli.* This medium can also be used for water filtration control work. Dipotassium phosphate and Monopotassium phosphate are buffering agents.

Formula*

Ingredients in grams per liter

Tryptose	15.00
Lactose	3.00
Dipotassium phosphate	4.00
Monopotassium phosphate	1.50
Sodium chloride	5.00

Final pH (at 25°C) 6.8 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 28.5gms of powder in 1000 ml distilled water.
2. For examination of 10 ml portion of water samples, use 57 grams per 1000 ml distilled water.
3. Dispense into tubes with inverted Durham's fermentation tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 45.5-46°C.

Organisms (ATCC)	Growth	Gas
Escherichia coli (25992)	Luxuriant	+
Enterobacter aerogenes (13048)	Poor	-

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

EMB Agar AM10391/AM50391

EMB Broth AM10401/AM50401

Use

Eosin Methylene Blue (EMB) Agar and Eosin Methylene Blue (EMB) Broth are slightly selective and differential media recommended for the isolation, cultivation and differentiation of gram-negative enteric bacilli from clinical and non-clinical specimens.

Summary

Eosin Methylene Blue (EMB) media were originally developed by Holt-Harris and Teague. Eosin Y and Methylene Blue are the two dyes incorporated in these media. This formulation gives a sharp and distinct differentiation between the colonies of lactose fermenting and non-lactose fermenting microorganisms.

Principle

The media contain Eosin Y and Methylene Blue dyes that inhibit gram-positive bacteria to a limited degree. In addition, these dyes also serve as differential indicators in response to lactose/sucrose fermentation by the microorganisms. Sucrose is added to the media as an alternative carbohydrate source for typical lactose fermenting, gram-negative bacilli, which may not ferment lactose or may do so slowly.

Formula*

Ingredients in grams per liter	EMB Agar	EMB Broth
Tryptone	10.0	10.0
Dipotassium Phosphate	2.0	2.0
Lactose	5.0	5.0
Sucrose	5.0	5.0
Eosin Y	0.4	0.4
Methylene Blue	0.065	0.065
Agar	13.5	-

Final pH (at 25° C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   EMB Agar - 36 grams

   EMB Broth - 22.5 grams
2. Mix thoroughly until the suspension is uniform.
3. Heat with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 20 minutes.
5. Cool to 50° C and shake the medium to oxidize the methylene blue and to suspend the flocculent precipitate.
6. Pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Light purple coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish-purple coloured, opalescent gel or solution having greenish cast forms in petriplates / tubes.

Cultural Response

Cultural response after 18 - 24 hours at 35° C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Escherichia coli (25922)	Luxuriant	Purple with black center with green metallic sheen	More than 70%
Proteus mirabilis (25933)	Luxuriant	Colourless	More than 70%
Salmonella serotype *Typhimurium* (14028)	Luxuriant	Colourless	More than 70%
Enterobacter aerogenes (13048)	Good	Pink, without sheen	More than 70%
Klebsiella pneumoniae (13883)	Good	Pink, mucoid	More than 70%
Staphylococcus aureus (25923)	Inhibited	----	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Obtain isolated colonies from specimens using standard procedures.
2. Allow plates to attain room temperature. Ensure that the agar surface is dry before inoculation.
3. Incubate plates, protected from light, at 35 ± 2° C for 18-24 hours.
4. Examine plates for colonial morphology.
5. Plates can be incubated for an additional 24 hours if no growth is observed.

Interpretation of Results

1. Coliforms produce blue-black colonies due to the taking up of an eosin-methylene blue dye complex by the bacterial cells when the pH drops.
2. *Salmonella* and *Shigella* colonies appear colourless or have a transparent amber colour.
3. *Escherichia coli* colonies may show a characteristic green metallic sheen due to rapid fermentation of lactose.
4. Some gram-positive bacteria, such as fecal *streptococci, staphylococci* and yeasts, usually form pinpoint colonies.

Precautions / Limitations

1. Store the prepared medium away from light to avoid photo oxidation.
2. If EMB Agar is inoculated on the same day, as it is prepared, it may be used without autoclave sterilization.
3. A number of non-pathogenic, lactose non-fermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.
4. Serial inoculation may be required to assure adequate isolation of mixed flora samples.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

EMB Agar, Levine — AM1040/AM5040

EMB Agar, Levine IP (Levine Eosin-Methylene Blue Agar Medium) — AM104011/AM504011

EMB Agar, Levine USP — AM104012/AM504012

EMB Agar, Levine BIS — AM104013/AM504013

Use

EMB Agar, Levine is a slightly selective and differential medium used for the isolation, enumeration and differentiation of members of *Enterobacteriaceae*.

Summary

Holt-Harris and Teague (45) developed a culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes. Levine (70) brought about a modification of their formulation, which he claimed gave a better differentiation between *Escherichia* and *Enterobacter* species. This Levine EMB Agar differs from the other formulation in not containing sucrose. The original medium could not discriminate between which carbohydrate (lactose or sucrose) was being fermented. Also, *Y.enterocolitica,* which ferments sucrose but not lactose, produced same colonies as that of lactose fermenters. Levine modified the formula by omitting sucrose and doubling the level of lactose. This medium was mainly developed to improve upon the differentiating properties of Endo Agar.

EMB Agar, Levine is mainly used for the differentiation of *Escherichia coli* and

Enterobacter aerogenes and also for the rapid identification of *Candida albicans*. It is recommended for use in microbial examination of dairy products (39), foods (20) and water (36) by APHA and for use in the performance of microbial limit test by USP and IP (114, 46). It is also included in the Bacteriological Analytical Manual for food testing (113).

Weld proposed the use of EMB Agar, Levine with added chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *C.albican s* can be made after 24-48 hours incubation at 35-37^{0}C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scrapings. Menolasino et al used this medium for identification of coagulase positive *staphylococci*, which grew, as characteristic colourless pinpoint colonies.

Principle

Peptone provides essential nutrients while lactose is the fermentable carbohydrate. Dipotassium phosphate is the buffer. The Eosin Y and methylene blue dye in this medium inhibit gram-positive organisms to a limited degree making the medium only slightly selective besides; it also helps in differentiating between lactose fermenters and non-lactose fermenters. Lactose fermenters produce blue-black colonies due to taking up of the dye when the pH drops. Lactose non-fermenters like *Salmonella* and *Shigella* probably raise the pH of the surrounding medium by oxidative deamination of the protein, solubilising the methylene blue-eosin complex and appear as colourless or transparent colonies. Some gram-positive bacteria like faecal *streptococci, staphylococci* and yeasts grow on this medium to form pinpoint colonies.

Formula*

Ingredients in grams per liter

Peptone	10.0
Lactose	10.0
Dipotassium Phosphate	2.0
Eosin Y	0.4
Methylene Blue	0.065
Agar	15.0

Final pH (at 25^{0}C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.
5. AVOID OVERHEATING.
6. Cool to 45-50^{0}C and shake the medium in order to restore the blue colour (i.e. oxidize the methylene blue) and to suspend the precipitate, which is an essential part of the medium.

Quality Control

Dehydrated Appearance

Light purple coloured, homogeneous free flowing powder.

Prepared Appearance

Reddish purple coloured, slightly opalescent gel with greenish cast and finely dispersed precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37^{0}C.

Organisms (ATCC)	Growth	Colour of colony	RGI
*Candida albicans** (10231)	Good to luxuriant	Colourless	More than 70%
Enterobacter aerogenes (13048)	Good	Pink to red	More than 70%
Enterococcus faecalis (29212)	Inhibited	-	0%
Escherichia coli (25922)	Luxuriant	Blue black with metallic sheen	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Colourless	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	None to poor	Colourless	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Key * = incubation in 10 % CO_2

Procedure

1. Allow the agar surface to dry before inoculating.
2. Inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Use standard procedures like the streak plate method to obtain isolated colonies.
5. A non-selective medium should also be streaked simultaneously to increase the chances of recovery when the population of gram-negative organisms is low and to detect other organisms present in the medium.
6. Incubate plates aerobically protected from light at 35-37^{0}C for 24 hours.
7. If negative, reincubate for an additional 24 hours.

Interpretation of Results

Typical colonial morphology on EMB Agar, Levine

Escherichia coli---------- Large, blue-black with green metallic sheen

Enterobacter/Klebsiella----------	Large, mucoid, blue-black
Proteus----------	Large, colourless
Salmonella----------	Large, colourless
Shigella----------	Large, colourless
Pseudomonas----------	Irregular, colourless
Gram-positive bacteria----------	No growth to slight growth
Candida albicans----------	After 24-48 hours, at 35°C in 10% CO_2, colonies are spidery or feathery

Precautions / Limitations

1. STORE THE MEDIUM AWAY FROM LIGHT TO AVOID PHOTO OXIDATION.
2. Some strains of *Salmonella* and *Shigella* will not grow in the presence of eosin Y and methylene blue.
3. Some gram-positive bacteria such as staphylococci, enterococci and yeasts may grow on this medium.
4. Non-pathogenic, non-lactose fermenting organisms may also grow on this medium.
5. Serial inoculation may be required to assure adequate isolation of mixed flora samples.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Endo Agar AM1041/AM5041

Use

Endo Agar is a differential and slightly selective culture medium for the detection of coliforms and other enteric microorganisms.

Summary

Endo (25) developed a culture medium for the differentiation of lactose fermenters from non-lactose fermenters in which no bile salts were used. Inhibition of gram-positive organisms was achieved by the combination of sodium sulphite and basic fuchsin. Endo's Fuchsin Sulphite Infusion Agar was the original name for this medium, which is known today as Endo Agar. This medium is used for the microbial examination of potable water and wastewater (36) and food (20); however, the current compendia of standard methods recommend alternative media formulations for the examination of these materials.

Principle

Peptone provides nitrogen and carbon while lactose is the fermentable carbohydrate. Dipotassium phosphate is the buffer. Sodium sulphite and basic fuchsin inhibit gram-positive organisms to a limited degree. This medium is classified as only slightly selective because other media contain more potent inhibitors of gram-positive organisms. Lactose fermenting coliforms produce aldehyde and acid. The aldehyde in turn liberates the fuchsin from the fuchsin-sulphite complex, giving rise to red colouration of the colonies and similar colouration of the medium. Non-lactose fermenters form faint to colourless colonies against the pink background of the medium. With *E.coli,* this reaction is very pronounced as the fuchsin crystallizes, exhibiting a permanent greenish metallic luster to the colonies.

Formula*

Ingredients in grams per liter

Lactose	10.0
Peptone	10.0
Sodium Sulphite	2.5
Dipotassium Phosphate	3.5
Basic Fuchsin	0.5
Agar	15.0

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C.
6. Re-suspend precipitate by gently mixing before use.

Warning:

Basic fuchsin is a potential carcinogen. Avoid inhalation of the powder and contact with skin.

Quality Control

Dehydrated Appearance

Light purple coloured, homogeneous, free flowing powder.

Prepared Appearance

Orange pink coloured, clear to slightly opalescent gel with fine precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink, mucoid	More than 70%
Enterococcus	None to	Pink, small	0%

faecalis (29212)	poor		
Escherichia coli (25922)	Luxuriant	Pink to rose red with metallic sheen	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant	Pink mucoid	More than 70%
Proteus vulgaris (13315)	Luxuriant	Colourless to pale pink	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Colourless, irregular	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	Colourless to pale pink	More than 70%
Shigella sonnei (25931)	Luxuriant	Colourless to pale pink	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. Incubate plates protected from light, at 35-37^{0}C for 18-24 hours.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is suspected to be low and to provide an indication of other organisms present in the specimen.
4. If negative, reincubate an additional 24 hours.

Interpretation of Results

Typical colony morphology

Escherichia coli---------- Pink to rose red, green metallic sheen

Enterobacter/ Klebsiella---------- Large, mucoid, pink

Proteus---------- Colourless to pale pink

Salmonella---------- Colourless to pale pink

Shigella---------- Colourless to pale pink

Pseudomonas---------- Irregular, colourless

Gram-positive bacteria---------- No growth to slight growth

Precautions / Limitations

1. Store the medium away from light to avoid photo oxidation.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Emerson Agar AM504101

Use

Emerson Agar is used for isolation and cultivation of *Actinomycetaceae , Streptomycetaceae,* fungi and moulds.

Summary

Emerson Agar was originally formulated by Emerson et al (24.3) and is used for the cultivation of moulds and bacterial species resembling moulds (45.6). This medium was further modified by Gottlieb et al (34.2) and is used for screening potent antibiotic-producing organisms (102.2). In their study, they stored Streptomyces in soil for long time and transferred them as needed, to slants of Emerson Agar. The slant cultures were incubated for 3-7 days. The spores were gently scraped from the cultures surface to form a spore inoculum.

Principle

Yeast extract provides a source of trace elements, vitamins and amino acids. For the selective isolation of *Streptomyces* species, cycloheximide is incorporated in the medium, which limits the growth of moulds. This medium is also used for routine cultivation and maintenance of pure cultures.

Formula*

Ingredients in grams per liter

Beef extract	4.0
Yeast extract	1.0
Peptic digest of animal tissue	4.0
Dextrose	10.0
Sodium chloride	2.5
Agar	20.0

Final pH (at 25°C) 7.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Dissolve 41.5 gms of medium in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Add 0.05 grams / litre cycloheximide, or 0.005 gm/l captan if desired..
4. Sterilize by autoclaving at 15lbs pressure (121ºC) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 48-72 hours at 30ºC.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Streptomyces albus subsp albus (3004)	Luxuriant	More than 70%
Streptomyces lavendulae (8664)	Luxuriant	More than 70%
Streptomyces achromogenes (12767) (13048)	Luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Eugonic Agar AM50410111

Use

Eugonic Agar is recommended for the cultivation of fastidious microorganisms like *Haemophillus, Neisseria, Posteurella, Brucella* and *Lactobacillus* species.

Summary

Eugonic Agar was developed by Pelczar and Vera (87.2) for cultivation of fastidious organisms like Brucella . These media can also be used to grow Mycobacteria and various pathogenic fungi including *Nocardia, Histoplasma* and *Blastomyces* , Eugonic Agar was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms like Brucella that are otherwise difficult to cultivate (77.1). The unnourished medium supports rapid growth of lactobacilli associated with cured meat products, dairy products and other foods. APHA recommends Eugonic agar, which is also used in germinating anaerobic spores

Principle

Casein enzymic hydrolysate and papaic digest of soyabean meal provide the nitrogen, vitamins and amino acids, which supports the growth of fastidious microbial species. The high concentration of dextrose is the energy source for rapid growth of bacteria. L-Cystine and sodium sulphite are added to stimulate growth. Sodium chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	15.0
Papaic digest of soyabean meal	5.0
Dextrose	5.0
Sodium chloride	4.0
Sodium sulphite	0.2
L-Cystine	0.2
Agar	15.0

Final pH (at 25°C) 7.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Dissolve 44.4 gms in 1000 ml distilled water.
2. Heat to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to 45°C and add 5 -10% v/v sterile defibrinated blood if desired. The blood may be chocolated by heating, if chocolate agar plates are required.

Quality Control

Dehydrated Appearance

Cream to Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured clear to slightly opalescent gel forms in petri plates

Cultural Response

Cultural characteristics after 48 hours at 35-37ºC for bacteria and 20-25ºC for fungi with added 5-10%

Organisms (ATCC)	Growth	RGI
Bacillus pumilus ATCC (14884)	Good	More than 70%
Candida albicans ATCC (26790)	Good	More than 70%
Lactobacillus fermentum ATCC (9338)	Good	More than 70%
Neisseria meningitidis ATCC (13090)	Good	More than 70%
Streptococcus pneumoniae ATCC (6303)	Luxuriant	More than 70%
Streptococcus pyogenes ATCC (19615)	Luxuriant	More than 70%
Brucella abortus (4315)	Good	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Casein Digest Soya Lecithin Medium (Twin Pack) IP AM10411/AM50411

Fluid Casein Digest Soya Lecithin Medium (Twin Pack) USP AM10412/AM50412

Use

Fluid Casein Digest Soya Lecithin Medium (Twin Pack) is used a medium for detection of microbes on

sanitized surfaces in compliance with IP and USP.

Summary

Fluid Casein Digest Soya Lecithin Medium is recommended for sanitary examination of surfaces in compliance with IP and USP . NASA also recommends this medium for the microbiological sampling of environmental surfaces sanitized with quaternary ammonium compounds(84.2).

Principle

Casein enzymic hydrolysate provides the essential nutrients for the growth of bacteria. Soya lecithin neutralizes the quaternary ammonium compounds whereas polysorbate 20 neutralizes phenolic disinfectants, hexachlorophene and formalin.

Formula*

Ingredients in grams per liter

Part A:	
Casein enzymic hydrolysate	20.0
Soy lecithin	5.0
Part B:	
Polysorbate 20	40.0 ml

Final pH (at 25°C) 7.3 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Dissolve 25 gms of Part A powder in 960 ml distilled water.
2. Heat gently to dissolve the medium completely. Do not boil.
3. Add 40 ml of Part B, mix well.
4. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A: Yellow coloured, homogeneous, free flowing powder.

Part B: Colourless, clear, viscous liquid.

Prepared Appearance

Yellow coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth
Bacillus subtilis (6633)	Good-luxuriant
**Candida albicans* (10231)	Good-luxuriant
Escherichia coli (25922)	Good-luxuriant
Staphylococcus aureus (25923)	Good-luxuriant

Key: * Incubate at 30°C for 24-48 hours.

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Lactose Medium AM1042/AM5042

Fluid Lactose Medium IP AM10421/AM50421

Fluid Lactose Medium USP AM104211/AM504211

Use

Fluid Lactose Medium is used for the detection of coliforms and the study of lactose fermentation by common bacteria.

Summary

Fluid Lactose Medium is used for testing water, dairy products and foods. It is also used in the performance of microbial limit test for *Escherichia coli* and *Salmonella* and in the Completed Test for coliforms in dairy products (113, 46).

Principle

Beef extract and pancreatic digest of gelatin provide the essential nutrients while lactose is the fermentable carbohydrate. Growth with gas formation is a presumptive test for coliforms. Multiple strength Fluid Lactose Broth can be used for larger inocula. The final concentration of the ingredients is maintained at a constant level. i.e. 13 grams per litre.

Formula*

Ingredients in grams per liter

Pancreatic Digest of Gelatin	5.0

Lactose	5.0
Beef Extract	3.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 13 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Distribute into tubes containing inverted Durham's tube.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. For large inocula, prepare multiple strength Fluid Lactose Broth.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms	Growth	Gas
Enterobacter aerogenes (13048)	Good to luxuriant	+
Enterococcus faecalis (29212)	Good to luxuriant	-
Escherichia coli (25922)	Good to luxuriant	+
Pseudomonas aeruginosa (27853)	Good to luxuriant	-

Procedure

1. The amount of sample added to Fluid Lactose Medium is dependent on the concentration of the medium. Regardless of the sample size, Fluid Lactose Medium must have a concentration of 13 grams per litre. For example, if 10 ml sample is to be added to 10 ml of Lactose Broth, the broth must be double strength.
2. Allow the medium to warm to room temperature before inoculation.
3. Inoculate tubes of Fluid Lactose Medium with the dilutions of the sample.
4. Incubate aerobically for 24 hours at 35-37°C.
5. Examine for turbidity and gas formation.
6. Reincubate the tubes for additional 24 hours (48 hours) if the results are negative at 24 hours.

Interpretation of Results

1. Turbidity in the medium accompanied by formation of gas in any amount in the Durham's tubes within 48 hours is a positive presumptive test for the presence of coliforms.

Precautions / Limitations

1. Durham's tube must be free from air bubbles before inoculation.
2. Avoid overheating multiple strength broth as inhibitory products may be formed.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Lactose Medium with Lecithin And Tween 80* AM50422

Use

Fluid Lactose Medium with Lecithin And Tween 80 is used for detection of coliform and lactose fermentation by common bacteria.

Summary

Fluid Lactose Medium is formulated in accordance with the recommendation of APHA and used for testing water, dairy products(78.1) and foods (115.1). Lactose Broth is used in the completed test for coliforms in dairy products. It is also used in the performance of microbial limit test for *Salmonella* species and *Escherichia coli* (111.1).

Principle

Beef extract and pancreatic digest of gelatin provide essential nutrients for bacterial metabolism. Lactose is the source of fermentable carbohydrate. Growth with gas formation is a presumptive test for coliforms. Tween 80 and lecithin act as neutralizers to inactivate the residual disinfectants where the samples are collected. Lecithin inactivates quaternary ammonium compounds whereas tween 80 neutralizes formalin, phenolic disinfectants, hexachlorophene etc.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	5.0
Lactose	5.0
Beef extract	3.0
Polysorbate 80 (Tween 80)	5.0
Lecithin	0.70

Final pH (at 25°C) 6.9± 0.2

*Formula adjusted to suit performance parameters

Directions

1. suspend 18.7 gms of the powder in 1000 ml distilled water. Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Distribute into tubes with inverted Durham's tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogenous, free flowing powder.

Prepared Appearance

Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37ºC.

Organisms (ATCC)	Growth	Gas
Enterobacter aerogenes (13048)	Good-luxuriant	+
Escherichia coli (25922)	Good-luxuriant	+
Pseudomonas aeruginosa (27853)	Good-luxuriant	-
Enterococcus faecalis (29212)	Good-luxuriant	-

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for result

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Sabouraud Medium AM1043/AM5043

Use

Fluid Sabouraud Medium is a liquid medium used for sterility testing of moulds and lower bacteria in pharmaceutical preparations.

Summary

Fluid Sabouraud Medium is a modification of the formulation of Sabouraud (100). It is a mycological sterility test medium conforming to the medium described in the USP (114) and FDA's Bacteriological Analytical Manual (113) for the determination of fungistatic activity of pharmaceutical and cosmetic products in order to avoid false sterility tests. It is also used for the cultivation of yeasts, moulds and aciduric microorganisms. In clinical microbiology, this medium has shown to increase the isolation rate of *Candida albicans* in blood culture. The acid reaction of the final medium is inhibitory to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and aciduric microorganisms.

Principle

Peptone and Tryptone provide nitrogen and carbon. Dextrose is the carbohydrate source.

Formula*

Ingredients in grams per liter

Dextrose	20.0
Peptone	5.0
Tryptone	5.0

Final pH (at 25°C) 5.7 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics at 25-30°C for 48-72 hours or up to 10 days if necessary.

Organisms (ATCC)	Growth
Aspergillus niger (16404)	Luxuriant
Candida albicans (10231)	Luxuriant
* *Escherichia coli* (25922)	Luxuriant
**Lactobacillus casei* (9595)	Luxuriant
Saccharomyces cerevisiae (9763)	Luxuriant

Key:

* = incubated at 35°C

Procedure

1. Incubate the positive control and the product to be tested at 25-30°C for 10 days.

Interpretation of Results

1. If growth in both the tubes is comparable, then the product is non fungistatic.
2. If the product is fungistatic, add a suitable sterile inactivating agent, or use a larger ratio of medium to product in order to determine the ratio of product to medium in which growth of the test organism is not affected.

Precautions / Limitations

1. Some fungi may be inhibited by the acidic pH of the medium.
2. Transfer of growth from broth to plated medium may be required in order to obtain pure cultures of fungi.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Selenite Cystine Medium (Twin Pack) AM1044/AM5044

Fluid Selenite Cystine Medium IP (Twin Pack) AM10441/AM50441

Fluid Selenite Cystine Medium USP (Twin Pack) AM10442/AM50442

Fluid Selenite Cystine Medium ISO (Twin Pack) AM50443

Use

Fluid Selenite Cystine Medium is used as a selective enrichment medium for the isolation of salmonellae from faeces, foods, pharmaceutical articles, water and other materials of sanitary importance.

Summary

Klett (56) first demonstrated the selective inhibitory effects of selenite and Guth (38) used it to isolate *Salmonella typhi*. Leifson found that selenite inhibited faecal streptococci and coliforms during the first 12 hours of incubation, allowing *Salmonella* to replicate. Fluid Selenite Cystine Medium is a modification of Leifson's formula (65) with added cystine. This medium is included in the USP (114) and IP (46) for use in the performance of microbial limit test for *Salmonella* species and is recommended by the FDA's Bacteriological Analytical Manual (113), AOAC International and APHA for detecting *Salmonella* in foods (20), particularly in egg products, milk (39) and water (36).

Principle

Tryptone provides nitrogen and other amino acids. Lactose is the carbohydrate source and also maintains the pH in the medium as selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of the selenite and results in the overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation helps to maintain a neutral pH. Sodium phosphate buffers the medium to maintain the pH and also lessens the toxicity of selenite. Sodium selenite inhibits gram-positive bacteria and suppresses the growth of most gram-negative bacteria other than *Salmonella*. L-cystine improves the recovery of *Salmonella* and also acts as a reducing agent.

Formula*

Ingredients in grams per liter

DisodiumPhosphate	10.0
Tryptone	5.0
Sodium Hydrogen Selenite	4.0
Lactose	4.0
L-Cystine	0.01

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23 gms of the powder in 1000 ml distilled water and mix well.
2. Warm or just boil to dissolve the medium completely and dispense in tubes as required. AVOID OVERHEATING. DO NOT AUTOCLAVE.
3. Sterilize in a boiling water bath or free flowing steam for 10 minutes.
4. Discard if large amount of selenite is reduced which is indicated by a red precipitate at the bottom of the tube.

Warning: Sodium Hydrogen Selenite (Sodium biselenite) is very toxic, corrosive agent and causes teratogenicity and should be handled with care. Upon contact with skin, wash with plenty of water.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C when subcultured on MacConkey Agar (AM1059/AM5059).

Organisms (ATCC)	Recovery	Colour of colony
Escherichia coli (25922)	Little to none	Pink with bile precipitate
Salmonella serotype *Choleraesuis* (12011)	Good to excellent	Colourless
Salmonella serotype Typhi (6539)	Good to excellent	Colourless

Procedure

1. For faeces, food sample or other materials, suspend 1-2 gms of the specimen in the broth (approximately 10-15% by volume) and emulsify if necessary.
2. Solid material is added to the normal strength broth.
3. Liquid samples are mixed with double strength medium in the ratio of 1:1.
4. Incubate for 12-24 hours at 35-37°C.
5. Sub-culture after 12-18 hours of incubation.

Interpretation of Results

1. After incubation, there must be an increase in the number of pathogens that the medium is designed to select for and enrich.
2. Subculture onto any combination of greater and lesser inhibitory, selective and differential media for *Enterobacteriaceae*. e.g. MacConkey Agar, XLD Agar, etc to isolate pathogens for identification.

Precautions / Limitations

1. Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate at the bottom of the tube.

2. Do not incubate for longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation and coliforms may overgrow the pathogens.
3. Take subcultures from the upper third of the broth column , which should be at least 5 cm in depth.
4. Enrichment broths should not be used as the sole isolation medium. Use in conjunction with selective and non-selective plating media to increase the chances of isolating pathogens, particularly when they may be present in small numbers.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Tetrathionate Medium USP AM504431

Use

An enrichment broth for isolation of *Salmonellae* from specimens suspected to be contaminated with Salmonellae in compliance with USP.

Summary

Tetrathionate broth base was originally described by Muller (81.2) and found that the medium selectively inhibit coliforms and permit unrestricted growth of enteric pathogens. The medium is now formulated according to USP which specify this medium as enrichment medium for Salmonella species

Principle

Fluid Tetrathionate Medium USP as enrichment medium for *Salmonella* species. Bile salts inhibit gram-Positive microorganisms. The selectivity depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organisms. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. For further confirmation, streak the enriched cultures after incubation, on the plates of Brilliant Green Agar, MacConkey agar, Bismuth Sulphite Agar.

Formula *

Pancreatic digest of casein	2.5g
Peptic digest of animal tissue	2.5g
Bile salts	1.0g
Calcium carbonate	10.0g
Sodium thiosulphate	30.0g

*Formula adjusted, standardized to suit performance parameters.

Directions

1. Suspend 46 grams in 1000 ml of distilled water.
2. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.
3. Cool below 45^{0}C and add 20 ml Iodine solution (Iodine 6 grams and Potassium Iodide-5 grams in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution.
4. Mix well and dispense in 10 ml quantities.

   (This complete medium should be used on the same day of preparation. Do not heat after the addition of Iodine solution. Use the medium immediately after addition of Iodine).

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Complete medium with added brilliant green and iodine solution forms light green coloured, opalescent solution with heavy white precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 37ºC when subcultured on MacConkey Agar after enrichment in Tetrathionate medium.

Organisms (ATCC)	Recovery	Colony
Escherichia coli (25922)	Little or on increase in number	white to pink with bile precipitate
S. serotype Choleraesuis (12011)	Good to excellent	Colourless
S. serotype Typhi (6539)	Good to excellent	Colourless
S. serotype Typhimurium (14028)	Good to excellent	Colourless

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Fluid Thioglycollate Medium AM1045/AM5045
Fluid Thioglycollate Medium IP AM10451/AM50451
Fluid Thioglycollate Medium USP AM10452/AM50452
Fluid Thioglycollate Medium EP AM10453/AM50453
Fluid Thioglycollate Medium BP AM10454/AM50454

Use

Fluid Thioglycollate Medium is used for sterility testing of biologicals and for cultivation of aerobes, anaerobes and microaerophiles.

Summary

Falk, Bucca and Simmons (29) showed the advantage of using small quantities of agar in detecting contaminants during sterility testing of biologicals. Brewer (9) demonstrated that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate and therefore formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes by adding a reducing agent and a small amount of agar.

Fluid Thioglycollate Medium is recommended by APHA (20) and the AOAC International for the examination of food, and for determining the phenol coefficient and sporicidal effect of disinfectants . This medium is also specified for sterility checks on banked blood. It is recommended in the USP (114) and IP (46) for use in sterility testing of articles supposed to be sterile and is also included in the Bacteriological Analytical Manual for food testing (113).

Principle

Tryptone, yeast extract and L-cystine provide sources of nitrogen, carbon and other growth factors while dextrose is the carbohydrate source. Sodium chloride provides essential ions and maintains the osmotic balance. Sodium thioglycollate is a reducing agent, which prevents the accumulation of peroxides that is lethal to bacterial growth and neutralizes the antibacterial effect of mercurial preservatives. L-cystine is also a reducing agent, since it contains sulphydryl groups that inactivate heavy metal compounds, which exert a bacteriostatic effect in the materials under examination, and also maintains a low redox potential, thereby maintaining anaerobiosis. Resazurin is the oxidation-reduction indicator; increased oxidation raises the Eh, causing resazurin to change colour to red. The small amount of added agar assists in maintaining a low redox potential by stabilizing the medium, thereby maintaining anaerobiosis in the lower depths of the medium.

Formula*

Ingredients in grams/liter	FTM	FTM IP	FTM USP	FTM EP	FTM BP
Tryptone	15.00	–	–	–	–
Pancreatic digest of casein	-	15.00	15.00	15.00	15.00
Yeast extract	5.00	5.00	5.00	5.00	5.00
Glucose monohydrate	–	–	–	5.50	5.50
Dextrose	5.50	–	–	–	–
Dextrose monohydrate	–	5.50	5.50	–	–
Sodium chloride	2.50	2.50	2.50	2.50	2.50
L-Cystine	0.50	0.50	0.50	0.50	0.50
Sodium thioglycollate	0.50	0.50	0.50	0.50	0.50
Resazurin Sodium	0.001	0.001	0.001	0.001	0.001
Agar	0.75	0.75	0.75	0.75	0.75

Final pH (at 25ºC) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29.75 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense as desired into containers.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Tighten lids of the containers immediately (while still warm) to reduce oxidation.
7. Cool to 25°C and store in a cool dark place preferably below 25°C.

   Note: If more than the upper one third of the medium is pink prior to use, reheat once (100°C) in a water bath to drive off absorbed oxygen (till pink colour disappears).

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light straw coloured, clear to very slightly opalescent solution with upper 10% or less medium turning pink on standing.

Cultural Response

Cultural characteristics after 48-72 hours at 35°C.

Organisms (ATCC)	Growth
**Bacillus subtilis* (6633)	Luxuriant
**Bacteroides vulgatus* (8482)	Luxuriant
**Candida albicans* (10231)	Luxuriant
**Clostridium sporogenes* (11437)	Luxuriant

**Micrococcus luteus* (9341)	Luxuriant
Streptococcus pyogenes (19615)	Luxuriant
Staphyloccocus aureus (6538)	Luxuriant

Key:

* These cultures may be incubated at 25-30°C for 2-7 days.

Interpretation of Results

1. After incubation, growth is indicated by the presence of turbidity compared to an un-inoculated control.
2. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in the portion of the broth that is below the upper oxidized layer.

Precautions / Limitations

1. Some dextrose fermenting organisms, which are able to reduce the pH of the medium to a critical level, may not survive in this medium. Early subculture is required to isolate these organisms.
2. In test samples, the proper surface to volume ratio of the medium must be maintained to avoid oxidation of the medium, which is unsuitable for microaerophilic and anaerobic growth.
3. A slight turbidity or haziness may be present due to the small amount of agar present in the medium. When the medium has been boiled, generally it appears clear.
4. Anaerobes can be overgrown by more rapidly growing facultative organisms. Gram stain and examine broth if plating medium reveals no growth.
5. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
6. Do not rely on broth cultures exclusively for isolation of anaerobes.
7. Do not reheat the medium more than once as it may give rise to toxicity.
8. If more than one third of the medium is oxidized (pink), it must be discarded.
9. Store the prepared medium at room temperature away from light.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fraser Broth Base AM50455

Fraser Broth Base ISO AM50456

Use

Fraser Broth Base is used for the isolation, cultivation and enrichment of *Listeria monocytogenes* from food and environmental samples.

Summary

Listeria monocytogenes is a Gram-positive, non-sporeforming, aerobic to facultatively anaerobic, rod shaped bacterium, which exhibits pathogenicity towards humans and other animals. Although not generally recognized as a food-borne pathogen, three recent outbreaks of listeriosis may indicate that this organism is becoming more prevalent as an agent of food-borne disease.

Fraser Broth Base and Fraser Supplements are based on the formulation of Fraser and Sperber. Fraser supplements results in a higher detection rate of *Listeria monocytogenes.*

Principle

Casein enzyme hydrolysate, pancreatic digest of casein, beef extract and yeast extract serves as a source of carbon, nitrogen, vitamins and minerals. Disodium phosphate and mono potassium phosphate are buffering agents. Addition of ferric ammonium citrate in the medium helps to differentiate the esculin hydrolysis, resulting in the blackening of the medium by *Listeria* species. Lithium chloride and high salt concentration makes the medium selective for *Listeria* species.

Formula*

Ingredients in grams per liter

Sodium chloride	20.0
Disodium phosphate $2H_2O$	12.0
Meat peptone	5.0
Tryptone	5.0
Yeast extract	5.0
Beef extract	5.0
Lithium chloride	3.0
Mono potassium phosphate	1.30
Esculin	1.00
Acriflavin	0.025
Nalidixic acid	0.020

Final pH (at 25°C) 7.2 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend the 57.35 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

5. Cool the medium to -50°C.
6. Add 2 vial of rehydrated Fraser Enrichment Supplement (AS0114).
7. After addition, the medium must be gently but thoroughly mixed to ensure that the antibiotics are uniformly distributed throughout the medium.

Warning: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Tan coloured, homogeneous, free flowing powder.

Prepared Appearance

Amber coloured, clear to slightly opalescent with a fine precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35ºC.

Organisms (ATCC)	Growth	Esculin hydrolysis
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	Inhibited	-
Listeria monocytogenes (19117)	Good	+
Listeria monocytogenes (19115)	Good	+
Staphylococcus aureus (25923)	Inhibited	-

Key : + = Black zones around colonies

Procedure

1. For fecal and biological specimens, the sample is homogenized in 0.1% Peptone water (AM1079/5079) and transfer 0.1 ml of the incubated broth to Fraser broth base
2. Incubate the medium at 35ºC for 26 ± 2 hours and examine the result.
3. After 24-48 hours, streak the Fraser Broth culture to Listeria Oxford Medium Base (AM 105512 / AM 505512).
4. Incubate the plates at 35ºC for 24-48 hours.

Interpretation of Results

Examine the agar plates for suspected colonies. For further identification and confirmation of Listeria species, consult appropriate references.

Precautions / Limitations

1. Since Listeria spp. other than L. monocytogenes can grow on these media, biochemical and serological testing should be done to identify L. monocytogenes.
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Fraser Secondary Enrichment Broth Base AM50457

Use

Fraser Secondary Enrichment Broth Base with added supplement is recommended for isolation, cultivation and enrichment of *Listeria monocytogenes* from food and environmental samples.

Summary

Listeria monocytogenes is a Gram-positive, non-sporeforming, aerobic to facultatively anaerobic, rod shaped bacterium, which exhibits pathogenicity towards humans and other animals. Although not generally recognized as a food-borne pathogen, three recent outbreaks of listeriosis may indicate that this organism is becoming more prevalent as an agent of food-borne disease.

Fraser secondary enrichment broth base is based on the original formulation described by Catherine W. Donnelly and Gregory J. Baigent (19.1), and its later modifications of United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) UVM Secondary Enrichment Broth. Fraser Secondary Enrichment Broth Base and Fraser supplements are based on the formulation of Fraser and Sperber. (32.2) This modification and two-step selective enrichment method developed (USDA-FSIS method) results in a higher detection rate of *Listeria monocytogene.*

Principle

Proteose peptone, casein enzyme hydrolysate, beef extract and yeast extract serves as a source of carbon, nitrogen, vitamins and minerals. Lithium chloride inhibits the growth of Enterococci. All *Listeria* species hydrolyze esculin to esculatin which with ferric ions results in forming dark-brown to black complex. Ferric ammonium citrate enhances the growth of *Listeria monocytogenes.* Fraser Secondary Enrichment Broth is inoculated with primary Enrichment Broth. All Fraser Broth Enrichment cultures should be subcultured on plating medium for confirmation of presence or absence of Listeria species.

Formula*

Ingredients in grams per liter

Proteose peptone	5.0
Casein enzyme hydrolysate	5.0
Yeast extract	5.0
Beef extract	5.0
Sodium chloride	20.0
Lithium chloride	3.0
Disodium phosphate	12.0
Mono potassium phosphate	1.35
Esculin	1.00

Ferric ammonium citrate	0.50

Final pH (at 25ºC) 7.2 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend the 57.85 gms of powder in 990 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool the medium to -50°C.
6. Add 1 vial of rehydrated Fraser Selective Supplement (AS0112) to prepare selective medium for *Listeria*.
7. After addition, the medium must be gently but thoroughly mixed to ensure that the antibiotics are uniformly distributed throughout the medium.

Warning: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly precipitate. With addition of supplement, fluorescent yellow coloured solution forms with slight precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35ºC.

Organisms (ATCC)	Growth	Esculin hydrolysis*
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	Inhibited	-
Listeria monocytogenes (19117)	Luxuriant	+
Staphylococcus aureus (25923)	Inhibited	-

Key : + = Black zones around colonies

* = Subcultured on Listeriaidentification Agar Base (PALCAM) ((AM1055/5055)

Procedure

1. For fecal and biological specimens, the sample is homogenized in 0.1% Peptone water (AM1079/5079) and transfer 0.1 ml of the incubated broth to Fraser broth base
2. Incubate the medium at 35ºC for 26 ± 2 hours and examine the result.
3. After 24-48 hours, streak the Fraser Broth culture to Listeria Oxford Medium Base (AM 105512/AM 505512).
4. Incubate the plates at 35ºC for 24-48 hours.

Interpretation of Results

1. Examine the agar plates for suspected colonies. For further identification and confirmation of *Listeria* species, consult appropriate references.

Precautions / Limitations

1. Since *Listeria* spp. other than *L. monocytogenes* can grow on these media, biochemical and serological testing should be done to identify *L. monocytogenes*.
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some *Enterococci*.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

G.C. Agar Base AM1046/AM5046

Use

G.C. Agar Base with various additives is used to isolate and cultivate gonococci and other fastidious organisms.

Summary

G.C. Agar Base with added blood or haemoglobin and other supplements is recommended for selective isolation and cultivation of fastidious organisms like *Neisseria gonorrhoeae*. Johnston (49) developed a medium that could produce colonies of *N.gonorrhoeae* in 24 hours rather than 48 hours. The accelerated growth rates were primarily due to the decreased agar content. Carpenter and Morton (13) later on improved the medium with the addition of haemoglobin.

Principle

Peptone special provides nitrogen, vitamins and amino acids. Corn starch absorbs and neutralizes the toxic metabolites and phosphates buffers the medium and prevents change in the pH due to amine production that can effect the survival of the organisms. Sodium chloride maintains the osmotic balance.

Chocolate Agar is prepared from G.C. Agar Base with the addition of 2% Haemoglobin. Haemoglobin provides hemin, which enhances growth of *Neisseria*. The medium can also be made selective with the addition of selective supplements.

Formula*

Ingredients in grams per liter

Peptone Special	15.0
Sodium Chloride	5.0
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.0
Corn Starch	1.0
Agar	10.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18 gms of the medium in 235 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and aseptically add separately prepared Haemoglobin (250 ml sterile 2% solution) (AS014) and 1 vial of G.C. Supplement (AS012).
5. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium - Light yellow coloured, clear to slightly opalescent gel.

With addition of haemoglobin - Chocolate brown coloured opaque gel.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C on Chocolate Agar prepared from GC Agar Base incubated in 5-10% CO_2 and 70% humidity.

Organisms (ATCC)	Growth	RGI
Haemophilus influenzae (19418)	Luxuriant	More than 70%
**Neisseria meningitidis* (13090)	Luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key:

*with antibiotic supplement

Procedure

1. Infectious material should be submitted directly to the laboratory protected from excessive heat and cold.
2. If there is to be delay in processing, the specimen should be inoculated onto an appropriate transport medium.
3. Specimens should be streaked on the surface of the plates so as to get some areas heavily seeded and other areas lightly seeded.
4. Incubate at 37°C in an atmosphere of 5-10% CO_2 and 70% humidity.

Precautions / Limitations

1. Avoid cotton wool for specimen collection.
2. Any suspected *Neisseria* containing specimen should be inoculated onto a primary isolation medium immediately on collection. If this is not possible, then *N.gonorrhoea* swabs are better held at 40°C for not more then 3 hours.
3. It is seen that the usual transport media are not totally reliable for *N.gonorrhoea*. Inoculation of the sample onto the surface of the medium slants is preferable.
4. Humidity is essential for the successful isolation of gonococci. If the plates look dry, moisten the surface with a few drops of sterile broth and allow it to soak into the agar before inoculation. Do not flood the plate with broth. Place damp gauze or paper towels in the CO_2 chamber before incubation.
5. Agar varies widely in their toxicity for *N.gonorrhoea* and may be a major factor in preventing the growth of gonococci on solid media.
6. Enrichments including haemoglobin and coenzymes must preferably be added to obtain a good growth of *Neisseria* species.
7. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely effect the growth and viability of the organisms.
8. Inactivation or deterioration of antibiotics in the selective medium may allow the growth of contaminants.
9. The medium has sufficient buffering capacity to offset the very low pH of the small amounts of nutritive enrichments added. However, the pH of some media may have to be adjusted with 1% NaOH after the addition of enrichments.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Glucose Agar AM50461

Use

Glucose agar is used for determining the fermentation reaction of presumptive *Enterobacteriaceae.*

Summary

Glucose agar is used for the differentiation of *Enterobacteriaceae* in urine, water and food. It differentiates species on the basis of glucose fermentation. Glucose agar is used for deep stab and shake cultures of anaerobes (77.2).

Principle

Tryptone provides carbon and nitrogen compounds glucose is the sole source of fermentable carbohydrate while sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Tryptone	10.0
Glucose	10.0
Sodium chloride	5.0

Yeast extract	1.5
Bromocresol purple	0.015
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 41.52 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured clear to slightly opalescent gel forms in petri plate.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	Colour of colony/Medium	RGI
Enterobacter aerogenes (13048)	Luxuriant	Yellow	More than 70%
Escherichia coli (25922)	Luxuriant	Yellow	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Colourless colony with no change in medium.	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Gelatin Broth AM504611

Use

Gelatin Broth is used for isolation and *enumeration* of *Bacillus cereus.*

Summary

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen. Collagens are a class of albuminiod found abundantly in bones, skin, tendon, cartilage and similar animal tissues.

Koch introduced gelatin into bacteriology when he invented the gelatin tube method in 1875 and the plate method in 1881.

Principle

The nitrogen, carbon, vitamins, and amino acids are provided by Yeast Extract for general growth requirements in Gelatin Broth. Gelatin is the substrate for determining if microorganisms elaborate the proteolytic enzyme to hydrolyze (liquefy) gelatin.

Formula*

Ingredients in grams per liter

Gelatin	30.0
Peptone	5.0
Yeast extract	5.0
Potassium dihydrogenphosphate	5.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 45.0 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance:

Dark yellow coloured clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth
Bacillus cereus (10876)	Luxuriant
Bacillus subtilis (6633)	Luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Glucose Broth AM1047/AM5047

Use

Glucose Broth is used in glucose fermentation studies where a pH indicator is not desired.

Summary

Waisbren, Carr and Dunnett used Glucose Broth for the study of glucose fermentation and for testing the sensitivity of microorganisms to antibiotics by the tube dilution method. This medium being highly nutritious, hastens the early recovery of injured cells. Glucose Broth is included in the Bacteriological Analytical Manual for food testing (113).

Principle

Tryptone provides carbon and nitrogen compounds, glucose is the sole source of fermentable carbohydrate while sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Glucose	5.0
Tryptone	10.0
Sodium Chloride	5.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 118°C (12 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	Gas
Escherichia coli (25922)	Luxuriant	+
Salmonella serotype Typhi (6539)	Luxuriant	-

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Glucose Salt Teepol Broth (Twin Pack) AM10471/AM50471

Use

Glucose Salt Teepol Broth is used for selective enrichment and enumeration of *Vibrio parahaemolyticus* from marine isolates in compliance with BIS specification IS: 5887.

Summary

Glucose Salt Teepol Broth is recommended by APHA for isolation of *Vibrio parahaemolyticus* from marine isolates (32.1). It is a selective enrichment medium for *Vibrio parahaemolyticus* that enables faster growth of the organism (40.1). Medium contains teepol, inhibits the migration of halophilic organisms and the growth of the Gram-positive organism while the organism utilizes glucose of the medium. It is also used to enumerate the bacteria by MPN technique. Glucose Salt Teepol Broth should be used as an enrichment broth in conjunction with TCBS Agar (AM1095/5095).

Principle

Peptic Digest of Animal Tissue supply nutrients, nitrogen compounds and amino acids. Beef extract also serves as an essential source of nitrogen. High percentage of chloride enhances the growth of halophilic *Vibrio parahaemolyticus*. It also provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium.

Formula*

Ingredients in grams per liter

Part A: Peptic digest of animal tissue	10.0
Beef extract	3.0
Sodium chloride	30.0
Glucose	5.0
Methyl violet	0.002
Part B: Teepol	4.0 ml

Final pH (at 25°C) 8.8 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Dissolve 48gms of Part A in 1000ml distilled water and add 4ml of Part B.
2. Mix thoroughly.
3. Heat gently to dissolve the medium completely. Do not boil.
4. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A: Light yellow coloured, homogeneous, free flowing powder.

Part B: Light yellow coloured viscous liquid.

Prepared Appearance

Purple colour clear solution with very slight precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35ºC.

Organisms (ATCC)	Growth
Vibrio parahaemolyticus (17802)	Good-luxuriant
Vibrio alginolyticus (17749)	Good-luxuriant

Procedure

1. Inoculate the Glucose Salt Teepol Broth with marine or other food specimens.
2. Mix well the inoculated medium.
3. Incubate at 35-37°C for 18-24 hours.
4. Transfer 0.1 ml of incubated broth to TCBS agar. Incubate at 35-37°C for 18-24 hours.

Interpretation of Results

Examine agar plates for suspect colonies. For further identification and confirmation of V. parahaemolyticus, consult appropriate references.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Glucose Yeast Extract Agar — AM1048/AM5048

Use

Glucose Yeast Extract Agar is used for enumeration of lactobacilli in pharmaceutical preparations.

Summary

Glucose Yeast Extract Agar was formulated by Evans and Niven (27) and Rogosa et al (95).

Principle

Glucose is the carbohydrate source. Peptone and yeast extract provides essential nutrients while salts like sulphates and phosphates support the growth of lactobacilli. The metallic salts are sources of ions essential for the replication of lactic acid bacteria.

Formula*

Ingredients in grams per liter

Yeast Extract	5.0
Peptone	5.0
Glucose	2.0
Dipotassium Phosphate	0.5
Monopotassium Phosphate	0.5
Magnesium Sulphate	0.3
Manganese Sulphate	0.01
Sodium Chloride	0.01
Cobalt Sulphate	0.0016
Copper Sulphate	0.0016
Zinc Sulphate	0.0016
Agar	15.0

* Formula adjusted to suit performance parameters

Directions

1. Suspend 28.4 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35°C.

Organisms (ATCC)	Growth	RGI
Lactobacillus casei (9595)	Good to luxuriant	More than 70%
Lactobacillus bulgaricus (11842)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Heart Infusion Agar — AM10481/AM50481

Heart Infusion Broth — AM10482/AM50482

Use

Heart Infusion Agar is a general-purpose medium used in the cultivation of a wide range of microorganisms from a variety of clinical and non-clinical specimens.

Heart Infusion Broth is used for cultivating fastidious microorganisms.

Summary

It has long been known that the growth of bacteria on culture media is facilitated by the addition of certain supplements, soluble in water, thermostable at 100°C and readily adsorbed from solution by filtration through paper or cotton. Huntoon demonstrated that pathogenic microorganisms could be grown on an infusion agar without supplements. Heart infusion media are specified for the isolation of *Vibrio cholerae* and *Vibrio* species.

Heart Infusion Agar is one such medium, which due to its nutritious composition is used as a basal medium for primary isolation of pathogenic organisms such as meningococci and pneumococci.

Heart Infusion Broth is a non-selective general-purpose medium used for the isolation of nutritionally fastidious microorganisms. Huntoon using fresh Beef Heart and Peptone prepared a "hormone" broth to retain growth-promoting substances. However, the formulation contains tryptose, which is better suited to the nutritional requirements of pathogenic bacteria than peptone.

Principle

Beef Heart Infusion and Tryptose provide nitrogenous compondounds, sulphur, vitamins, amino acids and trace ingredients. Sodium Chloride maintains the osmotic equilibrium. Agar is the solidifying agent. Heart Infusion broth can be modified by adding dextrose, 5% sheep blood or other ingredients to determine hemolytic reactions and many other purposes.

Formula*

Ingredients in grams per liter	Agar	Broth
Beef Heart, Infusion from	500.0	500.0
Tryptose	10.0	10.0
Sodium Chloride	5.0	5.0
Agar	15.0	--

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Heart Infusion Agar - 40 grams

   Heart Infusion Broth - 25 grams
2. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C.
5. Mix well and pour into sterile petriplates or tubes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured basal medium, cherry red coloured opaque gel.

Cultural Response

Cultural response after 18 - 48 hours at 35°C.

Organisms (ATCC)	Growth on HI Agar	Growth on HI Agar	Growth on HI Broth	Haemolysis With 5% sheep blood	RGI
Escherichia coli (25922)	Good to Luxuriant	Luxuriant	Luxuriant	Beta	More than70%
Neisseria meningitidis (13090)	Good to Luxuriant	Luxuriant	Luxuriant	None	More than 70%
Streptococcus pneumoniae (6303)	Good to Luxuriant	Good to Luxuriant	Luxuriant	Alpha	More than 70%
Streptococcus pyogenes (19615)	Good to Luxuriant	Good to Luxuriant	Luxuriant	Beta	More than 70%

Procedure

1. Obtain isolated colonies from specimens using standard procedures.
2. Since many pathogens require carbon dioxide on primary isolation, incubate plates in an atmosphere containing approximately 3-10% CO_2 at 35±2°C for 18-48 hours.
3. Examine plates for colonial morphology.

Interpretation of Results

1. Refer to U.S.P. and other appropriate references for interpretation of results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Hektoen Enteric Agar — AM104821/AM504821

Use

Hektoen Enteric Agar is used for isolation and differentiation of gram-negative enteric pathogens, particularly *Shigella* species from clinical and nonclinical specimens.

Summary

King and Metzger developed H E Agar at the Hektoen institute in Chicago for selective isolation of *Shigella* and other pathogenic species from clinical specimens(55.1). They found in their comparative study that H E medium was more superior than SS agar for recovery of Salmonella and *Shigella* species (55.2). The present formulation of H E agar has less amount of bile salt and deoxycholate is absent. High level of peptones and sugars reduce the inhibitory effect of bile salt and enable it to be moderately selective for Salmonella and

Shigella species. H E agar is suitable for isolation of *Salmonella* and *Shigella* species from food, clinical, dairy and other specimens.

Principle

Peptone and Yeast extract serve as a source of nitrogen. Bile salts act as a selective agent by inhibiting gram-positive and other than enteric organisms. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Salicin, Sucrose and Lactose provide differentiation of gram-negative enteric pathogens. Bromothymol blue and Acid fuchsin are acid-base indicator. The additions of ferric ammonium sulphate and sodium thiosulphate enable the detection of H_2S production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Proteose peptone	12.00
Yeast extract	3.00
Lactose	12.00
Sucrose	12.00
Salicin	2.00
Bile salts	9.00
Sodium chloride	5.00
Sodium thiosulfate	5.00
Ferric ammonium citrate	1.50
Bromthymol blue	0.065
Acid fuchsin	0.10
Agar	15.00

Final pH (at 25°C) 7.5 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 76.67 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. AVOID OVERHEATING. DO NOT AUTOCLAVE.
5. Cool the medium to approximately 45-50°C, pour in to sterile petriplates.

Quality Control

Dehydrated Appearance

Light greenish coloured, may have a slight green cast, homogeneous, free flowing powder.

Prepared Appearance

Green coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	Colour of colony	RGI
S. serotype Enteritidis (13076)	Luxuriant	Greenish blue with black center	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Greenish blue to green with black center	More than 70%
S. Choleraesuis (12011)	Luxuriant	Greenish blue colonies with black center	More than 70%
Shigella flexneri (12022)	Luxuriant	Green to blue-green	More than 70%
Enterobacter aerogenes (13048)	fair to Good	Salmon orange to yellowish orange	More than 70%
Enterococcus faecalis (29212)	Inhibited	-	0%
Escherichia coli (25922)	fair	Orange (may have bile precipitate)	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small area of the agar surface and streak for isolation with a sterile loop.
3. Incubate plates aerobically, protected from light, at 35-37°C for 18-24 hours.
4. If negative, incubate for an additional 24 hours.
5. Examine colony morphology.
6. **Note:** A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Interpretation of Results

Typical colonial morphology on H E Agar

E. coli	Large, yellow to salmon color; some strains may be inhibited
Salmonella	Blue-green to blue; most strains with black center
Enterobacter/Klebsiella	Large, yellow to salmon color
Shigella	Green and moist, raised
Proteus	Variable, blue-green to blue or salmon, most strains with black center
Pseudomonas	Irregular, green to brown
Gram-positive bacteria	No growth to slight growth

Limitations

1. Colonies of proteus may resemble Salmonella or Shigella.
2. It is preferable that biochemical and / or serological tests be performed on colonies from pure culture for complete identification.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Hottinger Broth AM104822

Use

Hottinger Broth is used for cultivation of less fastidious microorganisms and determination of indole production.

Summary

Hottinger Broth is recommended for the determination of indole production. Indole is a component of amino acid tryptophan. Some bacteria have the ability to breakdown tryptophan for nutritional needs using the enzyme tryptophanase and indole is produced as end product.

Principle

Fish peptone and yeast extract provides the essential nutrients. Tryptophan present in the medium is utilized by some of the bacteria and produced indole.

Formula*

Ingredients in grams per liter

Fish peptone	20.00
Tryptophan	1.00
Yeast extract	2.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23 gms in 1000 ml distilled water. Soak for 5 minutes.
2. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics observed after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Indole
Escherichia coli (25922)	Good	+
Pseudomonas aeruginosa (27853)	Good	-
Staphylococcus aureus (25923)	Good	-
Streptococcus pyogenes (19615)	Good	-

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Hoyle Medium Base AM104823/AM504823

Use

Hoyle Medium Base is used for the selective isolation and differentiation of Corynebacterium diphtheriae.

Summary

Hoyle Medium is the modification (45.2) of Neill's medium for the cultural isolation and differentiation of Corynebacterium diphtheriaetypes. Hoyle medium does not exert the inhibitory effect manifested by Neill's on some mitis types, but supports very rapid growth with all types of Corynebacterium diphtheriae, so that diagnosis is possible after 18 hours incubation.

Principle

Peptic digest of animal tissue and beef extract supply nutrients, nitrogen compounds and amino acids. Potassium tellurite is a selective agent which inhibits most of the normal flora of the upper respiratory tract except Corynebacterium.chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.0
Beef extract	10.0
Sodium chloride	5.0
Agar	15.0

Final pH (at 25°C) 7.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 40 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and aseptically add 50 ml of laked blood and 10 ml of

3.5% Potassium Tellurite Solution (AS023).

6. Mix thoroughly, but gently and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium yields amber coloured gel.

With addition of laked blood and tellurite, brownish red coloured, opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colony Characteristics	RGI
Corynebacterium diphtheriae type intermedius (11913)	Good to Luxuriant	Grey colonies with Darker centers.	More than 70%
Corynebacterium diphtheriae type intermedius (14779)	Good to Luxuriant	Grey colonies with Darker centers.	More than 70%
Enterococcus faecalis (29212)	Good to Luxuriant	black minute colonies	More than 70%
Escherichia coli (25922)	Inhibited	-	0%

Note: It should be noted that not all corynebacteriaproduce the typical colonies described above - so in all cases it is advisable to use Hoyle medium in conjunction with the non-selective media such as Loeffler Medium Base (AM1056/5056) and Blood Agar Base (AM 1014/5014).

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Inactivator Broth (Twin Pack) AM104824/AM504824

Use

This media is recommended for the detection and isolation of microbial contamination present on clean surfaces in environmentally controlled areas and accidentally contaminated raw material samples of pharmaceutical formulations.

Summary

This media may be employed to establish and monitor cleaning techniques in 'clean' rooms. This media is also used for detection and isolation of microorganisms present in raw material samples of pharmaceutical formulations which may have accidentally contaminated by various disinfectants.

Principle

Casein and Soya Peptone are sources of carbon and nitrogen. Dextrose is a source of fermentable Carbohydrate. Sodium Chloride maintains the osmotic equilibrium while mono and dipotassium phosphates are the buffers. Lecithin and Tween 80 inactivate many residual disinfectants. Lecithin neutralizes quaternary Ammonium compounds and Tween 80 neutralizes Phenols, hexachlorophene & formalin. Cysteine acts as detoxicant, it neutralizes toxic chemicals.

Formula*

Part A

Ingredients Gms/lit

Pancreatic digest of casein	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	1.25
Potassium dihydrogen phosphate	1.25
Dextrose	2.5
Soya lecithin	3.0
Histidine	1.0
Cysteine	1.0
Part B	
Tween 80	30 ml.

Final pH (at 25°C) 7.3 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35gms of Powder (Twin Pack A) in 970 ml distilled water.
2. Add 30 ml of Tween 80 (Twin Pack B)
3. Heat with frequent agitation to dissolve the powder completely
4. Dispense the media in tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A-Yellow coloured, homogeneous, free flowing powder.

Part B- Yellowish viscous liquid.

Prepared Appearance

Yellow coloured slight opalescent solution.

Cultural Response

Cultural characteristics observed after an incubation of 24-48 hrs at 35-37°C for bacteria and 48-72 hrs at 25-30°C for fungi.

Organisms(ATCC)	Growth
Staphylococcus aureus (25923)	Good
Bacillus subtillis (6633)	Good
Streptococcus pyogenes (19615)	Good
Bacteroides vulgatus (8482)	Good
Candida albicans (10231)	Good

Pseudomonas aeruginosa (9027)	Good
Aspergillus niger (16404)	Good

Procedure

1) If specimen is being cultured from a swab (rolled on clean surface), dip the swab directly in the medium.
2) Incubate tubes at 37°C ± 2°C for 48 hrs and 25°C-30°C for 72 hrs.
3) When incubation has been completed check for growth.

Interpretation of Results

Growth in the broth is indicated by the presence of turbidity compared to an uninoculated control.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Iron Sulphite Agar AM10483/AM50483

Use

Iron Sulphite Agar is recommended for the detection of thermophilic anaerobic organisms causing sulphide spoilage in foods.

Summary

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America. The medium was modified and improved by reducing the concentration of sodium sulphite. But Beerens showed that some 0.1% sulphite concentration was inhibitory to some strains of *Clostridium sporogenes*. This observation was later confirmed by Mossel et. al. who showed that a concentration of 0.05% sulphite was not inhibitory to the organisms.

Principle

Mossel et al. (1956) and Mossel (1959), reported an iron-sulfite agar medium containing 0.05 % sodium sulfite which yielded quantitative recovery of pure cultures of several species of clostridia in Miller-Prickett tubes. Iron Sulphite Agar is particularly suitable for the detection of thermophilic anaerobic organisms causing sulphide spoilage in foods. For detection of such organisms, two methods can be used: (a) Deep-Shake Culture Method and (b) Attenborough and Scarr Method.

Formula*

Ingredients in grams per liter

Tryptone	10.0
Sodium Sulphite	0.5
Iron (III) Citrate	0.5
Agar	15.0

Final pH (at 25° C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 26 grams in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, slightly opalescent gel forms in petriplates / tubes.

Cultural Response

Cultural response after 24 - 78 hours at 55 - 56° C under anerobic conditions.

Organisms (ATCC)	Growth	Colour of colonies	RGI
Clostridium sporogenes (19404)	Luxuriant	Black	More than 70%
Clostridium botulinum (25763)	Luxuriant	Black	More than 70%
Desulfotomaculum nigrificans (19858)	Luxuriant	Black	More than 70%
Escherichia coli (25922)	Good	No blackening	More than 70%

Procedure

Deep-Shake Culture Method

1. Dispense the medium in 10 ml amounts in tubes.
2. Inoculate the sample on the medium when it is about 50°C.
3. Allow to set and incubate at 55°C for 24-48 hours.

Attenborough and Scarr Method

1. Filter diluted samples of sugar or any other food particles though membrane filters.
2. Roll up the filters and place in tubes containing melted Iron Sulphite Agar sufficient enough to cover them at 50°C.
3. Allow the medium to set and incubate at 55-56°C for 24-48 hours.

Interpretation of Results

Deep-Shake Culture Method

1. Desulfotomaculum nigrificans, a typical thermophilic species, produces distinct black spherical colonies in the depth of the medium.

Attenborough and Scarr Method

1. After incubation, count the number of black colonies formed.

Precautions / Limitations

1. A number of thermophilic anaerobic organisms like Proteus and Salmonella grow on this medium and must be confirmed by additional biochemical tests.
2. The membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Iron Sulphite Agar ISO AM504831

Use

Iron Sulphite Agar is recommended for the detection of thermophilic anaerobic organisms causing sulphide spoilage in foods in compliance with ISO.

Summary

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America. The medium was modified and improved by reducing the concentration of sodium sulphite. But Beerens showed that some 0.1% sulphite concentration was inhibitory to some strains of *Clostridium sporogenes*. This observation was later confirmed by Mossel et al., who showed that a concentration of 0.05% sulphite was not inhibitory to the organisms.

Principle

Mossel et al., (1956) and Mossel (1959), reported an iron-sulfite agar medium containing 0.05 % sodium sulfite which yielded quantitative recovery of pure cultures of several species of clostridia in Miller- Prickett tubes. Iron Sulphite Agar is particularly suitable for the detection of thermophilic anaerobic organisms causing sulphide spoilage in foods. For detection of such organisms, two methods can be used: (a) Deep-Shake Culture Method and (b) Attenborough and Scarr Method.

Formula*

Ingredients in grams per liter

Enzymatic digest of casein	15.0
Pancreatic digest of soya	5.0
Yeast extract	5.0
Disodium disulfite	1.0
Iron (III) ammonium citrate	1.0
Agar	12.0

Final pH (at 25°C) 7.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 39 grams in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, slightly opalescent gel forms in petriplates.

Cultural Response

Cultural response after 24 - 78 hours at 55 - 56°C under anerobic conditions.

Organisms (ATCC)	Growth	Colour of colonies	RGI
Clostridium sporogenes (19404)	Luxuriant	Black	More than 70%
Clostridium botulinum (25763)	Luxuriant	Black	More than 70%
Desulfotomaculum nigrificans (19858)	Luxuriant	Bblack	More than 70%
Escherichia coli (25922)	Good	No blackening	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Deep-Shake Culture Method

1. Dispense the medium in 10 ml amounts in tubes.
2. Inoculate the sample on the medium when it is about 50°C.
3. Allow to set and incubate at 55°C for 24-48 hours.

Attenborough and Scarr Method

1. Filter diluted samples of sugar or any other food particles though membrane filters.
2. Roll up the filters and place in tubes containing melted Iron Sulphite Agar sufficient enough to cover them at 50°C.
3. Allow the medium to set and incubate at 55-56°C for 24-48 hours.

Interpretation of Results

Deep-Shake Culture Method

1. Desulfotomaculum nigrificans, a typical thermophilic species, produces distinct black spherical colonies in the depth of the medium.

Attenborough and Scarr Method

1. After incubation, count the number of black colonies formed.

Precautions / Limitations

1. A number of thermophilic anaerobic organisms like Proteus and Salmonella grow on this medium and must be confirmed by additional biochemical tests.
2. The membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

IUT Medium Base — AM10484/AM50484

Use

IUT Medium Base with added glycerol and egg yolk emulsion is used for cultivation of *Mycobacterium tuberculosis.*

Summary

IUT Medium is recommended by the International Union Against Tuberculosis for the diagnosis of Mycobacterial infections (46.3). It differs from Lowenstein-Jensen medium since it does not contain potato flour/starch. It is reported to be giving higher proportion of positives. This medium supports rapid and luxuriant growth of primary cultures.

Principle

Malachite green inhibits the growth of other bacteria and also acts as a pH indicator. L-Asparagine serves as a source of nutrients. Inorganic salts provide ions for the metabolism of Mycobacteria.

Formula*

Ingredients Gms/600 ml	
L-Asparagine	3.60
Monpotassium phosphate	2.46
Mgnesium sulphate	0.24
Magnesium citrate	0.60
Malachite green	0.40

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 7.3gms of Powder in 600 ml distilled water containing 12 ml glycerol.
2. If necessary, heat to dissolve the medium completely
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 50°C and aseptically add 1 liter of sterile whole egg emulsion. Prepared under aseptic conditions.
5. Mix well and dispense in screw capped containers. Sterilize by inspissation at 85°C for 1 hour.

Quality Control

Dehydrated Appearance

Peacock blue coloured, homogeneous, free flowing powder.

Prepared Appearance

Pale blue coloured opaque smooth slants.

Cultural Response

Cultural characteristics observed after 2-4 weeks at 35°C.

Organisms (ATCC)	Growth
Mycobacterium tuberculosis H37RV (25618)	Luxuriant
Mycobacterium smegmatis (14468)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Jensen's Broth — AM50485

Use

Jensen's Broth is recommended for detection and cultivation of nitrogen fixing bacteria.

Summary

Nitrogen fixing bacteria are capable of taking gaseous nitrogen and combining it with hydrogen to make ammonia. The plant for growth can use fixed nitrogen. Thus nitrogen-fixing bacteria increases the soil productivity. To isolate the nitrogen fixing bacteria nitrogen free Jensen's medium has been formulated and used (115.2).

Principle

Sucrose serves as energy source. Different salts support the bacterial growth. Sodium chloride maintains the osmotic of the medium. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter	
Sucrose	20.0
Calcium carbonate	2.0
Dipotassium phosphate	1.0
Sodium chloride	0.50

Ferrous sulphate	0.10
Sodium molybdate	0.005
Magnesium Sulphite	0.50

Final pH (at 25°C) 7.5± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.1 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Cream coloured, clear to slightly opalescent with a slightly precipitated gel forms in petri plates.

Cultural Response

Cultural characteristics after 7 days at 30°C.

Organisms(ATCC)	Growth
Rhizobium leguminosarum (10004)	Luxuriant
Rhizobium meliloti (9930)	Luxuriant

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Jensen's Medium — AM10486/AM50486

Use

Jensen's Medium is recommended for detection and cultivation of nitrogen fixing bacteria.

Summary

Nitrogen fixing bacteria are capable of taking gaseous nitrogen and combining it with hydrogen to make ammonia. The plant for growth can use fixed nitrogen. Thus nitrogen-fixing bacteria increases the soil productivity. To isolate the nitrogen fixing bacteria nitrogen free Jensen's medium has been formulated and used (115.2).

Principle

Sucrose serves as energy source. Different salts support the bacterial growth. Sodium chloride maintains the osmotic of the medium. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Sucrose	20.0
Calcium carbonate	2.0
Dipotassium phosphate	1.0
Sodium chloride	0.50
Ferrous sulphate	0.10
Sodium molybdate	0.005
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 39 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Cream coloured, clear to slightly opalescent with a slightly precipitated gel forms in petri plates.

Cultural Response

Cultural characteristics after 7 days at 30°C.

Organisms (ATCC)	Growth
Rhizobium leguminosarum (10004)	Luxuriant
Rhizobium meliloti (9930)	Luxuriant

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Karmali Campylobacter Agar Base AM1049/AM5049

Use

Karmali Campylobacter Agar Base is a blood free medium for selective isolation of thermotolerant *Campylobacter* species from clinical and non-clinical specimens.

Summary

Karmali et al (50) formulated Karmali Campylobacter Agar Base, a highly nutritious basal medium, which after the addition of antimicrobial agents, is used for the selective isolation of *Campylobacter* species from fecal specimens, food and environmental specimens. The addition of antimicrobial agents suppresses the growth of normal enteric flora.

Principle

Campylobacter species, which are microaerophilic, are inhibited by the normal atmospheric oxygen levels as microaerophilic bacteria are more sensitive to toxic forms of oxygen (super oxide, peroxide) that occur in aerobic culture media. Special peptone and corn starch provide essential vitamins. The selective supplement contains hemin, which provides essential nutrients, sodium pyruvate, which enhances the aero tolerance of microaerophilic bacteria by quenching the toxic forms of oxygen, vancomycin, which suppresses gram-positive organisms, amphotericin B, which inhibits yeasts, and cefoperazone, which inhibits gram-negative organisms other than *Campylobacter.*

Formula*

Ingredients in grams per liter

Peptone Special	23.0
Sodium Chloride	5.0
Charcoal Activated	4.0
Corn Starch	1.0
Agar	12.0

pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 22.5 gms of the powder in 490 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 40-50°C and aseptically add 1 vial of Campylobacter Selective Supplement with Hemin, Karmali Modified (AS007).
6. Mix well before pouring into sterile petri plates.

Quality Control

Dehydrated Appearance

Greyish black coloured free flowing, homogeneous powder.

Prepared Appearance

Black coloured opalescent gel.

Cultural Response

Cultural characteristics after 42-48 hours at 42°C.

Organisms (ATCC)	Growth	RGI
Campylobacter jejuni (29428)	Good to luxuriant	More than 70%
Escherichia coli (25922)	None to poor	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. If immediate inoculation of Karmali Campylobacter Agar Base cannot be performed, the use of a suitable holding medium is recommended.
3. Incubate inoculated plates at 42°C for 42-48 hours.

Precautions / Limitations

1. Since *C.jejuni* is thermophilic, it is important to incubate the plates at 42°C; otherwise, growth will be delayed. The higher temperature also improves selectivity by inhibiting the normal flora.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

King's Medium A Base AM50491

Use

King's Medium A Base is used for isolation, cultivation and pigment production of *Pseudomonas* species.

Summary

King's Medium A Base is recommended for *Pseudomonas* species. King first formulated this medium (33.1).

Principle

Proteose peptone supplies the essential carbon and nitrogen source to the medium. Metallic salts are the source of ions, which support the growth of the bacteria. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Proteose peptone	20.0

Potassium sulphate	10.0
Magnesium chloride, Anhydrous	1.64
Agar	15.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 46.64 gms of powder in 1000ml distilled water.
2. Add 10 ml of glycerol and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured clear gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Pseudomonas aeruginosa (17934)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

King's Medium B Base — AM50492

Use

King's Medium B Base is used for isolation, cultivation and pigment production of *Pseudomonas* species.

Summary

King's Medium B Base is recommended for *Pseudomonas* species. King first formulated this medium (33.1).

Principle

Proteose peptone provides the essential carbon and nitrogen. Dipotassium hydrogen phosphate acts as a buffering agent. Metallic ion supports the growth of bacteria.

Formula*

Ingredients in grams per liter

Proteose peptone No. 3	20.0
Magnesium Sulphate $7H_2O$	1.5
Dipotassium hydrogen phosphate	1.5
Agar	20.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 43 gms of powder in 1000ml of distilled water.
2. Add 15 ml of glycerol and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Pseudomonas aeruginosa (27853)	Luxuriant

Procedure

Refer to appropriate references for specific procedures

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Kirschner Medium Base, Modified — AM50493

Use

Kirschner Medium Base with added glycerol and enrichment is used for cultivation of *Mycobacterium tuberculosis.*

Summary

Kirschner Medium was first developed by Kirchner based on the formulation of Long's Medium (4.1) and further modified with addition of glycerol and

enrichments for the cultivation of *Mycobacterium tuberculosis*. It is agents and some time in differential culture of *Mycobacterium tuberculosis* from unhealthy materials.

Principle

Krichner medium contains two phosphates, a sulphate and citrate which buffers the medium well. Hence direct inoculum can be adopted without any neutralization. L-asparagine in the medium supports the growth of *Mycobacterium tuberculosis* as it is a good nutrient for the organism. Horse serum also promotes the growth of the organism. Penicillin inhibits the growth of contaminating bacteria.

Formula *

Ingredients in grams per liter

Disodium phosphate	3.00
Monopotassium phosphate	4.00
Magnesium sulphate	0.60
Sodium citrate	2.50
L-Asparagine	5.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15.1 gms of powder in 700 ml of distilled water. Add 200 ml glycerol.
2. Heat to boiling to dissolve the powder completely.
3. Dispense in 9 ml aliquotes.
4. Sterilize by autoclaving at 115°C (10lbs pressure) for 15 minutes.
5. Just before use, aseptically add 1 ml of Horse serum and 100 IU Penicillin per 9 ml medium.

Quality Control

Dehydrated Appearance

White coloured, homogeneous free flowing powder.

Prepared Appearance

Colourless solution having slight white precipitate at the bottom..

Cultural Response

Cultural characteristics after 2-4 weeks at 35-37°C.

Organisms (ATCC)	Growth
Mycobacterium tuberculosis H37 RV (25618)	Luxuriant
Mycobacterium smegmatis (14468)	Luxuriant
Mycobacterium fortuitum (6841)	Luxuriant

Procedure

Refer to appropriate references for specific procedures

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Kligler Iron Agar — AM1050/AM5050

Use

Kligler Iron Agar is a differential medium used for the differentiation of members of *Enterobacteriaceae* on the basis of their ability to ferment dextrose and lactose and to produce hydrogen sulphide.

Summary

Russel (97) described a double sugar tubed medium for the isolation of typhoid bacilli from urine and faeces. Some years later, Kligler (57) developed a simple lead acetate medium for the differentiation of typhoid-paratyphoid group. Subsequently, he evaluated culture media used in the isolation of typhoid, dysentery and allied bacilli. Bailey and Lacey substituted phenol red for the Andrade indicator previously used in the formulation, enabling the differentiation of gram-negative bacilli based on their ability to ferment dextrose, lactose and hydrogen sulphide production. Kligler Iron Agar is recommended by APHA for the examination of foods (20).

Principle

Tryptone and peptone provide essential growth nutrients. Yeast extract is a source of B group vitamins while sodium chloride maintains the osmotic balance. Lactose and dextrose enable the differentiation of enteric bacilli due to change in colour of the phenol red pH indicator in response to the acid produced during the fermentation of these sugars. Ferrous sulphate and sodium thiosulphate enable the detection of hydrogen sulphide production. Fermentation of dextrose is indicated by yellow butt and that of lactose by yellow slant.

Non-lactose fermenters (*Salmonella and Shigella*) initially produce acid (yellow slant) as a result of dextrose fermentation. The concentration of dextrose being very small is rapidly exhausted. Once the dextrose is depleted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt where an acidic environment is maintained. Lactose fermenting organisms produce yellow slants and butts. Organisms incapable of fermenting either of the carbohydrates produce red slants and butts. H_2S production results in the blackening of the medium, either throughout the butt or in a ring formation near the top of the butt. Gas production is demonstrated by the presence of bubbles or cracks in the medium.

Formula*

Ingredients in grams per liter

Peptone	15.0
Yeast Extract	3.0
Beef Extract	3.0
Proteose Peptone	5.0
Sodium Chloride	5.0
Lactose	10.0
Dextrose	1.0
Sodium Thiosulphate	0.3
Ferrous Sulphate	0.2
Phenol Red	0.024
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 57.5 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Mix well and distribute into tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Allow to set as slopes with 1 inch butts.
6. Best reactions are obtained on freshly prepared medium.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Escherichia coli (25922)	Luxuriant	Acid	Acid	+	-
Enterobacter aerogenes (13048)	Luxuriant	Acid	Acid	+	-
Klebsiella pneumoniae (13883)	Luxuriant	Acid	Acid	+	-
Pseudomanas aeruginosa (9027)	Luxuriant	Alkaline	Alkaline	-	-
Salmonella serotype Enteritidis (13076)	Luxuriant	Alkaline	Acid	+	+
Salmonella serotype Typhimurium (6539)	Luxuriant	Alkaline	Acid	-	+
Shigella flexneri (12022)	Luxuriant	Alkaline	Acid	-	-

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Touch the center of an isolated colony with an inoculating needle.
3. Stab the center of the medium into the deep of the butt to within 3-5 mm of the bottom.
4. Withdraw the inoculating needle, and streak the surface of the slant.
5. Incubate tubes with loosened caps for 18-24 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

1. An alkaline slant-acid butt (red / yellow) indicates fermentation of dextrose only.
2. An acid slant-acid butt (yellow / yellow) indicates fermentation of both dextrose and lactose.
3. An alkaline slant-alkaline butt (red / red) indicates that neither dextrose nor lactose was fermented.
4. Cracks, splits or bubbles in the medium indicate gas production.
5. A black precipitate in the butt indicates H_2S production.

Typical reactions produced by members of the *Enterobacteriaceae* are

	Slant	Butt	Gas	H_2S
Citrobacter	Alkaline	Acid	+	±
Edwardsiella	Alkaline	Acid	+	+
E. coli	Acid	Acid	+	-
Enterobacter	Acid (may revert to alkaline even though lactose fermented- *E. aerogenes*)	Acid	+	-
Morganella	Alkaline	Acid	±	-
Proteus	Alkaline or acid	Acid	+	+
Providencia	Alkaline	Acid	±	-
Salmonella	Alkaline	Acid	+	+
Shigella	Alkaline	Acid	-	-

Precautions / Limitations

1. It is essential that Kligler Iron Agar be examined and reported after 18-24 hours. Early or late readings may give false results.
2. Kligler Iron Agar will grow both oxidative and fermentative organisms and care should be taken to distinguish between the two groups.
3. It is essential that air is freely available for growth on the slant and therefore do not use screw cap bottles or tubes for testing.
4. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
5. It is important to stab the butt of the medium but the integrity of the medium must be maintained while stabbing. Use a straight wire for inoculation to avoid splitting the agar.
6. An organism that produces H_2S may mask acid production in the butt of the medium. However, H_2S production requires an acid environment, thus the butt portion should be considered acidic if H_2S is produced.
7. Certain species of organisms may give delayed reactions or completely fail to

ferment the carbohydrate. However, in most cases, if the organism fails to ferment dextrose within 48 hours and growth is definitely present, the organism, most likely does not belong to the family *Enterobacteriaceae*.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Kligler Iron Agar ISO — AM50501

Use

Kligler Iron Agar ISO is a differential medium recommended for identification of *Pseudomonas* species and members of *Enterobacteriaceae* on the basis of their ability to ferment dextrose and lactose and to produce hydrogen sulphide in compliance with ISO specification ISO/DIS/13720:1995.

Summary

Kligler Iron agar ISO is the modification of Kligler medium. ISO committee has recommenced for identification of *Pseudomonas* species.

Principle

Casein and meat peptones provide essential growth nutrients. Yeast extract is a good source of B vitamins. Lactose and dextrose are the fermentable sugars which enable the differentiation of species due to the colour changes of phenol red – the pH indicator. Sodium thiosulphate accelerate H_2S production which is evidenced by a black colour either throughout the butt or in a ring formation near the top of butt. Gas production is detected as individual bubbles or splitting or displacement of agar. Fermentation of dextrose is indicated by yellow butt and that of lactose by yellow slant and H_2S production is indicated by blacking in the butt.

Formula*

Ingredients in grams per liter

Beefextract	3.00
Yest extract	3.00
Casein enzymic hydrolysate	20.00
Sodium chloride	5.00
Lactose	10.00
Glucose anhydrous	1.00
Ferrous ammonium sulphate, $6H_2O$	0.50
Sodium thiosulphate, pentahydrate	0.50
Phenol red	0.025
Agar	15.00

Final pH (at 25°C) 7.4 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 57.7 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil to dissolve the powder completely.
4. Dispense in tubes and sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool in slanted position for use as slants.
6. Best reactions are obtained on freshly prepared media.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to slightly opalescent gel form as slants in tubes.

Cultural Response

Cultural characteristics after 24-48 hours at 37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Escherichia coli (25922)	Luxuriant	A	A	+	–
Enterobacter aerogenes (13047)	Luxuriant	A	A	+	–
Protus vulgaris (13315)	Luxuriant	K	A	–	+
S. serotype Typhi (6539)	Luxuriant	K	A	–	+
S. serotype Enteridis (13076)	Luxuriant	K	A	+	+
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	–
Shigella flexneri (12022)	Luxuriant	K	A	–	–
Pseudomonas aeruginosa (27853)	Luxuriant	K	K	–	–

Key: A = acid production (yellow)
K= alkaline reaction
+= positive or blackening

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactobacillus MRS Agar — AM1051/AM5051

Use

Lactobacillus MRS Agar is used for the isolation, enumeration and cultivation of all *Lactobacillus* species.

Summary

Lactobacillus MRS Agar is based on the formulation of deMann, Rogosa and Sharpe (19) with slight modification. It supports luxuriant growth of *lactobacilli* from oral cavity, faeces, foods (20) and dairy products (39).

Principle

Proteose peptone and beef extract provide carbon and nitrogen compounds. Yeast extract provides vitamin B complex while dextrose is the fermentable carbohydrate. Polysorbate 80, sodium acetate, magnesium sulphate and manganese sulphate provide growth factors. Sodium acetate and ammonium citrate inhibits *streptococci*, moulds and many other microorganisms. Dipotassium phosphate is the buffer.

Formula*

Ingredients in grams per liter

Dextrose	20.0
Beef Extract	10.0
Proteose Peptone	10.0
Yeast Extract	5.0
Sodium Acetate	5.0
Ammonium Citrate	2.0
Dipotassium Phosphate	2.0
Polysorbate 80	1.0
Magnesium Sulphate	0.1
Manganese Sulphate	0.05
Agar	12.0

Final pH (at 25°C) 6.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 67.15 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics at 18-24 hours or longer at 35°C.

Organisms (ATCC)	Growth	RGI
Lactobacillus leichmanni (7830)	Luxuriant	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	More than 70%
Lactobacillus fermentum (9338)	Luxuriant	More than 70%

Procedure

1. For quantitative test follow the pour plate method using 1 ml volumes of diluted test sample.
2. Alternatively, the streak plate method can be used for recovery of organisms.
3. Incubate the plates at 35°C for 3 days, or at 30°C for 5 days in an aerobic atmosphere supplemented with carbon dioxide.

Interpretation of results

1. *Lactobacilli* appear as large, white colonies in or on the surface of the medium.
2. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. Organisms other than *lactobacilli* may grow in these media and therefore isolates must be confirmed as *lactobacilli* by appropriate biochemical testing.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactobacillus MRS Agar ISO — AM50511

Use

Lactobacillus MRS Agar is used for isolation and enumeration of lactic acid bacteria from meat and meat products in compliance with ISO.

Summary

Lactobacillus MRS media are based on the formulation of deMan, Rogosa and sharpe with slight modification. It support luxuriant growth of all *Lactobacilli* from oral cavity, dairy products, foods faeces and other sources.

Principles

Proteose peptone and beef extract supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of *Lactobacilli*. Sodium acetate and ammonium citrate inhibit *streptococci*, moulds and many other microorganisms.

Formula*

Dextrose	20.0
Beef extract	10.0
Proteose peptone	10.0
Yeast extract	5.0
Sodium acetate	5.0
Ammonium citrate	2.0
Dipotassium phosphate	2.0
Polysorbate 80	1.0
Magnesium sulphate	0.1
Manganese sulphate	0.05
Agar	12.0

Final pH (at 25°C) 6.5 ± 0.2
* Formula adjusted to suit performance parameters

Directions

1. Suspend 67.15 gms of powder in 1000 ml of distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium to amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours or longer at 35°C.

Organisms (ATCC)	Growth	RGI
Lactobacillus fermentum (9338)	Luxuriant	More than 70%
Lactobacillus leichmanii (7830)	Luxuriant	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 2- 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactobacillus MRS Broth — AM10512/AM50512

Use

Lactobacillus MRS Broth is used for isolation and enumeration and cultivation of all *Lactobacillus* species.

Summary

Lactobacillus MRS Broth is based on the formulation of deMann, Rogosa and Sharpe with slight modification. It supports luxuriant growth of lactobacilli from oral cavity, faeces, foods and dairy products.

Principle

Proteose peptone and beef extract provide carbon and nitrogen compounds. Yeast extract provides vitamin B complex while dextrose is the fermentable carbohydrate. Polysorbate 80, sodium acetate, magnesium sulphate and manganese sulphate provide growth factors. Sodium acetate and ammonium citrate inhibits *streptococci,* moulds and many other microorganisms. Dipotassium phosphate is the buffer.

Formula*

Ingredients in grams per liter

Dextrose	20.0
Beef extract	10.0
Proteose peptone	10.0
Yeast extract	5.0
Sodium acetate	5.0
Ammonium citrate	2.0
Dipotassium phosphate	2.0
Polysorbate 80	1.0
Magnesium sulphate	0.1
Manganese sulphate	0.05

Final pH (at 25°C) 6.5 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 55.15 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium amber coloured, clear to slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics at 18-24 hours or longer at 35°C.

Organisms (ATCC)	Growth
Lactobacillus leichmanni (7830)	Luxuriant
Lactobacillus plantarum (8014)	Luxuriant
Lactobacillus fermentum (9338)	Luxuriant

Procedure

1. For quantitative test follow the pour plate method using 1 ml volumes of diluted test sample.
2. Alternatively, the streak plate method can be used for recovery of organisms.
3. Incubate the plates at 35°C for 3 days, or at 30°C for 5 days in an aerobic atmosphere supplemented with carbon dioxide.

Interpretation of results

1. Lactobacilli appear as large, white colonies in or on the surface of the medium.

2. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

Organisms other than lactobacilli may grow in these media and therefore isolates must be confirmed as lactobacilli by appropriate biochemical testing.

Storage

Store at 2- 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactobacillus Selection agar AM50513

Use

Lactobacillus Selection Agar Base is recommended for isolation and enumeration of *Lactobacilli* from foods.

Summary

Lactobacillus Selection Agar is used for isolation and enumeration of *Lactobacilli*. Rogosa et al (95, 95.1) developed LBS Agar as a selective medium for isolation and enumeration of *Lactobacilli* from oral, faecal specimens (24.2), food (103.4) and dairy products (91.2). Lactobacillus Selection Medium was demonstrated to be more suitable for growth of *lactobacilli* than Tomato Juice Medium traditionally used to isolate *lactobacilli*. *Lactobacilli* Selection Media can be further enriched by addition of tomato juice (100.1).

Principle

Casein enzymic hydrolysate, yeast extract and dextrose are the nitrogen and carbon sources. Polysorbate 80 provides fatty acids required for the metabolism of *Lactobacilli*. Selectivity of the medium is obtained due to the presence of ammonium citrate and sodium acetate. Addition of acetic acid lowers the pH which is inhibitory to many microorganisms but favours the growth of *Lactobacilli*.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	10.00
Yeast extract	5.00
Glucose	20.00
Sodium acetate	25.00
Monopotassium hydrogen phosphate	6.00
Ammonium citrate	2.00
Polysorbate 80	1.00
Magnesium sulphate	0.575
Manganese sulphate	0.12
Ferrous sulphate	0.034
Agar	15.00

Final pH (at 25°C) 5.5±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 84.7 grams in 1000 ml distilled water containing 1.32 ml glacial acetic acid.
2. Heat with frequent stirring.
3. Boil for 1-2 minutes to dissolve the medium completely. DO NOT AUTOCLAVE.
4. If storage is necessary, autoclave at 12 lbs pressure (118°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Yellow coloured slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 48 hours at 35-37°C.

Organisms (ATCC)	Growth	Growth	RGI Recovery
Enterococcus faecalis (29212)	Inhibited	0%	0%
Lactobacillus acidophilus (4356)	Luxuriant	>=50%	More than 70%
Lactobacillus casei (9595)	Luxuriant	>=50%	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	>=50%	More than 70%
Proteus vulgaris (13315)	Inhibited	0%	0%
Staphylococcus aureus (25923)	Inhibited	0%	0%
Escherichia coli (25922)	Inhibited	0%	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactose Broth AM1052/AM5052

Lactose Broth IP AM10521/AM50521

Lactose Broth BIS AM10524/AM50524

Use

Lactose Broth is used for the detection of coliforms, as a pre-enrichment broth for salmonellae and in the study of lactose fermentation in general.

Summary

Lactose Broth is recommended by APHA in the performance and confirmation of the presumptive test for coliform bacteria in milk (39). This medium is recommended in the Compendium of Methods for the Microbiological Examination of foods for pre-enrichment when *Salmonella* is suspected in foods (20) and is included in the USP (114) and IP (46) for use in the performance of Microbial Limit Test for salmonellae and *E. coli.* It is also included in the Bacteriological Analytical Manual for food testing (113).

Principle

Peptone and beef extract provide nitrogen and carbon compounds while lactose is the fermentable carbohydrate. In processed foods and in debilitated conditions *Salmonella* may be present in low numbers. The medium provides for repair of cell damage, dilutes toxic substances that may be present and favours the growth of *Salmonella*. Growth with the production of gas is a presumptive test for coliforms.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	5.0
Beef extract	3.0
Lactose	5.0
Peptone	5.0
Beef Extract	3.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 13 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Dispense in test tubes containing inverted Durham's tubes, in 10 ml amounts for testing samples of 1 ml or less. For testing larger samples (10 ml), prepare double strength broth (26 gms in 1000 ml) and distribute in 10 ml amounts.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35°C.

Organisms (ATCC)	Growth	Gas
Enterobacter aerogenes (13048)	Luxuriant	+
Enterococcus faecalis (29212)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	+
Pseudomonas aeruginosa (10145)	Luxuriant	-
Salmonella serotype Typhi (6539)	Luxuriant	-

Procedure

1. The amount of sample added to Lactose Broth is dependent on the concentration of the medium. Regardless of the sample size, Lactose Broth must have a concentration of 13 grams per liter. For example, if 10 ml samples are to be added to 10 ml of Lactose Broth, the broth must be double strength.
2. Inoculate tubes of Lactose Broth with the dilutions of the sample.
3. Incubate aerobically for 24 hours at 35°C.
4. Examine for turbidity and gas formation.
5. Reincubate the tubes for a further 24 hours (48 hours) if the results are negative at 24 hours.

Interpretation of Results

1. A positive test for coliforms is the production of turbidity in the medium and gas in the Durham's tube within 48 hours.

Precautions / Limitations

1. The results should be confirmed by additional standard testing.
2. The Durham's tubes must be free from air bubbles before inoculation.
3. Avoid overheating double strength broth as inhibitory products may be formed.
4. When used for the pre-enrichment of samples for the recovery of *Salmonella*, the growth from the medium should be subcultured to an appropriate medium for the identification of *Salmonella* species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactose Broth EP AM10522/AM50522

Lactose Broth BP AM10523/AM50523

Use

Lactose Broth is used for the detection of coliforms, in water, food and dairy products.

Summary

Lactose Broth is recommended by APHA in the performance and confirmation of the presumptive test for coliform bacteria in milk . This medium is recommended in the Compendium of Methods for the Microbiological Examination of foods for pre-enrichment when *Salmonella* is suspected in foods and is included in the USP and IP for use in the performance of Microbial Limit Test for salmonellae and E. coli. It is also included in the Bacteriological Analytical Manual for food testing.

Principle

Pancreatic digest of gelatin and beef extract provide nitrogen and carbon compounds while lactose is the fermentable carbohydrate. In processed foods and in debilitated conditions *Salmonella* may be present in low numbers. The medium provides for repair of cell damage, dilutes toxic substances that may be present and favours the growth of *Salmonella*. Growth with the production of gas is a presumptive test for coliforms.

Formula*

Ingredients in grams per liter

Lactose monohydrate	5.0
Pancreatic digest of gelatin	5.0
Beef Extract	3.0

Final pH (at 25°C) 6.9 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 13 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Dispense in test tubes containing inverted Durham's tubes, in 10 ml amounts for testing samples of 1 ml or less. For testing larger samples (10 ml), prepare double strength broth (26 gms in 1000 ml) and distribute in 10 ml amounts.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35°C.

Organisms (ATCC)	Growth	Gas
Enterobacter aerogenes (13048)	Luxuriant	+
Enterococcus faecalis (29212)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	+
Pseudomonas aeruginosa (10145)	Luxuriant	-
Salmonella serotype Typhi (6539)	Luxuriant	-

1. The amount of sample added to Lactose Broth is dependent on the concentration of the medium. Regardless of the sample size, Lactose Broth must have a concentration of 13 grams per liter. For example, if 10 ml samples are to be added to 10 ml of Lactose Broth, the broth must be double strength.
2. Inoculate tubes of Lactose Broth with the dilutions of the sample.
3. Incubate aerobically for 24 hours at 35°C.
4. Examine for turbidity and gas formation.
5. Reincubate the tubes for a further 24 hours (48 hours) if the results are negative at 24 hours.

Interpretation of Results

1. A positive test for coliforms is the production of turbidity in the medium and gas in the Durham's tube within 48 hours.

Precautions / Limitations

1. The results should be confirmed by additional standard testing.
2. The Durham's tubes must be free from air bubbles before inoculation.
3. Avoid overheating double strength broth as inhibitory products may be formed.
4. When used for the pre-enrichment of samples for the recovery of Salmonella, the growth from the medium should be subcultured to an appropriate medium for the identification of Salmonella species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lactose Monohydrate Sterile (g irradiated) AM505241/AM505241-5K

Use

Lactose Monohydrate Sterile (g irradiated) used for the media fill runs in dry filling injectable.

Summary

Routine sampling for sterility testing is not sensitive enough to detect any low level contamination in sterile pharmaceutical formulations. Sample numbers are too small and only gross contamination is likely to be detected. Pharmaceutical manufactures therefore need other means of guaranteeing the quality of their product. This is why process stimulations (Media Fill Run) supported by environmental monitoring is must in pharmaceutical industry.

The FDA guidelines have recommended using SCDM for liquid injectable and Lactose for dry injectable. Regular dehydrated culture media or Lactose is usually supplied in non sterile form which carries high bioburden and should not be directly taken into a controlled area therefore irradiated sterile SCDM/Lactose is used for Media Fill Run. g irradiation also assumes that sterile products is free from Mycoplasma.

Principle

During Media Fill Run for validation of dry injectable g irradiated Lactose is dispersed into individual vial/ampules. After completion of filling process individual vial is reconstituted with sterile distilled water (As per label claim of injection). Reconstituted vials an incubated at 35-37°C and monitored till 14 days.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Lactose Sulphite Broth Base AM50525

Lactose Sulphite Broth Base (Medium R) EP AM50526

Lactose Sulphite Broth Base (Medium R) BP AM50527

Use

Lactose sulphite Medium is recommended for detection and enumeration of *Closterium perfringens* in pharmaceutical products.

Summary

Lactose sulfite broth base is a selective medium used to detect and enumerate *Clostridium perfringens* based on lactose fermentation and production of hydrogen sulfide.

The Eruopean Pharmacopoeia recommends to prepare samples using 1:100 and 1:1000 dilutions with Buffered Peptone Water (AM10211). Mix sample with medium with minimum shaking and incubate at 45.5 - 46.5°C for 24 – 48 hours. Colonies producing hydrogen sulfide are characterized by blackening due to the reaction of Sodium bisulfite and the Ferric ammonium citrate salt. The containers showing a blackening and abundant formation of gas in the Durham tube (at least 1/10 of the volume) indicate the presence of *C. perfringens.*

The principle of the medium relies on the ability of *Clostridium perfringens* to ferment lactose whlie producing gas (observable in Durham bell jar) and to reduce sulphite to sulphide at 46 °C (black iron sulphide precipitate in base of the tube.

Principle

The nutrient base provides optimal conditions for the development of *Clostridia.* Casein peptone and Yeast extract provide the basic nutrients of the medium: nitrogen, vitamins, minerals and amino acids. Lactose is a complex carbohydrate energy source. Cysteine hydrochloride is the reducing agent.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	5.0
Yeast extract	2.5
Sodium chloride	2.5
Lactose monohydrate	10.0
Cysteine hydrochloride	0.3

Final pH (at 25°C) 7.1 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20.3 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Dispense 8 ml in test tubes containing inverted Durham's tubes, 16 mm x 160 mm of size.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 45-46°C.

Organisms (ATCC)	Growth	Gas	Blackening
Closterium perfringens (12924)	luxuriant	+	+
Closterium paraperfringens (27639)	inhibited	-	-

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lauryl Tryptose Broth (Lauryl Sulphate Broth) AM1053/AM5053

Use

Lauryl Tryptose Broth is used for the detection of coliform organisms in materials of sanitary importance.

Summary

Lauryl Tryptose Broth is based on the formulation of Mallmann and Darby (78). This medium is recommended by APHA for the presumptive detection of coliforms in water, effluent or sewage by Most Probable Number (MPN) technique (36) and for detection of coliforms in foods (20) and milk (39). Mallmann and Darby found that Lauryl Tryptose Broth gave higher colon index than confirmatory standard methods media and that gas production served not only as a presumptive test but also confirmatory of the presence of coliforms for routine testing of water. Lauryl Tryptose Broth is listed in the Official Methods of Analysis of AOAC International and in the Bacteriological Analytical Manual for food testing (113). This medium is designed to promote rich growth and copious gas production from small inocula of coliform organisms, while inhibiting the aerobic spore formers, which may give a false reaction. This medium gave a higher colon index than most of the media and the gas production served not only as a presumptive test but also a confirmatory test for the presence of coliforms in routine testing of water. The advantage in using this medium is that in addition to giving the fermentation reaction typical of MacConkey Broth, it can also be directly used for the detection of indole.

Principle

Tryptose provides the essential growth factors, sulphur and trace elements while lactose is the carbohydrate source. Mono and dipotassium phosphates are the buffers while sodium chloride maintains the osmotic balance. Sodium lauryl sulphate selectively inhibits organisms other than coliforms.

Formula*

Ingredients in grams per liter

Tryptose	20.0
Sodium Chloride	5.0
Lactose	5.0
Monopotassium Phosphate	2.75
Dipotassium Phosphate	2.75
Sodium Lauryl Sulphate	0.1

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.6 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly to completely dissolve the powder.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural response after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	Gas Production	Indole (44°C)
Enterobacter aerogenes (13048)	Luxuriant	+	-
Enterococcus faecalis (29212)	Inhibited	-	-
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Typhimurium (14028)	Luxuriant	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Procedure

1. Use single strength medium for inocula of 1 ml or less. For inocula of 10 ml or more, double strength or proportionate medium must be used.
2. After inoculation, incubate the tubes at 37°C for 24-48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate at 37°C and 44°C respectively.

Interpretation of results

1. If fermentation occurs in the tube incubated at 44°C after 8-24 hours, perform indole test by adding Kovac's reagent.
2. A positive indole test in the broth tube showing gas production at 44°C indicates the presence of *E. coli.*
3. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms.

Precautions / Limitations

1. If stored at 2-8°C, the broth becomes cloudy or forms a precipitate. This should clear at room temperature.
2. Gas formation is the criterion of growth and not turbidity.
3. Prior to inoculation of the medium, it may be required to invert the tube to release the bubbles that may have formed in the Durham's tubes.
4. Since the nutritional requirements of the organisms vary, some strains may fail to grow or grow poorly on this medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lauryl Tryptose Mannitol Broth with Tryptophan AM505311

Use

Lauryl Tryptose Mannitol Broth with Tryptophan is a single tube medium used for confirmation of *Escherichia coli* in drinking water.

Summary

Lauryl Tryptose Mannitol Broth with Tryptophan has been recommended as a single tube confirmatory test in report 71 (19.2). It is also recommended by ISO Committee (46.4) and is a suitable medium for the requirements of the EC directive (49.1) related to the quality of drinking water.

Principle

This medium may be used parallel to Lauryl Tryptose Broth (AM1053) to detect non-lactose fermenting strains of Escherichia coli. Escherichia coli is confirmed by gas and indole production when incubated at 44°C for 24 hours. If the indole test is negative even if in a single tube medium, repeat the test in Tryptone water (AM1104). Each tube showing acid and gas in the multiple tube test is subcultured to a tube of Lauryl Tryptose Mannitol Broth with Tryptophan and incubated at 44°C.

Formula*

Ingredients in grams per liter

Tryptose	20.00
Mannitol	5.00
Sodium chloride	5.00
Dipotassium phosphate	2.75
Monopotassium phosphate	2.75
Sodium lauryl sulphate	0.10
L-Tryptophan	0.20

Final pH (at 25°C) 6.8 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.8 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm gently to dissolve the medium completely.
4. Dispense in fermentation test tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 10 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24 hours at 44°C.

Organisms (ATCC)	Growth	Indole	Gas
Escherichia coli (25922)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	-	-
Staphylococcus aureus (25923)	Luxuriant	-	-

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lecithin Agar AM10531/AM50531

Use

Lecithin Agar is used for the detection of bacterial contamination of surfaces in unprotected and protected areas.

Summary

Monitoring the microbial flora of environmental surfaces, walls, ceilings, and equipment is an important stage in achieving good manufacturing practices in factories handling foods, cosmetics or pharmaceuticals. To maintain good hygiene standards in hotels and restaurant kitchens, microbiological contamination must also be monitored. Lecithin Agar is one such medium used for the detection of surface contaminants.

Principle

Tryptone and Papaic Digest of Soyabean Meal are sources of carbon and nitrogen. Sodium Thiosulphate neutralizes iodine and chlorine. Lecithin neutralizes quaternary ammonium compounds. Sodium Chloride maintains the osmotic equilibrium. Agar is a solidifying agent. Lecithin and Polysorbate 80 inactivate many residual disinfectants. Polysorbate 80 neutralizes phenols, hexachlorophene and formalin.

Formula*

Ingredients in grams per liter

Tryptone	15.0
L-Histidine	1.0
Lecithin	0.7
Papaic Digest of Soyabean Meal	5.0
Polysorbate 80	5.0
Sodium Chloride	5.0
Sodium Thiosulphate	1.0
Agar	20.5

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 53.2 grams of powder in 1000 ml distilled water.

2. Heat with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellowish brown coloured, homogeneous free flowing powder.

Prepared Appearance

Yellowish brown coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35±2° C aerobically.

Organisms (ATCC)	Growth	Pigment	Colony Colour	RGI
Staphylococcus aureus (25923)	Good to Luxuriant	--	Yellow to white	More than 70%
Pseudomonas aeruginosa (10145)	Good to Luxuriant	+	Green to blue	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Cool the medium to about 45°C, mix well and pour in petriplates or RODAC (Replicate Organism Detection and Counting) plates.
2. Inoculate onto the medium by spreading method.
3. Using RODAC plates for checking the cleanliness and disinfection efficiency of surfaces, press the plates with even pressure onto the surface.
4. Avoid rubbing to prevent damage of the agar bed. Clean the surface to remove any remains of the agar.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lee's Agar AM50532

Use

Lee Agar is used as differential enumeration of yoghurt starter bacteria i.e *lactobacillus bulgaris* & *streptococcus thermophilus.*

Summary

Lee's Agar is formulated as per APHA (115.1) for differential enumeration of yoghurt starter bacteria, homofermentative *Lactobacillus bulgaricus* and heterofermentative *Streptococcus thermophilus.* Yoghurt is made by the controlled fermentation of milk held at 43°C using a starter culture of *Streptococcus thermophilus* and Lactobacillus bulgaricus. Streptococci grow forst and produce a creamy buttery aroma from diacetyl and similar metabolites. Redox potential is also lowered by *streptococci* which enables *Lactobacilli* to grow thereby growth stimulatory products for streptococci are synthesized by *Lactobacilli.* Hence the typical sharp acetaldehyde flavour of mature yoghurt is formed (18.2).

Principle

Casein enzymic hydrolysate and yeast extarct provide the essential nitrogenous nutrients to the yoghurt (lactic) starter bacteria. Lactose and sucrose are the fermentable carbohydrates. Calcium carbonate is added to medium along with the dipotassium phosphate to buffer the medium and avoid the drastic drop in pH due to lactic acid formation. Bromo cresol Purple is the pH indicator which turns yellow in acidic condition and imparts the yellow colour of the colony. It is recommended to dry the media plates for 18-24 hours prior to use.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	10.0
Yeast extract	10.0
Lactose	5.0
Sucrose	5.0
Calcium carbonate	3.0
Dipotassium phosphate	0.5
Bromo cresol purple	0.0
Agar	18.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 51.52 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm gently to dissolve the medium completely.
4. Dispense and sterilize by autoclaving at 121°C, 15 lbs pressure for 20 minutes.
5. While dispensing, mix carefully to suspend calcium carbonate evenly.
6. Pour into sterile petri plates to obtain 4-5 mm thick gel.

Quality Control

Dehydrated Appearance

Light grey coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, opaque gel forms in petri plates.

Cultural Response

Cultural characteristics after 48 hours at 37°C in a CO_2 incubator.

Organisms (ATCC)	Growth	Colour of colony	RGI
Lactobacillus bulgaricus (11842)	Luxuriant	White	More than 70%

Streptococcus thermophilus (14486	Luxuriant	Yellow	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index)

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lee's Multi-differential Agar AM50533

Lee's Multi-differential Agar is used for the cultivation and identification of all brewing bacteria in the brewing industry.

Principle

Lee's Multidifferential Agar is used for cultivation and identification of brewing bacteria. The medium contains Tomato juice broth which provides nutrients and acid environment for the growth of acidophilic bacteria. Peptonized milk provides lactose as an energy source. The low pH of the medium inhibits bacteria other than acidophilic bacteria. Polysorbate 80 serves as a source of fatty acids. Bromo cresol green acts as a pH indicator. Acid producing bacteria produce a clear yellow halo around the colonies. Other bacteria produce colonies in colours ranging from colourless to yellow green and blue depending on species and strain. Further tests should be carried out for their identification.

Formula*

Ingredients in grams per liter

Tomato juice broth	41.00
Peptonized milk	20.00
Calcium pantothenate	2.00
Citric acid	1.10
Calcium carbonate	5.00
Polysorbate 80	0.50
Bromo cresol green	0.022
Cycloheximide	0.007
Agar	15.00

Final pH (at 25°C) 5.5 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

1. Suspend 85 grams in 1000 ml distilled water.
2. Heat gently to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Avoid overheating.
4. Stir the medium while dispensing to prevent settling of calcium carbonate.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light blue coloured, opaque gel forms in petri plates.

Cultural Response

Cultural characteristics observed after an incubation of 48-72 hours at 30°C.

Organisms (ATCC)	Growth	RGI
A. calcoaceticus (19606)	None-poor	0%
L. acidophilus (4356)	Luxuriant with clear yellow halo	More than 70%
Lact. Fermentum (9338)	Luxuriant with clear yellow halo	More than 70%
Lact. leichmannii (4797)	Luxuriant with clear yellow halo	More than 70%
Lact. Plantarum (8014)	Luxuriant with clear yellow halo	More than 70%
P. vulgaris (13315)	Inhibited	0%

Storage and Shelflife :

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Store below 8°C and the prepared medium at 2-8°C Use before expiry date on the label.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Legionella Agar Base AM1054/AM5054

Use

Legionella Agar Base with added supplements is used for cultivation of *Legionella* species.

Summary

Legionella Agar Base is used for isolation of *Legionella* species from clinical and non-clinical specimens. Feely et al (30) developed Legionella Agar as a modification of F-G Agar by replacing the starch with activated charcoal and casein hydrolysate with yeast extract. Pasculle (84) reported that the agar could be improved by buffering the medium with ACES (N- 2-acetamido-2-amino ethane sulphonic acid) buffer. Edelstein (22) further increased the sensitivity of the medium by adding a-Ketoglutarate.

Principle

Yeast extract provides nutrients for bacterial growth. a-Ketoglutarate meets the specific nutritional requirements of *Legionella* species. The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO_2 and modify surface tension. ACES buffer helps in maintaining proper pH of the medium for the optimal growth of *Legionella* species. Antibiotics in the Selective Supplement inhibit the growth of various contaminating bacteria and fungi.

Formula*

Ingredients in grams per liter

Yeast Extract	10.0
ACES Buffer	6.0
Potassium Hydroxide	1.5
Charcoal Activated	1.5
a-Ketoglutarate	1.0
Agar	17.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.5 gms of the powder in 500 ml distilled water and mix well.
2. DO NOT HEAT PRIOR TO STERILIZATION.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial each of Legionella Growth Supplement (AS016) and Legionella Selective Supplement (AS017).
5. Mix well and stir the medium while dispensing into sterile petri plates to prevent charcoal particles from settling down.

Warning: Potassium Hydroxide is corrosive. On contact with skin, wash immediately with plenty of water.

Quality Control

Dehydrated Appearance

Grey coloured, homogeneous, free flowing powder.

Prepared Appearance

Black coloured, opaque gel.

Cultural Response

Cultural characteristics after 48-72 hours at 35°C.

Organisms (ATCC)	Growth	Colour of the colony	RGI
Legionella pneumophila (33153)	Good to luxuriant	Light blue to grey white	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. Incubate the plates at 35°C for a minimum of 3 days.

Interpretation of results

1. Growth is usually visible after 3 days, but may take up to 2 weeks to appear.
2. *L.pneumophila* produce small to large, smooth, colourless to pale blue-grey, slightly mucoid colonies that fluoresce yellow-green when exposed to long-wave UV light.

Precautions / Limitations

1. *Legionella* species are highly pathogenic if inhaled. Handle liquid cultures in a protective cabinet and avoid creating aerosols.
2. Decontaminate working surfaces with 5% hypochlorite solution and autoclave all materials before discarding.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Letheen Agar — AM50541

Use

Letheen Agar is recommended to inactivate quaternary ammonium compounds and other preservatives when determining the number of bacteria present in cosmetics.

Summary

Letheen Agar is a highly nutritive medium with neutralizing agents for quaternary ammonium compounds. So this medium is very useful for sanitary and disinfectant testing. It neutralizes the disinfectants especially quaternary ammonium compounds and other water containing cosmetics. Letheen Agar is a modification of Tryptone Glucose Extract agar with the addition of lecithin and polysorbate 80.

american Society for Testing and Materials (ASTM) specifies Letheen Agar in the Standard TestMethod for Preservatives in Water-Containing (1.2)

Principle

Pancreatic digest of protein and beef extract provide the carbon and nitrogen source. Dextrose is the source of energy. Lecithin and polysorbate 80 are added to neutralize surface disinfectants.

Formula*

Ingredients in grams per liter

Part A

Casein enzymic hydrolysate	5.0
Beef extract	3.0
Dextrose	1.0
Lecithin	1.0
Agar	15.0
Part B	
Polysorbate 80	7.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 32 gms of the powder in 1000ml of distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Good to luxuriant
Staphylococcus aureus (6538)	Good to luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Letheen Agar, Modified — AM505411

Use

Letheen Agar, modified is recommended to inactivate quaternary ammonium compounds and other preservatives when determining the number of bacteria present in cosmetics.

Summary

Letheen Agar, Modified is based on Letheen Agar as described as in the U. S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (43.1). It is a highly nutritive medium with neutralizing agents for quaternary ammonium compounds. So this medium is very useful for sanitary and disinfectant testing. It neutralizes the disinfectants especially quaternary ammonium compounds and other water containing cosmetics.

Principle

Peptic digest of animal tissue, casein enzymic hydrolysate, yeast extract and beef extract provide the carbon and nitrogen source. Increased amount of peptone in the modified Letheen Agar serves as a better medium for microorganisms. Dextrose is the source of energy. Sodium chloride maintains the osmotic balance. Lecithin, polysorbate 80 and sodium bisulfite are added to neutralize surface disinfectants.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.0
Casein enzymic hydrolysate	10.0
Beef extract	3.0
Lecithin	1.0
Polysorbate 80	7.0
Dextrose	1.0
Agar	15.0
Yeast extract	2.0
Sodium chloride	5.0
Sodium bisulphite	0.1

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 54 gms powder in 1000ml-distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous free flowing powder..

Prepared Appearance

Yellow coloured, slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Letheen Broth, Modified (Twin Pack) AM50543

Use

Letheen Broth, Modified is used for determination of bactericidal activity of quaternary ammonium compounds using *Escherichia coli* and *Staphylococcus aureus*.

Summary

Letheen Broth, Modified is based on Letheen Broth as described as in the U. S. Food and Drug Administration (FDA) Bacteriological Analytical Manual. It is a highly nutritive medium with neutralizing agents for quaternary ammonium compounds. So this medium is very useful for sanitary and disinfectant testing. It neutralizes the disinfectants especially quaternary ammonium compounds and other water containing cosmetics.

Principle

Peptic digest of animal tissue and beef extract provide the carbon and nitrogen source. Sodium chloride maintains the osmotic balance. Lecithin, polysorbate 80 and sodium bisulfite are added to neutralize surface disinfectants.

Formula*

Ingredients in grams per liter

Part A	
Peptic digest of animal tissue	20.0
Beef extract	5.0
Lecithin	0.7
Sodium chloride	5.0
Sodium bisulfite	0.1
Yeast extract	2.0
Casein enzymic hydrolysate	5.0
Part B	
Polysorbate 80	5.0
Final pH (at 25°C) 7.2 ± 0.2	

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.8 gms of the powder in 995 ml-distilled water.
2. Add 5 gms of polysorbate 80 (Twin Pack B)
3. Mix thoroughly.
4. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A: Yellow coloured, homogeneous free flowing powder.

Part B: Yellow colour clear viscous liquid.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Good to luxuriant
Staphylococcus aureus (6538)	Good to luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Letheen Broth (Twin Pack) AM50542

Use

Letheen Broth is used for determining the phenol coefficient of cationic surface-active materials.

Summary

Letheen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Letheen Broth is recommended in the *Official Methods of Analysis of AOAC* International for use with disinfectants containing cationic surface active materials (45.2). American Society for Testing and Materials (ASTM) specifies Letheen Agar and Letheen Broth in the Standard Test Method for Preservatives in Water-Containing (1.2).

Principle

Peptic digest of animal tissue, and beef extract provide the carbon and nitrogen source. Sodium chloride maintains the osmotic balance. Lecithin and polysorbate 80 are added to neutralize surface disinfectants.

Formula*

Ingredients in grams per liter

Part A	
Peptic digest of animal tissue	10.0
Beef extract	5.0
Lecithin	0.7
Sodium chloride	5.0
Part B	
Polysorbate 80	5.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 20.7 gms (Twin pack) of the powder in 995 ml-distilled water.
2. Add 5 gms of polysorbate 80 (Twin Pack B)
3. Mix thoroughly.
4. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Good to luxuriant
Staphylococcus aureus (6538)	Good to luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Listeria Enrichment Broth AM50544

Use

Listeria Enrichment Broth is used for enrichment of Listeria from food.

Summary

Food borne listeriosis was first reported in 1985, since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes.*

Principle

Pancreatic digest of casein and yeast extract provide nitrogen, vitamins and minerals. Dextrose is a carbohydrate source, sodium chloride maintains the osmotic balance of the medium. Phosphate provides buffering capacity. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine Hcl suppresses the growth of gram-positive bacteria. Cycloheximide is incorporated to inhibit saprophytic fungi.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	17.0
Soytone	3.0
Dextrose	2.5
Sodium chloride	5.0
Dipotassium phosphate	2.5
Yeast extract	6.0
Cycloheximide	0.05
Acriflavine Hcl	0.0015
Nalidixic acid	0.04

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36.1 gms powder in 1000 ml distilled water.
2. Heat with frquent agitation to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellowish - amber coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 18-48 hours at 30 + 2°C.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Enterococcus faecalis (29212)	Inhibition
Escherichia coli (25922)	Inhibition
Listeria monocytogenes (19114)	Luxuriant
Saccharomyces cerevisiae (9080)	Inhibition

Procedure

For food samples, use Listeria Enrichment Broth in recommended laboratory procedures for isolating Listeria.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Listeria Identification Agar Base (PALCAM) AM1055/AM5055

Use

Listeria Identification Agar Base, PALCAM with added supplement is used for selective isolation and identification of *Listeria* species.

Summary

Van Netten et al (115) formulated Listeria Identification Agar Base, which is also called Polymixin Acriflavin lithium chloride Ceftazidime Esculin Mannitol (PALCAM) and is used for the isolation of *Listeria monocytogens* from foods. PALCAM is a highly selective medium due to the presence of Lithium chloride, and selective supplement containing Ceftazidime, Polymixin B and Acriflavin hydrochloride. It is a differential diagnostic medium utilizing two indicator systems as esculin-ferric citrate and mannitol-phenol red.

Listeria monocytogenes hydrolyze esculin, which is evidenced by blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7 dihydroxycoumarin, which reacts with ferric ions that are present in the medium as ammonium ferric citrate. It does not ferment mannitol but the contaminants such as enterococci and staphylococci ferment mannitol and is indicated by the change of colour from red to yellow. Under strict microaerophilic conditions strict aerobes such as *Bacillus* and *Pseudomonas* species are inhibited.

The addition of egg yolk (2.5%v/v) to Listeria Identification Agar Base, PALCAM has been reported to aid repair of damaged cells. A medium containing blood when overlaid on Listeria Identification Agar Base, PALCAM enables to differentiate and enumerate haemolytic *Listeria* species. Listeria Identification Agar Base is recommended by APHA for the examination of milk (39) and foods (20).

Principle

Peptone provides nitrogen, vitamins and other nutrients. Dextrose is the carbohydrate source while sodium chloride maintains the osmotic balance. High amounts of lithium chloride and added selective supplement inhibit accompanying micro flora and allow the growth of only *Listeria* species. The combination of mannitol and phenol red helps in the detection of mannitol fermentation while esculin and ammonium ferric citrate helps in the detection of esculin hydrolysis.

Formula*

Ingredients in grams per liter	
Peptone	23.0
Lithium Chloride	15.0
Mannitol	10.0
Sodium Chloride	5.0
Starch	1.0
Phenol Red	0.08
Esculin	0.80
Ammonium Ferric Citrate	0.50
Dextrose	0.50
Agar	13.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 69 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and aseptically add rehydrated contents of 2 vials of Listeria Selective Supplement (PALCAM) (AS018).
6. Mix well and pour into sterile petri plates.

Warning: Lithium chloride is harmful, bodily contact and inhalation of vapours must be avoided. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48 hours at 35°C, in microaerophilic environment.

Organisms (ATCC)	Growth	Colony characteristics	RGI
Listeria monocytogenes (19112)	Luxuriant	Grey-green with black center and a black halo.	More than 70%

Staphylococcus aureus (25923)	None to poor	Yellow colonies with yellow halo.	0%
Enterococcus faecalis (29212)	None to poor	Grey colonies with a brown green halo.	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. The technique for the isolation of *Listeria monocytogenes* depends on the material under test. The sample may be first inoculated into an enrichment broth to allow multiplication before isolation and identification.
2. Inoculate a loopful of the selective enrichment broth onto the Listeria Identification Agar Base, PALCAM plates.
3. Incubate at 37°C for 48 hours under microaerophilic conditions.

Interpretation of Results

1. Typical *Listeria* species form colonies that are approximately 2 mm in diameter, green-grey in colour with a black sunken center and a black halo against a red background.

Precautions / Limitations

1. Acriflavin Hydrochloride in the Listeria Selective Supplement should be protected from light because photo oxidation causes it to become inhibitory to *Listeria* growth.
2. Occasionally *Enterococcus* or *Staphylococcus* strains develop on Listeria Identification Agar Base, PALCAM to form grey colonies with a brown-green halo or yellow colonies with a yellow halo.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Listeria Identification Broth Base (PALCAM) AM105511/AM505511

Use

Listeria Identification Broth Base (PALCAM) with added supplement recommended for selective enrichment and identification of *Listeria* species

Summary

Listeria Identification Broth also known as Polymyxin Acriflavin Lithium-chloride Ceftazidime Aesculin Manitol (PALCAM) Broth is prepared as described by van Netten et al., for selective enrichment of *Listeria* species.

Listeria monocytogenes hydrolyze esculin, which is evidenced by blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7 dihydroxycoumarin, which reacts with ferric ions that are present in the medium as ammonium ferric citrate. It does not ferment mannitol but the contaminants such as *enterococci* and *staphylococci* ferment mannitol and is indicated by the change of colour from red to yellow. Under strict microaerophilic conditions strict aerobes such as *Bacillus* and *Pseudomonas* species are inhibited. The addition of egg yolk (2.5%v/v) to Listeria Identification Agar Base, PALCAM has been reported to aid repair of damaged cells. A medium containing blood when overlaid on Listeria Identification Agar Base, PALCAM enables to differentiate and enumerate haemolytic Listeria species. Listeria Identification Agar Base is recommended by APHA for the examination of milk and foods.

Principle

Peptone provides nitrogen, vitamins and other nutrients. Dextrose is the carbohydrate source while sodium chloride maintains the osmotic balance. High amounts of lithium chloride and added selective supplement (73.1) inhibit accompanying micro flora and allow the growth of only Listeria species. The combination of mannitol and phenol red helps in the detection of mannitol fermentation while esculin and ammonium ferric citrate helps in the detection of esculin hydrolysis.

Formula*

Ingredients Gms/Liter

Peptic digest of animal tissue	23.00
Yeast Extract	5.00
Lithium chloride	10.00
Esculin	0.80
Ammonium ferric citrate	0.50
D-Mannitol	5.00
Soya Lecithin	1.00
Polysorbate 80	2.00
Phenol red	0.08

Final pH (at 25°C) 7.4 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23.7 gms powder in 500 ml distilled water. Heat if necessary to dissolve the medium completely.
2. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

3. Cool to 45-50°C and aseptically add sterile reconstituted contents of vial of Listeria Selective Supplement (AS018).
4. Mix well before dispensing.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 48 hours at 30°C.

Organisms (ATCC)	Growth	Colour
Listeria monocytogenes (19118)	Good	Black
Staphylococcus aureus (25923)	Inhibited	–
Enterococcus faecalis (29212)	Inhibited	–
Micrococcus luteus (10240)	Inhibited	–

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. The technique for the isolation of Listeria monocytogenes depends on the material under test. The sample may be first inoculated into an enrichment broth to allow multiplication before isolation and identification.
2. Inoculate a loopful of the selective enrichment broth onto the Listeria Identification Agar Base, PALCAM plates.
3. Incubate at 37°C for 48 hours under microaerophilic conditions.

Interpretation of Results

1. Typical Listeria species form colonies that are approximately 2 mm in diameter, green-grey in colour with a black sunken center and a black halo against a red background.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Listeria Oxford Medium Base

AM105512/AM505512

Use

Listeria Oxford Medium Base is used for isolation of *Listeria* species from pathological specimens.

Summary

In 1926 Murray et al., first described that *Listeria monocytogenes* is a widespread problem in public health and the food industries. Identification of *Listeria* is based on successful isolation of the organism. According to Curtis *et al.,* description Listeria Oxford Medium Base is formulated for isolation of *Listeria monocytogenes* (18.1). Various techniques are followed to isolate the organism from different specimen. For all specimens selective and cold enrichment is recommended (33.2). Antimicrobial supplements are used for selective isolation of *Listeria* species.

Principles

Peptone serves as a source of nitrogen and amino acids. Lithium chloride inhibits Gram negative bacteria specially *enterococci*. Addition of various antimicrobial agents makes the base more selective. Sodium chloride maintains the osmotic balance. *Listeria monocytogenes* hydrolyzes esculin to esculatin and dextrose. Esculatin reacts with ferric ions and produces black zones around the colonies.

Formula*

Ingredients in grams per liter

Peptone, special	23.0
Lithium chloride	15.0
Sodium chloride	5.0
Corn starch	1.0
Esculin	1.0
Ammonium ferric citrate	0.50
Agar	10.0

Final pH (at 25°C) 7.0± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend the 55.50 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the medium to -50°C.
6. Add 1 vial of rehydrated Oxford Listeria Supplement (AS0201) to prepare Oxford's medium or add 1 vial of rehydrated Listeria Moxalactam Supplement (AS0171) to prepare selective medium for Listeria.
7. After addition, the medium must be gently but thoroughly mixed to ensure that the antibiotics are uniformly distributed throughout the medium.

Warning: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Dark yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Dark amber coloured, clear gel with blue cast, forms in petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 35ºC.

Organisms (ATCC)	Growth*	Esculin hydrolysis
Listeria monocytogenes (19117)	Luxuriant	+
Listeria monocytogenes (19111)	Luxuriant	+
Listeria monocytogenes (19112)	Luxuriant	+
Staphylococcus aureus (25923)	Good	-
Enterococcus faecalis (29212)	Inhibited	-
Enterococcus hirae (10541)	Inhibited	-
Bacillus subtilis (6633)	Inhibited	-

Key : + = Black zones around colonies

Procedure

For faecal and biological specimens, the sample is homogenized in 0.1% Peptone water (AM1079/5079) and 0.1ml amount is directly plated on Listeria Selective Medium. Incubate the plates at 35ºC for 24-48 hours and examine the plates.

Interpretation of Results

Black zone is found around esculin positive colonies. For further identification do biochemical testing. For additional information, refer to appropriate references.

Precautions / Limitations

Since *Listeria* species other than L. monocytogenes can grow on these media, biochemical and serological testing should be done to identify L. monocytogenes. Use freshly prepared antimicrobial agent solutions or aliquot portions and store at - 20ºC or below. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some *enterococci.*

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Liver Infusion Agar AM10551/AM50551

Liver Infusion Broth AM10552/AM50552

Use

Liver Infusion Agar is used for the cultivation of *Brucella* and other pathogenic bacteria.

Liver Infusion Broth is used for the cultivation of highly fastidious microorganisms, particularly *Brucella* species and anaerobes.

Summary

Brucellosis is a zoonotic disease and is usually transmitted by milk, milk products, meat and direct contact with infected animals. Most strains of *Brucella* grow on chocolate agar or blood agar. However, the nutritive factors of Liver Infusion media permit luxuriant growth of *Brucella* and other fastidious pathogens.

For isolating *Brucella* strains from contaminated milk, crystal violet can be added to Liver Infusion Agar to suppress the growth of gram-positive microorganisms and 5% heated horse or rabbit serum to enhance *Brucella* growth.

Liver Infusion Broth supports the growth of anaerobic microorganisms, especially Clostridium species.

Principle

Proteose Peptone and Beef Liver Infusion provide sources of nitrogen, amino acids, vitamins and carbon. Sodium Chloride maintains the osmotic equilibrium. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter	Agar	Broth
Beef Liver, Infusion from	500.0	500.0
Proteose Peptone	10.0	10.0
Sodium Chloride	5.0	5.0
Agar	20.0	--
Final pH (at 25^{0} C) 6.9 ± 0.2		

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.
   Liver Infusion Agar - 55 grams
   Liver Infusion Broth - 35 grams
2. Boil with frequent agitation to dissolve the medium completely.
3. Sterilize by autoclaving at 121^{0} C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Dark beige to light tan, homogeneous, free flowing powder.

Prepared Appearance

Medium to dark amber, slightly opalescent gel.

Cultural Response

Cultural response at 35 ± 2^{0}C after 18 - 48 hours up to 72 hours.

Organisms (ATCC)	Growth	RGI
Brucella abortus (4315)	Good	More than 70%
Brucella melitensis (4309)	Good	More than 70%
Brucella suis (4314)	Good	More than 70%
Clostridium sporogenes (11437)	Good	More than 70%
Streptococcus pneumoniae (6305)	Good	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Loeffler Medium Base

AM1056/AM5056

Use

Loeffler Medium Base with added horse serum is used for the cultivation of *Corynebacterium diphtheriae* from clinical specimens and in pure cultures.

Summary

Loeffler (72) devised Loeffler Medium Base, which was further modified by Pery and Petran (88) and Buck (11). This medium is used for the promotion of growth and morphological characterization of members of the genus *Corynebacterium*; formation of metachromatic granules is enhanced within the cells of these organisms. This medium is also useful for demonstrating colonial pigmentation, ascospore production and proteolytic activity of microorganisms.

Principle

Bovine serum and peptone provides amino acids and other nitrogenous substances necessary to support growth of corynebacteria. Dextrose is the carbohydrate source.

Formula*

Ingredients in grams per liter

Beef Extract	2.5
Peptone, Special	2.5
Dextrose	2.5
Sodium Chloride	1.25

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 8.8 gms of the powder in 250 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 115°C (10 lbs pressure) for 20 minutes.
5. Cool to 50-55°C, aseptically add 750 ml of sterile horse serum (AS 015).
6. Mix well and aseptically dispense into sterile tubes.
7. Inspissate the medium at 80-85°C for 2 hours in free flowing steam for at least 3 consecutive days.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium - Light amber coloured clear solution.

With added serum and after coagulation - Opalescent slants.

Cultural Response

Cultural characteristics after 3-4 days at 35°C.

Organisms(ATCC)	Growth
Corynebacterium diphtheriae (19113)	Fair to good
Pseudomonas aeruginosa (10145)	Good (green colonies)

Procedure

1. Inoculate the tubed medium as soon as possible after specimen collection, using direct inoculation of the specimen by swabs or by means of an inoculating loop.
2. Incubate the tubes with caps loosened for 18-24 hours and up to 45 days at 35-37°C in an aerobic atmosphere.

Interpretation of Results

1. Examine cultures and smears with Loefflers methylene blue after incubation.
2. Observe for typical cellular morphology of *Corynebacterium* species and for the presence of metachromatic granules, which take up the methylene blue dye.
3. Colonies that are catalase positive and exhibit typical morphology are subcultured on to blood agar plates to provide growth for identification procedures.
4. Observe for pigmentation of specific organisms; e.g. *Pseudomonas aeruginosa* (green) and *Staphylococcus aureus* (yellow to gold).
5. Proteolytic activity is observed by destruction of the integrity of the coagulated medium.

Precautions / Limitations

1. Though the production of metachromatic granules on this medium is characteristic of *Corynebacterium* genus, other organisms such as *Propionibacterium*, some *Actinomyces* and pleomorphic streptococcal strains display stained granules resembling those of the corynebacteria.
2. Loeffler Medium Base must be used in parallel with a tellurite-containing medium for the selective isolation of pathogens, particularly *C.diphtheria*.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lowenstein Jensen Medium Base

AM1057/AM5057

Use

Lowenstein Jensen Medium Base supplemented with eggs is used for the cultivation and isolation of mycobacteria, especially *M.tuberculosis*.

Summary

Lowenstein Jensen Medium is an inspissated, egg based medium developed from Jensen's modification (48) of Lowensteins (73) formula. Gruft (37) modified this medium by adding two antimicrobials penicillin and nalidixic acid for selective

isolation of mycobacteria. He also found that addition of ribonucleic acid (RNA) increased the percentage of tubercle bacilli recovered from clinical specimens compared to recovery on the standard Lowenstein Jensen Medium.

Principle

Lowenstein Jensen Medium Base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Glycerol and egg mixture is added prior to the inspissation process. They provide fatty acids and protein required for the metabolism of mycobacteria. Glycerol also serves as a carbon source and is favourable to the human type tubercle bacilli while being unfavourable to the bovine type. The coagulation of egg albumin during sterilization provides a solid medium for inoculation purposes. Malachite green serves as a pH indicator and inhibits majority of contaminants surviving decontamination of the specimen. RNA in the supplement acts as a stimulant and helps to increase the isolation rate of mycobacteria.

Formula*

Ingredients in grams per 600 ml

Potato Starch, Soluble	30.0
L-Asparagine	3.6
Monopotassium Phosphate	2.4
Magnesium Citrate	0.60
Malachite Green	0.4
Magnesium Sulphate	0.24

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.24 gms of the powder in 600 ml distilled water containing 12 ml glycerol and mix thoroughly. (Do not add glycerol if bovine tubercle bacilli or other glycerophobic organisms are to be cultivated).
2. Boil to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes. Cool to approximately 50°C.
4. Add 1000 ml sterile whole egg emulsion (AS010). Gruft Mycobacterial Supplement (AS 013) may be added if desired, 1 vial per 400 ml of above medium and mix gently to obtain a uniform mixture.
5. Dispense in sterile screw-capped tubes.
6. Arrange tubes in slanted position.
7. Coagulate and inspissate the medium in an inspissator water bath or autoclave at 85°C for 45 minutes.

Quality Control

Dehydrated Appearance

Greenish blue coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal medium with whole egg emulsion, on inspissation, coagulates to yield pale bluish green coloured, opaque, smooth slants.

Cultural Response

Cultural characteristics after 2-4 weeks at 35°C with 5-10% CO_2.

Organism (ATCC)	L-J Medium Base	L-J Medium with Gruft supplement
Mycobacterium tuberculosis (H37Rv strain)	Luxuriant growth, granular rough, warty, dry, friable colonies	Good to luxuriant
Mycobacterium smegmatis (14468)	Luxuriant growth, wrinkled, creamy white colonies	Good to luxuriant
Mycobacterium kansasii (12478)	Luxuriant growth, photochromogenic, smooth to rough	Good to luxuriant

Procedure

1. The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation of specimens containing mycobacteria. N-Acetyl-L-Cysteine-Sodium Hydroxide solution is recommended as a gentle but effective digesting and decontaminating agent.
2. Following inoculation, the test containers should be kept away from light and placed in a suitable system providing an aerobic atmosphere enriched with CO_2.
3. Incubate at 35-37°C. Slanted and bottled media should be incubated in a horizontal plane until the inoculum is absorbed. The screw caps of containers should be loose for the first three weeks to allow circulation of CO_2. Thereafter, to prevent dehydration, the caps should be tightened and loosened briefly once a week.

   Note: Cultures from skin lesions suspected to be *M.marinum* or *M.ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M.avium* or *M.xenopi* exhibit optimum growth at 40-42°C.

Interpretation of Results

1. Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.
2. Record the following observations: -
   a) Number of days required for colonies to become macroscopically visible.

      Rapid growers have mature colonies within 7 days; slow growers require more than 7 days.

   b) Pigment production

      White, cream to buff Non-chromogenic.

      Lemon, yellow, orange, red Chromogenic.

3. Stained smears may show acid fast bacilli, which are reported only as "acid fast bacilli" unless definitive tests are performed.

Precautions / Limitations

1. Biosafety Level 2 practices, containment equipment and facilities are required for non-aerosol producing activities such as preparation of acid-fast smears.
2. All aerosol generating activities must be conducted in a class 1 or 2 biological safety cabinet. Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M.tuberculosis* and *M.bovis.*
3. Lowenstein Jensen Medium Base requires incubation in a 5-10% CO_2 atmosphere in order to recover mycobacteria.
4. The medium should be protected from all sources of light, as malachite green is highly photosensitive.
5. Do not use media that have turned yellow, as it will interfere with interpretation of the pigmentation of mycobacteria. Formation of blue zones indicates a decrease in pH by gram-positive contaminants like streptococci and yellow zones of dye destruction by gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of the medium.
6. Negative culture results do not rule out active infection by mycobacteria. Some factors responsible for unsuccessful cultures are:

   The specimen was not representative of the infectious material i.e. saliva instead of sputum.

   The mycobacteria were destroyed during digestion and decontamination of the specimen.

   Gross contamination interfered with the growth of the mycobacteria. Proper aerobic conditions and increased CO_2 tension were not provided during incubation.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Luria Agar — AM10571/AM50571
Luria Broth — AM10572/AM50572

Use

Luria Agar and Luria Broth are recommended for the cultivation and maintenance of recombinant strains of *Escherichia coli* and may be used for routine cultivation of not particularly fastidious microorganisms.

Summary

Luria Agar / Luria Broth as described by Lennox is used for the cultivation and maintenance of recombinant strains of *Escherichia coli.*

Principle

Tryptone provides a source of peptones. Yeast Extract is a source of Vitamin B complex. Sodium Chloride maintains the osmotic equilibrium. Agar is the solidification agent.

Formula*

Ingredients in grams per liter	Agar	Broth
Tryptone	10.0	10.0
Yeast Extract	5.0	5.0
Sodium Chloride	5.0	5.0
Agar	15.0	--

Final pH (at 25^0 C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Luria Agar - 35 grams

   Luria Broth - 20 grams
2. Heat with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.
4. Dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow to amber coloured clear to slightly opalescent gel.

Cultural Response

Cultural response after 18 - 24 hours at 35^0 C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Luria Bertani Agar, Miller AM50573

Luria Bertani Broth, Miller AM50574

Use

Luria Bertani Agar, Miller and Luria Bertani Broth, Miller are used for maintenance and cultivation of *Escherichia coli* in molecular microbiology procedures.

Summary

Luria Bertani Agar, Miller and Luria Bertani Broth, Miller (1.1) are nutritionally rich media recommended for growth of pure cultures of recombinant strains of *E. coli*. The media are nutritionally rich suitable for the growth of pure cultures like recombinant strains. For example *Escherichia coli K12* and derived strains which are deficient in Vitamin B synthesis and modified by specific mutation to create auxotrophic organisms, that means they are not able to grow on nutritionally poor media. Luria Bertani Agar, Miller and Luria Bertani Broth, Miller contain double amount of sodium chloride of the Luria Agar (AM10571) (69.1) and Luria Broth(AM10572). This allows the researcher to select the optimal salt concentration for a specific strain.

Principle

Tryptone and Yeast extract serve as a source of nitrogen, sulfur and carbon while Yeast extract also contains Vitamin B complex. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter	Luria Bertani Agar, Miller	Luria Bertani Broth, Miller
Tryptone	10.0	10.0
Yeast Extract	5.0	5.0
Sodium Chloride	10.0	10.0
Agar	15.0	-

Final pH (at 25°C) 7.5 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water
   Luria Bertani Agar, Miller - 40.00gms
   Luria Bertani Broth, Miller 25.00gms
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Luria Bertani Agar, Miller - Yellow to amber coloured, slightly opalescent gel.
Luria Bertani Broth, Miller- Yellow to amber, slightly opalescent solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	Luria Bertani Broth, (RGI)	Luria Bertani Broth (RGI)
Escherichia coli (25922)	Luxuriant	More than 70%	Luxuriant
Escherichia coli (23724)	Luxuriant	More than 70%	Luxuriant

Procedure

Consult appropriate references for recommended test procedures.

Interpretation of Results

Growth is results in the form of isolated colonies and/or a confluent lawn on the surface of the agar medium or the appearance of turbidity in the broth medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

LPM Agar Base AM10575

Use

LPM Agar is recommended for isolation and cultivation of *Listeria monocytogenes* from food and dairy products.

Summary

LPM Agar is a modified McBride agar developed by Lee and McClain. This medium is recommended by APHA for the isolation of *Listeria monocytogenes.*

Principle

Casein enzymic hydrolysate and beef extract provides all essential nutrients for metabolism. Glycine anhydride, lithium chloride and phenylethyl alcohol suppress gram – positive cocci and gram – negative rods. Moxalactam inhibits both gram-positive and gram-negative bacteria including *Staphylococci, Proteus* and *Pseudomonas* species. *Listeria monocytogenes* show blue-green iridescence when examined with oblique transmitted light.

Formula*

Ingredients in grams per liter	
Casein enzymic hydrolysate	5.00
Peptic digest of animal tissue	5.00

Beef extract	3.00
Glycine anhydride	10.00
Lithium chloride	5.00
Sodium chloride	5.00
Phenylethyl alcohol	2.50
Agar	15.00

Final pH (at 25°C) 7.3 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 50.5 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to boiling to dissolve the medium completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 12 minutes.
5. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Moxalactam Supplements (AS0182).
6. Mix well before pouring into sterile petri plates.

Caution: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Listeria monocytogenes (19112)	Good - luxuriant	More than 70%
Staphylococcus aureus (25923)	Inhibited	0%
Pseudomonas aeruginosa (27853)	Inhibited	0%
Escherichia coli (25922)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lysine Iron Agar AM10576/AM50576

Use

Lysine Iron Agar is used for differentiation of enteric bacteria on the basis of hydrogen sulphide production and the decarboxylation or deamination of lysine.

Summary

Edwards P.R. and Mary A. Fife designed Lysine Iron Agar in 1961 (86.1). A Lysine Iron Agar is described and recommended for the detection of Arizona strains, which ferment lactose rapidly. *Salmonella* and *Arizona* cultures produce a distinctive reaction since they are only recognized groups of enteric bacteria, which regularly produce lysine decarboxylase rapidly and form large amount of hydrogen sulphide.

Principle

Peptic digest of animal tissue and yeast extract serves as a source of carbon, nitrogen, vitamins and minerals. Bromocresol purple act as a indicator. An alkaline reaction is seen by the presence of a purple colour, and an acidic reaction is indicated by the appearance of a yellow colour. Sodium thiosulphate is the source of hydrogen sulphide, and ferric ammonium citrate as the indicator, which turns the butt black in the presence of free hydrogen sulphide gas. Lysine is added to show the decarboxylation reaction, which causes an alkaline situation to occur, seen as a purple butt. The yellow colour is seen only if lysine decarboxylation does not occur, as this reaction overcomes any acidic (yellow) conditions. If lysine is deaminated in the presence of oxygen (the reaction seen in the presence of *Proteus* and *Providencia* species), a red colour change is seen on the slant.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Yeast extract	3.0
Dextrose	1.0
L-Lysine	10.0
Ferric ammonium citrate	0.50
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	15.0

Final pH (at 25°C) 6.7 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 34.56 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, slightly opalescent gel forms in tubes.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Butt	Slant	H_2S
Citrobacter freundii (8090)	Luxuriant	A	K	+
Escherichia coli (25922)	Luxuriant	K	K	_
Proteus mirabilis (25933)	Luxuriant	A	R	+
S.serotype typhimurium (14028)	Luxuriant	K	K	+
Shigella flexneri (12022)	Luxuriant	A	K	_
S. serotype arizonae (13314)	Luxuriant	K	K	+

Key:

A= Acid (yellow)

K= Alkaline (red purple, no change in colour)

R= Red (lysine deaminase)

H_2S + = Black precipitate

H_2S _ = No black precipitate

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulphide production.

Interpretation of Results

Lysine Decarboxylation is detected in the butt by an alkaline (Purple) reaction. Lysine deamination is detected by a red slant. Hydrogen sulphide production is detected by the formation of a black precipitate. A negative reaction (purple slant and yellow butt) indicates fermentation of dextrose only.

Hydrogen sulphide may not be detected in this medium by organisms that are negative for lysine decarboxylase activity since acid production in the butt may suppress its formation. Because of this, hydrogen sulphide producing *Proteus* species do not blacken this medium.

Precautions / Limitations

It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Lysine Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Lysine Medium Base

AM10577/AM50577

Use

Lysine Medium Base is recommended for isolation and enumeration of wild yeasts in pitching yeasts.

Summary

Lysine Medium Base is used for the isolation and enumeration of wild yeasts encountered in brewing. On this medium pitching yeasts are suppressed. Morris & Eddy first described this complex medium (80.1). Walters and thiselton developed a liquid synthetic medium containing lysine as the sole nitrogen source for yeasts (117.1). Later Morris & Eddy formulated a solid lysine medium.

Principle

Dextrose is the source of energy. Lysine serve as sole nitrogen source. Different salts and amino acids support the growth of bacteria.

Formula*

Ingredients in grams per liter

Dextrose	44.50
Monopotassium phosphates	1.78
Magnesium phosphates	0.89
Calcium chloride	0.178
Sodium chloride	0.089
Adenine	0.00178
DL-Methionine	0.000891
L-Histidine	0.000891
DL-Tryptophan	0.000891
Boric acid	0.0000089
Zinc sulphate	0.0000356
Ammonium molybdate	0.0000178
Manganese sulphate	0.0000356
Ferrous sulphate	0.0002225
L-Lysine	1.00
Inositol	0.02
Calcium pantothenate	0.002
Aneurine	0.0004
Pyridoxine	0.0004
p-Amino benzoic acid	0.0002
Nicotinic acid	0.0004
Riboflavin	0.0002
Biotin	0.000002
Folic acid	0.000001
Agar	17.80

Final pH (at 25°C) 5.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 6.6 gm of the powder in 100 ml-distilled water containing 1ml potassium lactate (50%) (AS0211).
2. Mix thoroughly.
3. Boil to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool the medium to 50°C, adjust pH to 5.0 with 10% lactic acid and pour into sterile petri dishes.

Quality Control

Dehydrated Appearance

White coloured, homogeneous, free flowing powder.

Prepared Appearance

Colourless, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics at 25°C for 7 days.

Organisms (ATCC)	Growth
Pichia fermentans (10651)	Luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M 17 Agar AM50578

Use

M 17 Agar used for cultivation of lactic *Streptococci* and plaque assay of lactic bacteriophages.

Summary

M17 media are based on the formulation described by Terzaghi and Sandine (109.1) for the cultivation and enumeration of lactic *Streptococci* and their bacteriophages. It is possible to study plaque morphology and lysogeny. M17 Agar is recommended by the International Dairy Federation (46.1.2) for selective enumeration of *Streptococcus thermophilus* from yoghurt. M17 Agar is recommended by APHA for the cultivation of lactic *Streptococci* (20).

Principle

Peptic digest of animal tissue, papaic digest of soyabean meal, yeast extract, beef extract provide carbonaceous, nitrogenous compounds, vitamin B complex and other essential growth factors. Lactose is the fermentable carbohydrate and ascorbic acid is stimulatory for the growth of lactic *Streptococci*. Magnesium sulphate provides essential ions to the organisms. Disodium-beta-glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as lower pH results in injury and reduced recovery of lactic Streptococci. Disodium glycerophosphate suppresses Lactobacillus bulgaricus .

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Papaic digest of soyaben meal	5.0
Yeast extract	2.5
Beef extract	5.0
Ascorbic acid	0.5
Magnesium sulphate	0.25
Lactose	5.0
Agar	10.0

Final pH (at 25°C) 7.1±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 33.25 gms powder in 1000 ml distilled water.
2. Add 19grams of Disodium ß-Glycerophosphate. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well and dispense as desired.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C with added Disodium ß-Glycerophosphate..

Organisms (ATCC)	Growth	RGI
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Lactobacillus bulgaricus (11842)	None-poor	0%
Lactobacillus leichmannii (4797)	Luxuriant	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	More than 70%
Streptococcus thermophilus (14485)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M 17 Broth AM50579

Use

MM 17 Broth used for cultivation of lactic *Streptococci* and their bacteriophages.

Summary

M17 broth is based on the formulation described by Terzaghi and Sandine (109.1) for the cultivation and enumeration of lactic *Streptococci* and their bacteriophages. M17 Broth is a modification of M16 Medium (64.1). Lactic *Streptococci* are nutritionally fastidious and require complex media for optimal growth (1.4 & 91.3)). Disodium glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as the lower pH results in injury and reduced recovery of lactic *Streptococci*. Glycerophosphate does not form precipitate with calcium which is needed for the plaque assay of lactic bacteriophages.

Principle

Peptic digest of animal tissue, casein enzymix hydrolysate, papaic digest of soyabean meal, yeast extract, beef extract, provide carbonaceous, nitrogenous compounds, vitamin B complex and other essential growth factors. Lactose is the fermentable carbohydrate and ascorbic acid is stimulatory for the growth of lactic *Streptococci*. Magnesium sulphate provides essential ions to the organisms. Disodium - ß – glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as lower pH results of in injury and reduced recovery of lactic *Streptococci*.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	2.5
Casein enzymic hydrolysate	2.5
Papaic digest of soyabean meal	5.0
Yeast extract	2.5
Beef extract	5.0
Lactose	5.0
Ascorbic acid	0.5
Disodium - ß - glycerophosphate	19.0
Magnesium sulphate	0.25

Final pH (at 25°C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 42.25 gms powder in 1000 ml distilled water and mix thoroughly.
2. Heat if necessary to dissolve the powder completely. AVOID OVERHEATING.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well and dispense as desired.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37°C

Organisms (ATCC)	Growth
Enterococcus faecalis (29212)	Luxuriant
Lactobacillus bulgaricus (11842)	None-poor
Lactobacillus leichmannii (4797)	Luxuriant
Lactobacillus plantarum (8014)	Luxuriant
Streptococcus thermophilus (14485)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Agar Base AM1058/AM5058

MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts AM1059/AM5059

MacConkey Agar without Crystal Violet and with 0.15% Bile Salts AM1060/AM5060

MacConkey Agar without Crystal Violet, NaCl and with 0.5% Sodium Taurocholate AM1061/AM5061

Use

MacConkey Agar Base is used for studying carbohydrate fermentation reactions of coliforms by adding the desired carbohydrate. MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts is a slightly selective and differential medium for the detection of coliforms and enteric pathogens. MacConkey Agar without Crystal Violet and with 0.15% Bile Salts is used for selective isolation and

differentiation of lactose fermenting and non-lactose fermenting enteric bacteria. MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate is used for the cultivation and differentiation of enteric bacteria and potentially pathogenic gram-positive organisms, while restricting swarming of *Proteus* species.

Summary

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of specimens like water, faeces and other sources suspected of containing these microorganisms (76, 77). The original MacConkey Agar was based on the bile salt-neutral red-lactose agar of MacConkey, which was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. The formula was further modified to be more selective. The presence of bile salts allows the growth of enteric gram-negative organisms.

MacConkey Agar Base is used with added carbohydrates in differentiating coliforms based on fermentation reactions.

MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts is designed to achieve more differentiation of lactose fermenters and non-lactose fermenters, for the promotion of superior growth of enteric pathogens and to improve the inhibition of swarming Proteus species.

MacConkey Agar without Crystal Violet and with 0.15% Bile Salts though less selective than the original MacConkey Agar is used for isolating and cultivating gram-negative enteric microorganisms.

The lack of crystal violet in MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate permits the growth of *Staphylococcus* and *Enterococcus*. It is a differential medium used for isolating and cultivating gram-negative enteric microorganisms and gram-positive cocci. Swarming of *Proteus* is reduced due to the lack of salt in this medium.

The above-mentioned MacConkey Agars except MacConkey Agar Base are recommended for use in microbiological examination of clinical specimens, foodstuffs (20) and for direct plating and inoculation of water samples (36) for coliform counts. These media are included in the Standard Methods for the Examination of Milk and Dairy Products (39), pharmaceutical preparations (46, 114) and industrial sources. They are also included in the Official Methods of Analysis of AOAC International as well as the Bacteriological Analytical Manual (113).

Principle

Peptone, proteose peptone and tryptone provide nitrogen and other nutrients, while lactose is the carbohydrate source. Bile salts and crystal violet are selective agents that inhibit the growth of gram-positive bacteria but allow enteric gram-negative bacteria to grow. Sodium taurocholate is also a selective agent that inhibits gram-positive bacteria except staphylococci and enterococci. Neutral red is the pH indicator.

Directions

1. Suspend the powder in 1000 ml distilled water and mix thoroughly.

   MacConkey Agar Base - 40 gms

Formula*

Ingredients in grams per liter	AM1058/AM5058	AM1059/AM5059	AM1060/AM5060	AM1061/AM5061
Peptone	17.0	1.5	17.0	20.0
Proteose Peptone	3.0	-	3.0	-
Tryptone	-	1.5	-	-
Pancreatic Digest of Gelatin	-	17.0	-	-
Lactose	-	10.0	10.0	10.0
Bile Salts	1.5	1.5	1.5	-
Sodium Chloride	5.0	5.0	5.0	-
Crystal Violet	0.001	0.001	-	-
Neutral Red	0.03	0.03	0.03	0.04
Sodium Taurocholate	-	-	-	5.0
Agar	13.5	15.0	15.0	20.0
Final pH (at 25°C)	7.1±0.2	7.1±0.2	7.1±0.2	7.4±0.2

*Formula adjusted to suit performance parameters

   MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts - 51.5 gms

   MacConkey Agar without Crystal Violet and with 0.15% Bile Salts - 51.53 gms

   MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate 55 gms

2. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and (For MacConkey Agar Base add sterile 1% w/v desired carbohydrate solution) pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pinkish beige coloured, homogeneous, free flowing powder.

Prepared Appearance

MacConkey Agar Base & MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts Red with purplish tinge, clear to slightly opalescent gel.

MacConkey Agar without Crystal Violet and with 0.15% Bile Salts & MacConkey Agar without Crystal violet, NaCl & with Sodium Taurocholate Orange red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts

Organisms (ATCC)	Growth	Colour of Colony
Enterobacter aerogenes (13048)	Luxuriant	Pink to red
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	Luxuriant	Pink to red with bile precipitate
Proteus vulgaris (13315)	Luxuriant	Colourless
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless
Shigella flexneri (12022)	Fair to good	Colourless
Staphylococcus aureus (25923)	Inhibited	-

Cultural characteristics after 18-24 hours at 35°C.

MacConkey Agar without Crystal Violet and with 0.15% Bile Salts

Organisms (ATCC)	Growth	Colour of Colony
Enterobacter aerogenes (13048)	Luxuriant	Pink
Enterococcus faecalis (29212)	Inhibited	-
Escherichia coli (25922)	Luxuriant	Pink to red
Proteus vulgaris (13315)	Luxuriant	Colourless
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless
Shigella flexneri (12022)	Fair to good	Colourless
Staphylococcus aureus (25923)	Inhibited	-

Cultural characteristics after 18-24 hours at 35-37°C.

MacConkey Agar without Crystal Violet, NaCl & with 0.5% Sodium Taurocholate

Organisms (ATCC)	Growth	Colour of Colony
Enterobacter aerogenes (13048)	Luxuriant	Pink to red
Enterococcus faecalis (29212)	Fair to good	Pale pink to red
Escherichia coli (25922)	Luxuriant	Pink to red with bile precipitate
Proteus vulgaris (13315)	Luxuriant	Colourless
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless
Shigella flexneri (12022)	Luxuriant	Colourless
Staphylococcus aureus (25923)	Fair to good	Pale pink to red

Procedure

1. The surface of the medium should be dry when inoculated.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Interpretation of Results

For MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts & MacConkey Agar without Crystal Violet and with 0.15% Bile Salts.

1. Lactose fermenting bacteria produce pink to brick-red colonies and may be surrounded by a zone of bile precipitation.
2. Non-Lactose fermenting bacteria produce colourless colonies.

For MacConkey Agar without Crystal violet, NaCl & with Sodium Taurocholate.

1. Lactose fermenting organisms grow as pink to brick red colonies with or without a zone of precipitated bile.
2. Non-Lactose fermenters grow as colourless or clear colonies.
3. Staphylococci produce pale pink to red colonies.
4. Enterococci produce tiny red colonies.
5. Rapid growers of the *M.fortuitum* complex usually grow in 5-11 days, while the commonly saphyrophytic species are inhibited.
6. The swarming of *Proteus* is reduced on this medium.

Precautions / Limitations

1. Incubation of plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.
2. Some strains of *M.smegmatis* from humans may grow on MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate, but these strains can be differentiated from *M.fortuitum* complex by the 3-day arylsulphatase test.
3. Not all strains of *E.coli* ferment lactose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Agar (Harmonized) AMH5059

MacConkey Agar Base Medium USP AM10581/AM50581

Use

MacConkey Agar Medium is a selective and differential medium for the detection of coliforms and enteric pathogens, in compliance with USP.

Summary

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of specimens like water, faeces and other sources suspected of containing these microorganisms. The original MacConkey Agar was based on the bile salt-neutral red-lactose agar of MacConkey, which was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts is designed to achieve more differentiation of lactose fermenters and non-lactose fermenters, for the promotion of superior growth of enteric pathogens. It is recommended by USP for microbial limit tests.

Principle

Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen and other nutrients, while lactose is the carbohydrate source. Bile salts and crystal violet are selective agents that inhibit the growth of gram-positive bacteria but allow enteric gram-negative bacteria to grow. Neutral red is the pH indicator.

Formula*

Ingredients in grams per liter	MacConkey Agar Medium USP	MacConkey Agar (Harmonized)
Pancreatic digest of casein	1.5	–
Peptic digest of animal tissue	1.5	–
Peptone (Meat and casein)	–	3.0
Bile salts mixture	1.5	1.5
Pancreatic digest of gelatin	17.0	17.0
Lactose	10.0	10.0
Sodium chloride	5.0	5.0
Crystal violet	0.001	0.001
Neutral red	0.03	0.03
Agar	13.5	13.5

Final pH (at 25°C) 7.1 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 50.03 gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and (For MacConkey Agar Base add sterile 1% w/v desired carbohydrate solution) pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pinkish beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Red with purplish tinge, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacer aerogenes (13048)	luxuriant	Pink to red	More than 70%
Enterococcus faecalis (29212)	Fair to good	Colourless to pink	0%
Escherichia coli (25922)	Luxuriant	pink to red with bile precipitate	More than 70%
Proteus vulgaris (13315)	Luxuriant	Colourless	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	More than 70%
Shigella flexneri (12022)	Fair to good	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

Procedure

1. The surface of the medium should be dry when inoculated.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organismspresent in the specimen.

Interpretation of Results

1. Lactose fermenting bacteria produce pink to brick-red colonies and may be surrounded by a zone of bile precipitation.
2. Non-Lactose fermenting bacteria produce colourless colonies.

Precautions / Limitations

1. Incubation of plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gramnegative bacilli.
2. Some strains of M.smegmatis from humans may grow on MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate, but these strains can be differentiated from M.fortuitum complex by the 3-day arylsulphatase test.

3. Not all strains of E.coli ferment lactose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Agar with CV, NaCl and 0.15% Bile Salts IP AM50591

MacConkey Agar with CV, NaCl and 0.15% Bile Salts USP AM50592

MacConkey Agar with CV, NaCl and 0.15% Bile Salts BP AM50594

Use

MacConkey Agar Medium is a selective and differential medium for the detection of coliforms and enteric pathogens.

Summary

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of specimens like water, faeces and other sources suspected of containing these microorganisms. The original MacConkey Agar was based on the bile salt-neutral red-lactose agar of MacConkey, which was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts is designed to achieve more differentiation of lactose fermenters and non-lactose fermenters, for the promotion of superior growth of enteric pathogens. It is recommended by USP for microbial limit tests.

Principle

Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen and other nutrients, while lactose is the carbohydrate source. Bile salts and crystal violet are selective agents that inhibit the growth of gram-positive bacteria but allow enteric gram-negative bacteria to grow. Neutral red is the pH indicator.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Bile salts mixture	1.5
Pancreatic digest of gelatin	17.0
Lactose	10.0
Sodium chloride	5.0
Crystal violet	0.001
Neutral red	0.03
Agar	13.5

Final pH (at 25°C) 7.1 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 50.03 gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50°C and (For MacConkey Agar Base add sterile 1% w/v desired carbohydrate solution) pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pinkish beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Red with purplish tinge, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink to red	More than 70%
Enterococcus faecalis (29212)	Fair to good	Colourless to pink	More than 70%
Escherichia coli (25922)	Luxuriant	Pink to red with bile precipitate	More than 70%
Proteus vulgaris (13315)	Luxuriant	Colourless	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	More than 70%
Shigella flexneri (12022)	Fair to good	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. The surface of the medium should be dry when inoculated.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Interpretation of Results

1. Lactose fermenting bacteria produce pink to brick-red colonies and may be surrounded by a zone of bile precipitation.

2. Non-Lactose fermenting bacteria produce colourless colonies.

Precautions / Limitations

1. Incubation of plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gramnegative bacilli.
2. Some strains of M.smegmatis from humans may grow on MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate, but these strains can be differentiated from M.fortuitum complex by the 3-day arylsulphatase test.
3. Not all strains of E.coli ferment lactose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts (Agar Medium H)EP AM50593

Use

MacConkey Agar Medium is a selective and differential medium for the detection of coliforms and enteric pathogens, in compliance with EP.

Summary

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of specimens like water, faeces and other sources suspected of containing these microorganisms. The original MacConkey Agar was based on the bile salt-neutral red-lactose agar of MacConkey, which was used to differentiate strains of *Salmonella typhosa* from members of the coliform group.

MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts is designed to achieve more differentiation of lactose fermenters and non-lactose fermenters, for the promotion of superior growth of enteric pathogens. EP recommends it for tests for microbial contaminations.

Principle

Pancreatic digest of gelatin and peptones provide nitrogen and other nutrients, while lactose is the carbohydrate source. Bile salts and crystal violet are selective agents that inhibit the growth of gram-positive bacteria but allow enteric gram-negative bacteria to grow. Neutral red is the pH indicator.

Formula*

Ingredients in grams per liter

Peptones (meat and casein)	3.0
Bile salts	1.5
Pancreatic digest of gelatin	17.0
Lactose monohydrate	10.0
Sodium chloride	5.0
Crystal violet	0.001
Neutral red	0.03
Agar	13.5

Final pH (at 25ºC) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 50.03 gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Pinkish beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Red with purplish tinge, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35ºC.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink to red	More than 70%
Enterococcus faecalis (29212)	Inhibited	Colourless to pink	0%
Escherichia coli (25922)	Luxuriant	Pink to red with bile precipitate	More than 70%
Proteus vulgaris (13315)	Luxuriant	Colourless	More than 70%
Salmonella enteritidis (13076)	Luxuriant	Colourless	More than 70%
Shigella flexneri (12022)	Fair to good	Ccolourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. The surface of the medium should be dry when inoculated.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Interpretation of Results

1. Lactose fermenting bacteria produce pink to brick-red colonies and may be surrounded by a zone of bile precipitation.
2. Non-Lactose fermenting bacteria produce colourless colonies.

Precautions / Limitations

Incubation of plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli. Some strains of M. smegmatis from humans may grow on MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate, but these strains can be differentiated from M. fortuitum complex by the 3-day arylsulphatase test. Not all strains of E. coli ferment lactose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Agar without Crystal Violet and with 0.5% Bile Salts — AM50601

Use

MacConkey Agar without CV and with 0.5% Bile salts is used for isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacteria.

Summary

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of specimens like water, faeces and other sources suspected of containing these microorganisms. The original MacConkey Agar was based on the bile salt-neutral red-lactose agar of MacConkey, which was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. MacConkey Agar without Crystal Violet and with 0.5% Bile Salts is less selective than the original MaConkey Agar is used for isolating and cultivating gram-negative enteric microorganisms.

Principle

Peptone provide nitrogen and other nutrients, while lactose is the carbohydrate source. Bile salts is selective agents that inhibit the growth of gram-positive bacteria but allow enteric gram-negative bacteria to grow. Neutral red is the pH indicator.

Formula*

Ingredients in grams per liter

Peptone	20.0
Bile salts mixture	5.00
Lactose	10.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

Final pH (at 25°C) 7.4 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 52 gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. AVOID OVERHEATING.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Pinkish beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Orange red coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Colour of Colony
Enterobacter aerogenes (13048)	Luxuriant	Ppink to red
Enterococcus faecalis (29212)	Fair to good	Pale pink to red
Escherichia coli (25922)	Luxuriant	Pink to red
Proteus vulgaris (13315)	Luxuriant	Colourless
Salmonella serotypeEnteritidis (13076)	Luxuriant	Colourless
Shigella flexneri (12022)	Luxuriant	Colourless
Staphylococcus aureus (25923)	Fair to Good	Pale pink to red

Procedure

1. The surface of the medium should be dry when inoculated.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Interpretation of Results

1. Lactose fermenting bacteria produce pink to brick-red colonies and may be surrounded by a zone of bile precipitation.
2. Non-Lactose fermenting bacteria produce colourless colonies.

Precautions / Limitations

1. Incubation of plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.
2. Some strains of M.smegmatis from humans may grow on MacConkey Agar without Crystal Violet, NaCl & with Sodium Taurocholate, but these strains can be differentiated from M.fortuitum complex by the 3-day arylsulphatase test.

3. Not all strains of E.coli ferment lactose.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Double Strength with Neutral Red — AM1062/AM5062

MacConkey Broth Purple with Bromocresol Purple — AM1063/AM5063

MacConkey Broth with Neutral Red — AM1064/AM5064

Use

MacConkey Broth Double Strength with Neutral Red is used for the primary isolation of coliforms from large samples such as water and wastewater.

MacConkey Broth Purple with Bromocresol Purple is used for the presumptive identification of coliforms and for cultivating gram-negative, lactose fermenting bacilli from a variety of samples like water, milk and food.

MacConkey Broth with Neutral Red is a standard medium for the primary isolation as well as presumptive identification of coli-aerogenes group in food and water.

Summary

MacConkey Broth Double Strength with Neutral Red, MacConkey Broth Purple with Bromocresol Purple and MacConkey Broth with Neutral Red are all modifications of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator. In later publications, MacConkey suggested variations of this formula using neutral red as the indicator instead of litmus. Bile salts in the medium replaces the original sodium taurocholate to inhibit growth of gram-positive organisms.

The above mentioned MacConkey Broths are recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. These media are also included in the Official Methods of Analysis as well as pharmaceutical preparations (46, 114) and industrial products.

Principle

Peptone provides amino acids and other growth factors. Lactose is a carbon and energy source. Bile salts inhibit the growth of gram-positive organisms. Neutral red and bromocresol purple are the pH indicators. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter	MacConkey Broth Double Strength with Neutral Red	MacConkey Broth Purple with Bromocresol Purple	MacConkey Broth with Neutral Red
Peptone	40.0	20.0	20.0
Lactose	20.0	10.0	10.0
Bile Salts	10.0	-	5.0
Sodium Chloride	10.0	5.0	5.0
Neutral Red	0.15	-	0.075
Sodium Taurocholate	-	5.0	-
Bromocresol Purple	-	0.01	-
Final pH (at 25°C)	7.4±0.2	7.4±0.2	7.4±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.
   MacConkey Broth Double Strength with Neutral Red - 80.15 gms.
   MacConkey Broth Purple with Bromocresol Purple - 40 gms.
   MacConkey Broth with Neutral Red - 40 gms.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

MacConkey Broth Double Strength with Neutral Red & MacConkey Broth with Neutral Red - Red coloured, clear solution without any precipitate.

MacConkey Broth Purple with Bromocresol Purple - Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

MacConkey Broth Double Strength with Neutral Red, MacConkey Broth Purple with Bromocresol Purple & MacConkey Broth with Neutral Red

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Double Strength with Neutral Red BIS — AM10621/AM50621

Use

MacConkey Broth Double Strength with Neutral Red is used for the primary isolation of coliforms from large samples such as water and wastewater.

Summary

MacConkey Broth Double Strength with Neutral Red is a modifications of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator. In later publications, MacConkey suggested variations of this formula using neutral red as the indicator instead of litmus. Bile salts in the medium replaces the original sodium taurocholate to inhibit growth of gram-positive organisms. The above mentioned MacConkey Broths are recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. These media are also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Peptone provides amino acids and other growth factors. Lactose is a carbon and energy source. Bile salts inhibit the growth of grampositive organisms. Neutral red the pH indicators. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Peptone	40.0
Lactose	20.0
Bile salts	10.0
Sodium chloride	10.0
Neutral red	0.15

Final pH (at 25°C) 7.5 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 80.15 of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth (Harmonized) — AMH5063

MacConkey Broth USP — AM50636

Use

MacConkey Broth is used for the presumptive identification of coliforms from a variety of specimens like water, milk and food.

Summary

MacConkey Broth is recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. This medium is also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Pancreatic digest of gelatin provides amino acids and other growth factors. Lactose is a carbon and energy source. Bile salts inhibit the growth of Gram positive organisms. Bromocresol purple are the pH indicators.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	20.0
Lactose monohydrate	10.0
Dehydrated Ox bile	5.0
Bromocresol purple	0.01

Final pH (at 25ºC) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.01 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Purple with Bromocresol Purple IP AM10631/AM50631

Use

MacConkey Broth Purple with Bromocresol Purple is used for the presumptive identification of coliforms and for cultivating gramnegative, lactose fermenting bacilli from a variety of samples like water, milk and food in compliance with IP.

Summary

MacConkey Broth Purple with Bromocresol Purple is a modification of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator. In later publications, MacConkey suggested variations of this formula using neutral red as the indicator instead of litmus. It is recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. This media is also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Pancreatic digest of gelatin provides amino acids and other growth factors. Lactose is a carbon and energy source. Dehydrated Ox bile inhibit the growth of gram-positive organisms. Bromocresol purple is the pH indicator. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Bromo cresol purple	0.01
Pancreatic digest of gelatin	20.0
Lactose	10.0
Dehydrated Ox bile	5.0

Final pH (at 25°C) 7.3 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.01 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Purple with BCP (Broth Medium G) EP AM10632/AM50632

MacConkey Broth Purple with BCP (Broth Medium G) BP AM10633/AM50633

Use

MacConkey Broth Purple with Bromocresol Purple is used for the presumptive identification of coliforms and for cultivating gram-negative, lactose fermenting bacilli from a variety of samples like water, milk and food.

Summary

MacConkey Broth Purple with Bromocresol Purple is a modification of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator.

It is recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. This media is also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Pancreatic digest of gelatin provides amino acids and other growth factors. Lactose is a carbon and energy source. Dehydrated Ox bile inhibits the growth of gram-positive organisms. Bromocresol purple is the pH indicator. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	20.0
Lactose monohydrate	10.0
Bromo cresol purple	0.01
Dehydrated Ox bile	5.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.01 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Purple with BCP BIS AM10634/AM50634

Use

MacConkey Broth Purple with Bromocresol Purple is used for the presumptive identification of coliforms and for cultivating gram-negative, lactose fermenting bacilli from a variety of samples like water, milk and food.

Summary

MacConkey Broth Purple with Bromocresol Purple is a modification of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator.

It is recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. This media is also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Pancreatic digest of animal tissue provides amino acids and other growth factors. Lactose is a carbon and energy source. Sodium taurocholate inhibits the growth of gram-positive organisms. Bromocresol purple is the pH indicator. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.00
Lactose	10.00
Sodium taurocholate	5.00
Sodium chloride	5.00
Bromocresol Purple	0.02

Final pH (at 25ºC) 7.3±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.02 gms of the powder in 1000 ml distilled water..
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth Purple with BCP ISO AM10635/AM50635

Use

MacConkey Broth Purple with Bromocresol Purple is used for the presumptive identification of coliforms and for cultivating gramnegative, lactose fermenting bacilli from a variety of samples like water, milk and food.

Summary

MacConkey Broth Purple with Bromocresol Purple is a modification of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator. In later publications, MacConkey suggested variations of this formula using neutral red as the indicator instead of litmus. It is recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. This media is also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Peptone provides amino acids and other growth factors. Lactose is a carbon and energy source. Sodium Taurocholate inhibit the growth of gram-positive organisms. Bromocresol purple is the pH indicator. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Bromo cresol purple	0.01
Peptone	20.0
Lactose	10.0
Sodium Chloride	5.0

Sodium Taurocholate	5.0

Final pH (at 25°C) 7.4±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted Durham tubes.
2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Broth with Neutral Red BIS — AM10641/AM50641

Use

MacConkey Broth with Neutral Red is a standard medium for the primary isolation as well as presumptive identification of coliaerogenes group in food and water in compliance with BIS.

Summary

MacConkey Broth with Neutral Red are all modifications of the original bile salt broth recommended by MacConkey, which contained 0.5% sodium taurocholate and litmus as the indicator. In later publications, MacConkey suggested variations of this formula using neutral red as the indicator instead of litmus. Bile salts in the medium replaces the original sodium taurocholate to inhibit growth of gram-positive organisms. The above mentioned MacConkey Broths are recommended for use in microbiological examination of clinical specimens, foodstuffs and for direct plating and inoculation of water samples for coliform counts. These media are also included in the Official Methods of Analysis as well as pharmaceutical preparations and industrial products.

Principle

Peptone provides amino acids and other growth factors. Lactose is a carbon and energy source. Bile salts inhibit the growth of grampositive organisms. Neutral red and bromocresol purple are the pH indicators. Sodium chloride maintains the osmotic balance.

Formula*

Peptone	20.0
Lactose	10.0
Bile Salts	5.0
Sodium chloride	5.0
Neutral Red	0.075

Final pH (at 25°C) 7.5±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.07 gms of the powder in 1000 ml distilled water. MacConkey Broth Double Strength
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense into tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Salmonella serotype Choleraesuis (12011)	Fair to good	-	-
Staphylococcus aureus (25923)	Inhibited	-	-

Interpretation of Results

1. Lactose fermenting organisms grow well producing acid, causing the medium to turn yellow. Gas is also produced which collects in the inverted

Durham tubes.

2. Non-lactose fermenting organisms produce good growth but will not produce acid or gas.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MacConkey Sorbitol Agar AM50642

Use

MacConkey Sorbitol Agar is recommended for isolation and identification of enteropathogenic *Escherichia coli* strains associated with infant diarrhea.

Summary

Escherichia coli O157:H7 is a human pathogen associated with hemorrhagic colitis (78.2). MacConkey Sorbitol Agar is a variant of traditional MacConkey Agar used in the detection of *E. coli* O157:H7. *Escherichia coli* O157:H7 differs from most other strains of E. coli in being unable to ferment sorbitol. In MacConkey Sorbitol Agar, lactose is replaced by sorbitol. Most strains of *E. coli* ferment sorbitol to produce acid: *E. coli* O157:H7 cannot ferment sorbitol, so this strain uses peptone to grow. This raises the pH of the medium allowing the O157:H7 strain to be differentiated from other E. coli strains through the action of the pH indicator in the medium.

Principle

Peptic digest of animal tissues and proteose peptone provide the carbon and nitrogen while sodium chloride maintains the osmotic balance. Sorbitol is the source of energy. Bile salt mixture and crystal violet inhibit the Gram- positive organisms. Neutral red is a pH indicator.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	17.0
Proteose peptone	3.0
D- Sorbitol	10.0
Sodium chloride	5.0
Bile salt mixture	1.5
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Final pH (at 25°C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 50 gms of the powder in 1000ml of distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow to pink coloured homogeneous free flowing powder.

Prepared Appearance

Purplish red coloured clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Escherichia coli O157:H7	Luxuriant	Colourless	More than 70%
Escherichia coli (25922)	Luxuriant	Pink	More than 70%
Escherichia coli serotype 011 & 055	Luxuriant	Colourless	More than 70%
S. serotype Typhi (6539)	Luxuriant	Pink	More than 70%
Shigella flexneri (12022)	Luxuriant	Colourless	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Malonate Broth Ewing Modified AM1065/AM5065

Use

Malonate Broth Ewing Modified is used for the differentiation of *Enterobacteriaceae* on the basis of malonate utilization.

Summary

Leifson (66) developed a synthetic liquid medium, which differentiated *Aerobacter (Enterobacter)* from *Escherichia* species based on their ability to utilize malonate. Ewing et al (28) devised the modification, in which dextrose and yeast extract were incorporated. The addition of yeast extract, a source of vitamins, and a relatively small amount of dextrose, a minimal carbon source, was included in Ewing's modification to stimulate the growth of some organisms. Hence, the

medium supports the growth of organisms that cannot utilize malonate or ammonium salt, but any alkalinization produced by such organisms is buffered by the phosphate system and counteracted by the acid produced in the fermentation of small amount of dextrose. Organisms capable of utilizing malonate and ammonium sulphate produce an alkaline result (blue colour) in this medium. Malonate Broth Ewing Modified is recommended by APHA for the examination of foods (20) and milk (39) and is included in the Bacteriological Analytical Manual for testing food and cosmetics (113).

Principle

Organisms that simultaneously utilize malonate as its carbon source and ammonium sulphate as its nitrogen source produce an alkalinity due to the formation of sodium hydroxide. The alkali changes the colour of the bromothymol blue indicator in the medium from green to blue. The colour of the medium remains unchanged in the presence of organisms that cannot utilize these substances. Yeast extract and the small amount of dextrose provides vitamins and carbon respectively stimulating the growth of organisms that are unable to utilize malonate or ammonium salts. Some malonate negative strains produce a yellow colour due to the fermentation of dextrose only, which results in increased acidity causing the pH indicator (bromothymol blue) to change to yellow at pH 6.0. Dipotassium and Monopotassium phosphate act as buffering agents. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sodium Malonate	3.0
Ammonium Sulphate	2.0
Sodium Chloride	2.0
Yeast Extract	1.0
Dipotassium Phosphate	0.60
Monopotassium Phosphate	0.40
Dextrose	0.25
Bromothymol Blue	0.025

Final pH (at 25°C) 6.7 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 9.28 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Dispense in desired containers as per requirements.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light green coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Malonate Utilization
Enterobacter aerogenes (13048)	Luxuriant	+
Escherichia coli (25922)	Poor to fair	-
Klebsiella pneumoniae (13883)	Luxuriant	+
Salmonella serotype Typhimurium (14028)	Fair to good	-

Procedure

1. Inoculate tubes, using a light inoculum, with growth from an 18 to 24 hour pure culture.
2. Incubate the tubes with loosened caps for 18-48 hours at 35°C ± 2°C in an aerobic atmosphere.

Interpretation of Results

1. The bacterial genera in which the majority of species produce a positive alkaline reaction i.e. light blue to Prussian blue colour throughout the medium are *Enterobacter, Klebsiella and Citrobacter.*
2. The bacterial genera in which the majority of species produce a negative reaction i.e. colour of the medium remains unchanged, green or yellow are *Escherichia, Salmonella, Shigella, Edwardsiella, Yersinia, Serratia, Morganella, Proteus* and *Providencia.*

Precautions / Limitations

1. Some malonate-positive organisms produce only slight alkalinity.
2. Compare the tubes with an un-inoculated malonate tube.
3. Any trace of blue colour after a 48-hour incubation period denotes a positive reaction.
4. Before making a final negative interpretation, be sure that test tubes have been incubated for 48 hours.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Malt Agar — AM1066/AM5066

Use

Malt Agar is used for isolating and cultivating yeasts and moulds from foods and dairy products and carrying stock cultures of yeasts and moulds.

Summary

Malt Agar is recommended by APHA (20) for use in antibiotic and acidified standard methods for the determination of yeast and mould counts in food and

also for maintaining stock cultures of fungi. Malt Agar is included in the Bacteriological Analytical Manual for food testing (113).

Principle

Malt extract provides nutrients required for growth of microorganisms. The acidic pH of the medium allows the optimal growth of yeasts and moulds while restricting bacterial growth.

Formula*

Ingredients in grams per liter

Malt Extract	30.0
Agar	15.0

Final pH (at 25°C) 5.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 118°C (12 lbs pressure) for 15 minutes.
5. DO NOT OVERHEAT, as it will give a softer and darker agar.
6. To lower the pH, add sterile 1:10 lactic acid, do not reheat the medium.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 40-48 hours at 30°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Precautions / Limitations

1. Do not reheat the medium after the addition of acid, as this will hydrolyze the agar and reduce its solidifying properties.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Malt Extract Agar AM1067/AM5067

Malt Extract Broth AM1068/AM5068

Use

Malt Extract Agar is used for the enumeration, cultivation and isolation of yeasts and moulds while Malt Extract Broth is used for detection of yeasts, moulds and aciduric organisms.

Summary

Malt Extract Agar is similar to the formula of Galloway and Burgess (33) used for the detection, isolation and enumeration of yeasts and moulds. The use of malt extract for the propagation of yeasts and moulds is quite common. Reddish (91) described a culture medium prepared from malt extract that was a satisfactory substitute for wort. Thom and Church (112), following the formula of Reddish, used malt extract as a base from which they prepared Malt Extract Agar and Malt Extract Broth. This media are included in the Bacteriological Analytical Manual for food and cosmetics testing (113) and are recommended by APHA in examination of foods (20).

Principle

Malt Extract provides the energy source. Mycological peptone serves as the nitrogen source and gives rapid luxuriant growth with typical morphology and pigmentation. Agar is the solidifying agent. For mycological count it is advisable to adjust the reaction of the medium to more acidic with the addition of 10% lactic acid or antibiotics as sterile solutions to the molten medium immediately before pouring into sterile petri plates. The acidic pH of Malt Extract Agar allows the optimal growth of moulds and yeasts while restricting the growth of bacteria.

Formula*

Ingredients in grams per liter	Malt Extract Agar	Malt Extract Broth
Malt Extract	30.0	17.0
Mycological Peptone	5.0	3.0
Agar	15.0	-
Final pH (at 25°C)	5.4 ± 0.2	5.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Malt Extract Agar - 50 gms

   Malt Extract Broth - 20 gms
2. Mix thoroughly. Soak for 15 minutes.
3. Sterilize by autoclaving at 115°C (10 lbs pressure) for 10 minutes. DO NOT OVERHEAT.
4. Mix well before dispensing.
5. To adjust acidic pH use 10% lactic acid.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Malt Extract Agar - Light brown coloured, slightly opalescent gel.

Malt Extract Broth - Light brown coloured, slightly opalescent solution.

Cultural Response

Cultural characteristics after 48-72 hours at 25-30°C.

Organisms (ATCC)	Growth on Malt Extract Agar & in Malt Extract Broth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Precautions / Limitations

1. Avoid overheating Malt Extract Agar as it results in a softer gel.

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Mannitol Motility Test Medium AM10681/AM50681

Use

Mannitol Motility Test Medium is a semisolid medium suitable for determining motility and mannitol fermentation.

Summary

Semi-solid media have been employed for many years in the study of bacterial motility. Tittsler and Sandholzer in their study have sited its importance in detecting bacterial motility (111). Mannitol Motility Test Medium is a semi-solid medium that has been developed for rapid identification of *Enterobacteriaceae* on the basis of motility and mannitol utilization.

Principle

Peptic digest of animal tissue provides the nitrogen, minerals and amino acid nutrients essential for bacterial growth. Mannitol is the fermentable carbohydrate for energy source. Potassium nitrate provides additional nutrients to the medium. Phenol red is the pH indicator and agar is the solidification agent.

Formula*

Ingredients in grams per liter

Peptic Digest of animal Tissue	20.0
Mannitol	2.0
Potassium Nitrate	1.0
Phenol Red	0.04
Agar	3.0

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 26 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense into sterile test tubes.
4. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.
5. Cool the tubed medium in an upright position.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red slightly opalescent gel.

Cultural Response

Cultural response after 18 - 24 hours at 35 ± 2° C.

Organisms (ATCC)	Motility	Mannitol
Escherichia coli (25922)	+	+
Klebsiella pneumoniae	-	+
Proteus mirabilis (25933)	+	-

Procedure

1. Inoculate the medium using standard procedure like stab inoculation.
2. Incubate the tubes at 35 ± 2° C for 18-24 hours.

Interpretation of Results

1. Motility is observed as diffused growth away from the stab inoculation line.
2. Non-motile organism growth is seen along the stab line.
3. Mannitol fermentation is indicated by a change in colour of the medium from red to yellow.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

D-Mannitol, A. R. Sterile (g irradiated) AM506811

Use

D-Mannitol, A. R. Sterile (g irradiated) used for the media fill runs in dry filling injectable.

Summary

Routine sampling for sterility testing is not sensitive enough to detect any low level contamination in sterile pharmaceutical formulations. Sample numbers are too small and only gross contamination is likely to be detected. Pharmaceutical manufactures therefore need other means of guaranteeing the quality of their product. This is why process stimulations (Media Fill Run) supported by environmental monitoring is must in pharmaceutical industry.

The FDA guidelines have recommended using SCDM for liquid injectable and D-Mannitol for dry injectable. Regular dehydrated culture media or D.Mannitol is usually supplied in non sterile form which carries high bioburden and should not be directly taken into a controlled area therefore irradiated sterile SCDM/D-Mannitol is used for Media Fill Run. g irradiation also assumes that sterile products is free from Mycoplasma.

Principle

During Media Fill Run for validation of dry injectable g irradiated D-Mannitol is dispersed into individual vial/ampules. After completion of filling process individual vial is reconstituted with sterile distilled water (As per label claim of injection). Reconstituted vials an incubated at 35-370C and monitored till 14 days.

Formula*

Quality Control

Dehydrated Appearance Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance Colourless solution without any precipitate.

Cultural Response

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Mannitol Salt Agar (Harmonized) AMH5069

Mannitol Salt Agar AM1069/AM5069

Mannitol Salt Agar Medium IP AM10693/AM50693

Mannitol Salt Agar Medium USP AM10694/AM50694

Use

Mannitol Salt Agar is a selective medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens.

Summary

Koch (60) reported that only *staphylococci* grow on agar media containing 7.5% sodium chloride. Chapman (14) studied this phenomenon in detail and concluded that the addition of 7.5% salt to phenol red mannitol agar results in an improved medium for the isolation of plasma coagulating *staphylococci.* Mannitol Salt Agar is recommended by the USP (114) and IP (46) for use in Microbial Limit Tests. It is used for the detection and enumeration of coagulase positive staphylococci in milk, food and other specimens. This medium is also included in the Bacteriological Analytical Manual for cosmetics testing (113).

Principle

Proteose peptone and beef extract supplies essential growth factors such as nitrogen, carbon, sulphur and trace nutrients. The 7.5% salt concentration results in partial or complete inhibition of bacteria other than staphylococci. Mannitol fermentation, results in change in the phenol red indicator, (from red to yellow) which helps in the differentiation of *staphylococcal* species. Coagulase-negative species of staphylococci and micrococci do not ferment mannitol and grow as small red colonies surrounded by red or purple zones. Yellow coloured colonies should be tested for production of coagulase. Addition of 5% v/v Egg Yolk Emulsion (AS009) enables the detection of lipase activity of staphylococci along with mannitol fermentation. The salt clears the egg yolk emulsion and lipase production is detected as yellow opaque zone around the colonies. Coagulase positive *staphylococci* produce colonies surrounded by bright yellow zones while non-pathogenic *staphylococci* produce colonies with reddish purple zones.

Formula*

Ingredients in grams per liter

	Mannitol salt agar	Mannitol salt agar USP	Mannitol salt agar IP	Mannitol salt agar (Harmonized)
Beef extract	1.0	1.0	1.0	1.0
Proteose peptone	10.0	–	–	–
Sodium chloride	75.0	75.0	75.0	75.0
D-Mannitol	10.0	10.0	10.0	10.0

Phenol red	0.025	0.025	0.025	0.025
Agar	15.0	15.0	15.0	15.0
Pancreatic digest of casein	–	5.0	5.0	5.0
Peptic digest of animal tissue	–	5.0	5.0	5.0

Final pH (at 25°C) 7.4 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 111 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. OPTIONAL: Add 5% v/v Egg Yolk Emulsion (AS010).
5. Mix well, dispense as desired.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of the Colony	RGI
Escherichia coli (25922)	Inhibited	-	0%
Staphylococcus aureus (25923)	Good to luxuriant	Good to Yellow	More than 70%
Staphylococcus epidermidis (12228)	Fair to good	Red	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate plates for 24-48 hours at 35 ±2°C in an aerobic atmosphere.

Interpretation of Results

Typical colony morphology on Mannitol Salt Agar is as follows:

Staphylococcus aureus---------- Small to large with yellow zones.

Staphylococci other than S.aureus--------- Small to large with red zones.

Streptococci---------- No growth to trace growth.

Micrococci---------- Large white to orange.

Gram-negative bacteria---------- No growth to trace growth.

Precautions / Limitations

1. Negative plates should be re-incubated overnight before discarding.
2. Presumptive Staphalococcus aureus should be confirmed with a coagulase test.
3. A few strains of S.aureus may exhibit delayed fermentation of mannitol.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Mannitol Salt Broth — AM10695/AM50695

Use

Mannitol Salt Broth is a selective medium for the isolation and enumeration of *Staphylococcus* aureus from clinical and non-clinical specimens.

Summary

Koch, reported that only *staphylococci* grow on media containing 7.5% sodium chloride. Chapman, studied this phenomenon in detail and concluded that the addition of 7.5% salt to phenol red mannitol salt results in an improved medium for the isolation of plasma coagulating *staphylococci*. Mannitol Salt is recommended by the USP and IP for use in Microbial Limit Tests. It is used for the detection and enumeration of coagulase positive *staphylococci* in milk, food and other specimens. This medium is also included in the Bacteriological Analytical Manual for cosmetics testing. Because of the amount of peptones and beef extract, Mannitol Salt is a nutrient rich medium. Most bacteria (other than staphylococci) are inhibited by the high concentration of sodium chloride. Organisms capable of fermenting mannitol e.g. *Staphylococcus* aureus, cause a pH change in the media. With phenol red as the pH indicator the colonies appear with a yellow coloration

Principle

Proteose peptone and beef extract supplies essential growth factors such as nitrogen, carbon, sulphur and trace nutrients. The 7.5% salt concentration results in partial or complete inhibition of bacteria other than *staphylococci*. Mannitol fermentation, results in change in the phenol red indicator, (from red to yellow) which helps in the differentiation of *staphylococcal* species. Coagulase-negative species of *staphylococci* and micrococci do not ferment mannitol and grow as small red colonies surrounded by red or purple zones. Yellow coloured colonies should be tested for production of coagulase.

Formula*

Ingredients in grams per liter

Beef extract	1.0

Pancreatic digest of casein	5.0
Peptic digest of animal tissue	5.0
Sodium chloride	75.0
D-Mannitol	10.0
Phenol red	0.025

Final pH (at 25°C) 7.4 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 96 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well, dispense as desired.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear transparent solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C and then recovery on Mannitol Salt Agar.

Organisms (ATCC)	Growth	Colour of the medium	Recovery on Mannitol Salt Agar
Staphylococcus aureus (25923)	Good to luxuriant	Yellow or Orange	Yellow colonies
E. coli	Inhibited	Red	Inhibited

Procedure

1. Use standard procedures for isolation of Staphylococcus aureus from specimens.
2. Incubate plates for 24-48 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

Typical colony morphology on Mannitol Salt Agar is as follows:

Staphylococcus aureus---------- Small to large with yellow zones.

Staphylococci other than S.aureus---------- Small to large with red zones.

Streptococci---------- No growth to trace growth.

Micrococci---------- Large white to orange.

Gram-negative bacteria---------- No growth to trace growth.

Precautions / Limitations

1. Negative tubes should be re-incubated overnight before discarding.
2. Presumptive Staphalococcus aureus should be confirmed with a coagulase test.
3. A few strains of S.aureus may exhibit delayed fermentation of mannitol.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Marine Agar 2216 (Zobell Marine Agar) AM10691/AM50691

Use

Marine Agar 2216 (Zobell Marine Agar) is used for isolation and enumeration of heterotrophic marine bacteria.

Summary

Zobell Marine media are designed, based on the formula suggested by Zobell (122.1). Marine bacteria are very much essential to the life cycle of nearly all marine flora and fauna. Marine bacteria are of prime importance in food industry and marine life conservation and hence activity of these bacteria can be studied by the use of this medium.

Principle

This medium contain the nutrients which are required for the growth of marine bacteria. Medium is consist not only of minerals as sea water (122.2) but also has peptic digest of animal tissue and yeast extract as the better sources of nutrients for the marine bacteria as reported by Jones (49.2).

Formula*

Ingredients in grams per liter

Yeast extract	1.0
Peptone	5.0
Ferric citrate	0.10
Sodium chloride	19.45
Magnesium chloride	8.80
Sodium sulphate	3.24
Calcium chloride	1.80
Potassium chloride	0.55
Sodium bicarbonate	0.16
Potassium bromide	0.08
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Dipotassium phosphate	0.008
Agar	15.00

Final pH (at 25°C) 7.6 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 55.25 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light beige with a few dark particles, free flowing powder.

Prepared Appearance

Light amber, slightly opalescent may have a slight precipitate, may contain dark particle.

Cultural Response

Cultural characteristics after 2 – 3 days (longer if required) at 20°C .

Organisms (ATCC)	Growth	RGI
Vibrio fischeri (7744)	Good-luxuriant	More than 70%
Vibrio harveyi (14126)	Good-luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Marine Broth 2216 (Zobell Marine Broth) AM10692/AM50692

Use

Marine Broth 2216 (Zobell Marine Broth) is used for isolation and enumeration of heterotrophic marine bacteria.

Summary

Zobell Marine media are designed, based on the formula suggested by Zobell. Marine bacteria are very much essential to the life cycle of nearly all marine flora and fauna. Marine bacteria are of prime importance in food industry and marine life conservation and hence activity of these bacteria can be studied by the use of this medium.

Principle

This medium contain the nutrients which are required for the growth of marine bacteria. Medium is consist not only of minerals as sea water but also has peptic digest of animal tissue and yeast extract as the better sources of nutrients for the marine bacteria as reported by Jones.

Formula*

Ingredients in grams per liter

Yeast extract	1.0
Peptone	5.0
Ferric citrate	0.10
Sodium chloride	19.45
Magnesium chloride	8.80
Sodium sulphate	3.24
Calcium chloride	1.80
Potassium chloride	0.55
Sodium bicarbonate	0.16
Potassium bromide	0.08
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Dipotassium phosphate	0.008

Final pH (at 25°C) 7.6 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.25 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light beige with a few dark particles, free flowing powder.

Prepared Appearance

Light amber, with slight precipitate.

Cultural Response

Cultural characteristics after 2 – 3 days (longer if required) at 20°C .

Organisms (ATCC)	Growth
Vibrio fischeri (7744)	Good-luxuriant
Vibrio harveyi (14126)	Good-luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-Endo Agar LES AM106921/AM506921

Use

M Endo Agar LES is recommended for enumeration of coliforms in water using a two step membrane filter technique.

Summary

The membrane filter (MF) been an method detection and enumeration coliform organisms water. , Delaney and GrassoEndo Agar LES (Lawrence Experimental

Station) for testing water for coliform bacteria by a two-step membrane filter procedure (79.3). High numbers of coliforms are recovered by this method compared with the one step technique using M Endo Broth. In two-step membrane filter procedure lauryl tryptose broth is used as a preliminary enrichment medium. The American Public Health Association specifies using M Endo Agar LES in the standard total coliform membrane filtration procedure for testing drinking waterbottled water (17.1 & 55.3).

Principle

Peptic digest of animal tissue, casein enzymic hydrolysate and yeast extract act as a source of carbon, nitrogen, vitamins and minerals. Lactose and tryptose are the source of energy. Sodium chloride maintains the osmotic balance. Monopotassium phosphate and dipotassium phosphate are the buffering agents. Sodium deoxycholate and sodium lauryl sulphate are added as inhibitors for the Gram positive organisms. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution. Agar is the solidifying agent. Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	3.7
Casein enzymic hydrolysate	3.7
Lactose	9.4
Sodium chloride	3.7
Tryptose	7.5
Dipotassium phosphate	3.3
Monopotassium phosphate	1.0
Sodium sulphite	1.6
Yeast extract	1.2
Basic fuchsin	0.8
Sodium deoxycholate	0.1
Sodium lauryl sulphate	0.05
Agar	15.0

Final pH (at 25ºC) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 51 gms of powder in 980ml of distilled water.
2. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
3. Cool to 45^{0}C and aseptically add 20 ml of 95% ethanol.
4. Dispense adequate amount into petri plates. DO NOT EXPOSE PLATES TO DIRECT SUNLIGHT.

Warning: Basic fuchsin is a potential carcinogen and care must be taken to avoid inhalation and contamination of the skin.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous free flowing powder.

Prepared Appearance

Red coloured, slightly opalescent gel with precipitate.

Cultural Response

Cultural characteristics after 20-24 hours at 35ºC.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Escherichia coli	Luxuriant	Red – black with metallic Sheen	More than 70%
Enterobacter aerogenes (13048)	Luxuriant	Red – black with metallic Sheen	More than 70%
S. serotype Typhi (6539)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

Procedure

1. Place a membrane filter absorbent pad inside the cover of a petridish.
2. Add 1.8-2 ml of Lauryl Tryptose Broth (AM 1053/ AM 5053) to each pad.
3. Run the water sample through a membrane filter.
4. Place the filter, topside up, onto the pad containing Lauryl Tryptose Broth.
5. Incubate at 35^{0}C for 1.5-2.5 hours. Transfer the membrane from the pad to the surface of the M Endo Agar LES medium I the petri dish bottom, keeping the side on which the bacteria have been collected facing upward.
6. Leave the filter pad in the lid and incubate the plates in the inverted position at 35^{0}C for 20-24 hours.

Interpretation of Results

Red colour colonies with characteristic metallic sheen are considered as coliform.

Precautions / Limitations

1. Non-coliform colonies may produce typical colonies with metallic sheen.
2. Coliform organisms may also occasionally produce atypical colonies without metallic sheen. It is advisable to verify both the colony types.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

M-Endo Broth AM106922/AM506922

Use

M- Endo Broth is used for estimation of coliforms in water using a two step membrane filter.

Summary

The membrane filter (MF) been an method detection and enumeration coliform organisms water. numbers of coliforms are recovered by using this medium .

Principle

Peptic digest of animal tissue and yeast extract act as a source of carbon, nitrogen, vitamins and minerals. Lactose is the source of energy. Sodium chloride maintains the osmotic balance. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.0
Yeast extract	6.0
Lactose	25.0
Dipotassium phosphate	7.0
Basic fuchsin	1.0
Sodium sulphite	2.5

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 61.50 gms powder in 1000ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. Do not boil.
4. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Warning: Basic fuchsin is a potential carcinogen and care must be taken to avoid inhalation and contamination of the skin.

Quality Control

Dehydrated Appearance

Purple coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish orange solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 37°C.

Organisms (ATCC)	Growth	Colour of colony
Escherichia coli (25922)	Good-luxuriant	Pink with metallic sheen
S. s erotype Typhimurium (14028)	Good-luxuriant	Colourless to very light pink
Staphylococcus aureus (25923)	Inhibited	-

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-FC Agar Base AM506923

Use

M-FC Agar media is used for the detection and enumeration of faecal coliforms using membrane filter technique at higher temperature.

Summary

M-FC Agar was designed by Geldreich, clark, Huff and Bert (33.3). It is recommended by APHA for the detection and enumeration of faecal coliforms using membrane filter technique.

Principle

Proteose peptone, tryptose and yeast extract provide necessary nutrients for the growth of faecal coliforms. Lactose is the carbon source as well as fermentable carbohydrate in the medium. Bile salts inhibit the growth of contaminating gram-positive microorganisms. Aniline blue is a triphenyl methane dye which suppressive the growth of many gram-positive microorganisms.

Formula*

Ingredients in grams per liter

Tryptose	10.00
Proteose peptone	5.00
Yeast extract	3.00
Lactose	12.50
Bile salt mixture	1.50
Sodium chloride	5.00
Aniline blue	0.10
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 52.1 gms powder in 1000ml distilled water containing 10 ml 1% Rosolic Acid.

2. Mix thoroughly.
3. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.
4. Cool to 45°C and add 2 ml of M-FC Broth on sterile absorbent pad placed in a sterile petri plate.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

With addition of rosolic acid, red coloured slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 22-24 hours at .....

Organisms (ATCC)	Growth at 35°C	Growth at 45.5°C	Colour of the colony	RGI at 35°C	RGI at 45.5°C
Escherichia coli (25922)	Luxuriant	luxuriant	Light blue	More than 70%	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Inhibited	Pinkish	More than 70%	0%
Shigella flexneri (12022)	Luxuriant	Inhibited	Pinkish	More than 70%	0%
Enterococcus faecalis (29212)	Inhibited	Inhibited	--	0%	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-FC Broth Base AM506924

Use

M-FC Broth media is used for the detection and enumeration of faecal coliforms using membrane filter technique at higher temperature.

Summary

M-FC Broth was designed by Geldreich, clark, Huff and Bert (33.3). It is recommended by APHA for the detection and enumeration of faecal coliforms using membrane filter technique.

Principle

Proteose peptone, tryptose and yeast extract provide necessary nutrients for the growth of faecal coliforms. Lactose is the carbon source as well as fermentable carbohydrate in the medium. Bile salts inhibit the growth of contaminating gram-positive microorganisms. Aniline blue is a triphenyl methane dye which suppressive the growth of many gram-positive microorganisms.

Formula*

Ingredients in grams per liter

Tryptose	10.00
Proteose peptone	5.00
Yeast extract	3.00
Lactose	12.50
Bile salt mixture	1.50
Sodium chloride	5.00
Aniline blue	0.10

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.1 gms powder in 1000ml distilled water containing 10 ml 1% Rosolic Acid (AS0232).
2. Mix thoroughly.
3. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

With addition of rosolic acid, red coloured slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 22-24 hours at .....

Organisms (ATCC)	Growth at 35°C	Growth at 45.5°C	Colour of the colony
Escherichia coli (25922)	Luxuriant	Luxuriant	Light blue
S. serotype Typhimurium (14028)	Luxuriant	Inhibited	Pinkish
Shigella flexneri (12022)	Luxuriant	Inhibited	Pinkish
Enterococcus faecalis (29212)	Inhibited	Inhibited	--

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-(HPC) Heterotrophic Plate Count Agar Base AM506925

Use

M-(HPC) Heterotrophic Plate Count Agar is used for enumeration of heterotrophic microorganisms from water samples using membrane filter technique.

Summary

MM-(HPC) Agar Base, with added glycerol is recommended for heterotrophic plate counts of potable water, swimming pool and other waters (108.1). It is used as an alternative medium to Standard Methods Agar and R2A Agar.

Principle

Peptic digest of animal tissue is the source of nutrients for organisms which are not highly fastidious. Gelatin is utilized by microorganisms through a proteolytic mechanism. The addition of glycerol to the basal medium provides a source of carbon and energy.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.00
Gelatin	25.00
Agar	15.00

Final pH (at 25°C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspended 60 grams in 1000 ml distilled water containing 10 ml glycerol.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15lbs pressure (121°C) for 10 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C

Organisms (ATCC)	Growth	RGI
Escherichia coli(25922)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-(HPC) Heterotrophic Plate Count Broth Base AM506926

Use

M-(HPC) Heterotrophic Plate Count Broth is used for enumeration of heterotrophic microorganisms from water samples using membrane filter technique.

Summary

M-(HPC) Broth Base, with added glycerol is recommended for heterotrophic plate counts of potable water, swimming pool and other waters (108.1). It is used as an alternative medium to Standard Methods Agar and R2A Agar.

Principle

Peptic digest of animal tissue is the source of nutrients for organisms which are not highly fastidious. Gelatin is utilized by microorganisms through a proteolytic mechanism. The addition of glycerol to the basal medium provides a source of carbon and energy.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.00
Gelatin	25.00

Final pH (at 25°C) 7.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45 grams in 1000 ml distilled water containing 10 ml glycerol.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15lbs pressure (121°C) for 5 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Pseudomonas aeruginosa (27853)	Luxuriant
Enterococcus faecalis (29212)	Luxuriant

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Middlebrook 7H9 Agar Base — AM506927

Use

Middlebrook 7H9 Agar Base enriched with OADC enrichment are recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis.*

Summary

Middlebrook 7H9 Agar Base developed developed by Middlebrook et al., (80.2) is used for cultivating *Mycobateria* and for assaying INH content of Patients sera. The medium can also be used for preparing inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

Principle

This medium contains many inorganic salts which support the growth of Mycobacteria. Sodium citrate becomes citric acid in the medium which retains certain inorganic cations in the solution. This medium is supplemented for the growth of *Mycobacteria.* Middlebrook OADC Growth Supplement (AS0181) contains oleic acid, bovine albumin, dextrose, catalase and sodium chloride. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to *Mycobacteria* but albumin binds to those fatty acids and prevents toxic action on *Mycobacteria.* Toxic peroxides present in the medium are destroyed by catalase. Dextrose supplies energy while sodium chloride maintains osmotic equilibrium. Glycerol enhances the growth.

Formula*

Ingredients in grams per liter

Ammonium sulphate	0.50
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	0.10
Magnesium sulphate	0.05
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.04
L-Glutamic acid	0.50
Pyridoxine	0.001
Biotin	0.0005
Agar	15.00

Final pH (at 25°C) 6.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 19.6 gms powder in 1000ml distilled water.
2. Add either 2 ml glycerol or 0.5 g polysorbate 80.
3. Mix thoroughly.
4. Heat if necessary to dissolve the medium completely.
5. Cool to 45°C and add 2 ml of M-FC Broth on sterile absorbent pad placed in a sterile petri plate.
6. Sterilize by autoclaving at 15lbs pressure (121°C) for 10 minutes.
7. Cool to 45°C or below and aseptically add 1 vial of reconstituted Middlebrook OADC Growth Supplement. Mix well before dispensing.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear gel with slight precipitate.

Cultural Response

Cultural characteristics after 2-4 week at 35-37°C

Organisms (ATCC)	Growth	RGI
Mycobacterium tuberculosis H37 RV (25618)	Good-luxuriant	More than 70%
Mycobacterium smegmatis (14468)	Good-luxuriant	More than 70%
Mycobacterium fortuitum (6841)	Good-luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M-TEC Agar — AM5069261

Use

M-TEC Agar is recommended for isolation, differentiation and rapid enumeration of thermotolerant *Escherichia coli* from water by membrane filtration

Summary

M-TEC Agar is recommended for rapid isolation, differentiation and rapid enumeration of thermotolerant E. coli from water by membrane filtration. TEC stands for thermotolerant *E. coli,* the presence of which is widely used as an

indicator of faecal contamination in water.

Principle

Proteose peptone and yeast extract act as source of nitrogen, carbon, amino acids and vitamins. Potassium phosphate salts help in buffering the medium. Lactose is the source of fermentable carbohydrate. Bromocresol purple and bromophenol red serve as indicator. Sodium lauryl sulphate and sodium deoxycholate inhibit gram-positive bacteria.

Formula*

Ingredients in grams per liter

Proteose peptone	5.00
Yeast extract	3.00
Lactose	10.00
Sodium chloride	7.50
Potassium dihydrogen phosphate	1.00
Dipotassium hydrogen phosphate	3.30
Sodium lauryl sulphate	0.20
Sodium deoxycholate	0.10
Bromocresol purple	0.08
Bromphenol red	0.08
Agar	15.00

Final pH (at 25°C) 7.3±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.26 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Cream to greenish yellow homogeneous free flowing powder

Prepared Appearance

Dark purple coloured with red cast clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 2 hours and at 44.5°±0.5°C for 22 hours.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Good (further testing usingurease substrate should be performed)	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Follow applicable membrane filter procedures.
2. Incubate inoculated plates for 2 hours at 35 ± 2°C to resuscitate injured cells.
3. Transfer the plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 22 ± 2 hours.
4. Transfer countable filters to pads saturated with urea substrate. Prepare urea substrate by combining 2 g urea and 10 mg phenol red in 100 mL of purified water and adjust the pH to 5.0 ± 0.2. Store at 2-8°C and use within 1 week.

NOTE: Other methods may recommend an alternative pH. Prepare substrate according to recommended guidelines.

5. After 15-20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Expected Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as E. coli.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

M 7 Hr FC Agar AM5069262

Use

M7 Hr FC Agar is recommended for examination of water and wastewater.

Summary

M7 Hr FC Agar is a modified method of Van Donsel et al (115.5) and Reasoner et al (90.4), which is recommended by APHA (22.1) for the examination of water and wastewater for the presence of faecal coliforms by the membrane filter technique. This medium has an advantage over other media to yield results in 7 hours that generally are comparable to those obtained by the standard coliform method. Thus this medium is accepted for assessment of the sanitary quality of water during emergencies involving water treatment plant failure or line breaks in a distribution network. It is reliable and has sensitivity levels equal to those of the standard tests routinely used.

Principle

Biopeptone and yeast extract provide nutritional requirement to a wide variety of organisms. Lactose and mannitol are energy sources and sodium chloride maintains osmotic equilibrium of the medium. Sodium lauryl sulphate and sodium deoxycholate help to restrict the gram-positive and gram-negative bacterial flora present in water. Bromocresol purple and phenol red help as

indicators in the detection of organisms. This is a solid culture medium for the rapid detection of faecal coliforms by membrane filtration method. After filtering a suitable or desired volume of water, the membrane is placed on the surface of plate and then incubated at 41.5°C for 7 hours. Faecal coliform form yellow colonies, indicating lactose fermentation.

Formula*

Ingredients in grams per liter

Biopeptone	5.00
Yeast extract	3.00
Lactose	10.00
D-Mannitol	5.00
Sodium chloride	7.50
Sodium lauryl sulphate	0.20
Sodium deoxycholate	0.10
Bromo cresol purple	0.35
Phenol red	0.30
Agar	15.00

Final pH (at 25°C) 7.3±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 46.45 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. DO NOT AUTOCLAVE.
4. Mix well and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Beige to purple homogeneous free flowing powder.

Prepared Appearance

Dark pinkish purple coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 41.5°C for 7-18 hours

Organisms (ATCC)	Growth	Colour	RGI of Colony
Escherichia coli (25922)	Luxuriant	yellow	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Enterococcus faecalis (29212)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MLCB Agar (Mannitol Lysine Crystal Violet Brilliant Green) AM5069263

Use

MLCB (Mannitol Lysine Crystal Violet Brilliant Green Agar) is used for the isolation of *salmonellae* (not Salmonella typhi or Salmonella paratyphi A.).

Summary

Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB Agar) is based on the formula of Inoue et al (106.2) for the selective isolation of salmonellae from faeces and foods. Visual detection of very small numbers of hydrogen sulphide-producing strains is easy because of the distinctive colonial appearance. The concentration of Magnesium ions appears to be critical for maximum growth of *salmonellae* on MLCB Agar. van Schothorst et al. (114.1) showed that MLCB Agar did not inhibit any of the *salmonellae* species investigated.

MLCB Agar is not suitable for Salmonella typhi and *Salmonella paratyphi* A because of the inhibitory concentration of brilliant green.

Principle

Biopeptone and yeast extract provide nutritional requirement to a wide variety of organisms. Lactose and mannitol are energy sources and sodium chloride maintains osmotic equilibrium of the medium. Sodium lauryl sulphate and sodium deoxycholate help to restrict the gram-positive and gram-negative bacterial flora present in water. Bromocresol purple and phenol red help as indicators in the detection of organisms. This is a solid culture medium for the rapid detection of faecal coliforms by membrane filtration method. After filtering a suitable or desired volume of water, the membrane is placed on the surface of plate and then incubated at 41.5°C for 7 hours. Faecal coliform form yellow colonies, indicating lactose fermentation.

Formula*

Ingredients in grams per liter

Yeast Extract	5.0
Pepton	10.0
"Lab-Lemco' Powder	2.0
Sodium chloride	4.0
Mannitol	3.0
L-lysine hydrochloride	5.0
Sodium thiosulphate	4.0
Ammnium iron (III) citrate	1.0
Brilliant green	0.0125

Crystal violet	0.01
Agar	15

Final pH (at 25°C) 6.8±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 49.0 gms powder in 1000 ml distilled water.
2. Mix and bringgently to the boil with frequent agitation to dissolve the medium completely.
3. Cool to 50°C and pour approximately 20ml into sterile Petri dishes.
4. DO NOT AUTOCLAVE OR OVERHEAT.

Quality Control

Dehydrated Appearance

Green/straw coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, gel forms in Petri plates

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C

Organisms (ATCC)	Growth	Colour of Colony	RGI
Salmonella typhimurium (23564)	Luxuriant	Purple colour with black center	More than 70%
Escherichia coli (25922)	Inhibition	-	0%
Salmonella enteritidis (13076)	Luxuriant	Purple colour with black center	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Minimal Broth AM506927

Use

Minimal media are recommended for isolation and enumeration of *Bacillus cereus*.

Summary

Minimal media described by Lederberg for the isolation of nutritional mutants of *E. coli* and B. subtilis. The minimal media contains the necessary nutrients only for the growth of wild type *E. coli* and *B. subtilis* strains. The random isolation and delayed enrichment method, described by Lederberg, can be used to isolate nutritional mutants derived from irradiated cultures of wild type *E. coli* (65.1). With added dextrose it also supports the growth of nutritional mutants of *Bacillus subtilis*. B. subtilis mutants can be isolated by the same techniques as *E. coli* or by the penicillin method described by Nester et al., (84.3).

Principle

Peptone and yeast extract provide nitrogenous nutrients. Glucose is the fermentable carbohydrate and carbon source in the medium. Acetyl methyl carbinol is produced from glucose by the members of *Bacillus cereus* groups. After the inoculation and incubation at 35°C for 48 hours, the presence of acetyl methyl carbinol is determined by adding 0.2 ml of 40% potassium hydroxide and 0.6 ml of 5% alcoholic alpha-naphthol solution to 1 ml of culture tube. This reaction is hastened by the addition of a few crystals of creatine by which the purple colour development take place within 15 minutes.

Formula*

Ingredients Gms/Liter

Glucose	10.0
Peptone	5.0
Yeast Extract	5.0
Potassium dihydrogen phosphate	5.0

Final pH (at 25°C) 7.0 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25.0 gms of the powder in 1000 ml distilled.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Dark yellow coloured clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C

Organisms (ATCC)	Growth	RGI
Bacillus cereus (10876)	luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Milk Agar AM10692711/AM50692711

Use

Milk Agar is recommended for enumeration of microorganisms in milk and milk products.

Summary

Milk Agar is formulated as per the official medium described by Dept. of Health Memo (18.3). EEC Commission also recommends it for the examination of ice creams (57.1).

Principle

Peptic digest of animal tissue and yeast extract provide the essential nutrient for the growth of the microorganisms. Milk solids are the source of carbohydrate. Agar is used as an solidifying agent.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Yeast extract	3.0
Milk solids	1.0
Agar	15.0

Final pH (at 25ºC) 7.2 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 24gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-48 hours at 35ºC.

Organisms (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Good to luxuriant	More than 70%
Lactobacillus casei (9595)	Good to luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	More than 70%
Serratia marcescens (8100)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Using either pour plates or surface counting techniques may carry out bacterial enumeration.

Prepare milk dilutions of 1/10, 1/100, 1/1000 in ¼ strength Ringer's salt (AM 108531) solution.

(a) Pour plate: 1 ml of each dilution is pipetted aseptically into sterile petri plates and 10 ml of sterile milk agar is added to it and mixed thoroughly.

(b) Spread plate: Spread 1 ml of milk dilution over the surface of the solidified medium in a petri plate. (C)Incubate the plates at 35ºC for 18-48 hours.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Middlebrook 7H9 Broth Base AM5069272

Use

Middlebrook 7H9 Broth Base with added enrichment is recommended for cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

Summary

Middlebrook 7H9 Broth Base was formulated by Middlebrook (80.2.1) and Middlebrook *et al* and Schaeffer (80.2, 80.2.2). This medium with Middlebrook ADC Growth Supplement (AS01811)) and glycerol or polysorbate 80 is also recommended for cultivation of Mycobacteria and for assaying the INH content of the patients sera. The medium can also be used for preparing inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

Principle

Middlebrook media consists of many inorganic salts, which help, in growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Middlebrook ADC Growth Supplement (AS01811) contains bovine albumin, dextrose, catalase and sodium chloride. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source.

Catalase neutralizes toxic peroxides. Mycobacteria grow more rapidly in broth media; therefore primary isolation of all specimens can be done in Middlebrook 7H9 Broth Base. After processing the sample as required, inoculate the media

with the test specimen. Mycobacteria are strict aerobes and therefore increased CO2 tension and aerobic conditions must be provided during incubation.

Formula*

Ingredients in grams per liter

Ammonium sulphate	0.50
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	0.10
Magnesium sulphate	0.05
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.50
Pyridoxine	0.001
Biotin	0.0005

Final pH (at 25°C) 6.6±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 2.35 grams in 450 ml distilled water.
2. Add either 2 ml glycerol or 0.5 g polysorbate 80.
3. Heat if necessary to dissolve the medium completely.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.
5. Cool to 45°C or below and aseptically add contents of 1 vial of Middlebrook ADC Growth Supplement (AS01811).
6. Mix well before dispensing.

Quality Control

Dehydrated Appearance

Cream to beige homogeneous free flowing powder

Prepared Appearance

Light amber coloured clear solution in tubes

Cultural Response

Cultural characteristics observed with added Middlebrook ADC Growth Supplement (AS01811) and glycerol or Polysorbate 80 after an incubation at 35-37°C for 2-4 weeks

Organisms (ATCC)	Growth
Mycobacterium fortuitum (6841)	Good-luxuriant
Mycobacterium smegmatis (14468)	Good-luxuriant
Mycobacterium tuberculosis (25618)	Good-luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Middlebrook 7H11 Agar Base AM5069273

Use

Middlebrook 7H11 Agar Base with the addition of supplement is recommended for isolation, cultivation and sensitivity testing of Mycobacterium

Summary

Middlebrook 7H11 Agar is a modification of Middlebrook 7H10 Agar (80.2) used for the isolation, cultivation and sensitivity testing of M. tuberculosis. It was shown by Cohn et al (17.4) that the addition of casein enzymic hydrolysate enhanced the growth of more fastidious M. tuberculosis strains, which in turn was helpful in drug susceptibility testing (77.1). The media is enriched by the addition of Middlebrook OADC Growth Supplement (AS0181) and glycerol.

Principle

Middlebrook media contain a variety of inorganic salts, which help, in growth of Mycobacteria. Citric acid formed fromsodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Middlebrook OADC Growth Supplement (AS0181) contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source. Catalase neutralizes toxic peroxides. Malachite green partially inhibits other bacteria.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	1.00
Ammonium sulphate	0.50
Monopotassium phosphate	1.50
Disodium phosphate	1.50
Sodium citrate	0.40
Magnesium sulphate	0.05
L-Glutamic acid	0.50
Ferric ammonium citrate	0.04
Pyridoxine	0.001
Biotin	0.0005
Malachite green	0.001
Agar	15.000

Final pH (at 25°C) 6.6±0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 10.25 gms of the powder in 450 ml distilled water containing 2.5 ml Glycerol.

2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 50°C. Aseptically add contents of 1 vial of Middlebrook OADC Growth Supplement (AS0181). Mix thoroughly before dispensing.

Quality Control

Dehydrated Appearance

Light yellow to light green homogeneous free flowing powder

Prepared Appearance

Light amber coloured clear to slightly opalescent gel with greenish tinge forms in Petri plates

Cultural Response

Cultural characteristics after 2-4 weeks at 35-37°C.

Organisms (ATCC)	Growth	Growth Recovery	RGI
Mycobacterium fortuitum (6841)	Good-luxuriant	>=50%	More than 70%
Mycobacterium smegmatis (14468)	Good-luxuriant	>=50%	More than 70%
Mycobacterium tuberculosis H37RV (25618)	Good-luxuriant	>=50%	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Modified Rappaport Vassiliadis Medium AM506928

Modified Rappaport Vassiliadis Medium for Water Testing BIS AM506929

Modified Rappaport Vassiliadis Medium for Water Testing ISO AM506930

Use

Modified Rappaport Vassiliadis Medium is used for the selective enrichment of *Salmonella* from environmental and foods specimens.

Summary

Rappaport F. developed a culture media for the selective enrichment of *Salmonella,* which enable the *salmonella* to multiply freely and inhibit accompanying coliform organism like *Proteus* and *pseudomonas* etc (90.1). Vassiliadis modified Rappaport Broth by lowering the concentration of malachite green and raising the incubation temperature to 43°C (115.3). This modified Rappaport Enrichment Broth is RV or Rappaport-Vassiliadis Medium and has been found to be superior to other salmonella selective enrichment media, especially with small inoculum.

Principle

Papaic digest of soyabean-meal provide nitrogen compounds, carbon compounds and nutrients. Magnesium chloride increases the osmotic pressure in the medium. Malachite green makes the medium selective for *Salmonella*. chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium.

The ingredients magnesium chloride & malachite green and the low pH of the medium inhibit the growth of coliform contaminants and permit unrestricted development of salmonellae.

Formula*

Ingredients in grams per liter

Papaic digest of soyabean meal	5.00
Sodium chloride	8.00
Mono-Potassium phosphate	1.60
Magnesium chloride.6H_2O	40.00
Malachite green	0.04

Final pH (at 25°C) 5.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 30 gms (the equivalent weight of dehydrated medium per liter) of powder in 1110 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Blue coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 24-48 hours at 37°C and 43°C.

Organisms (ATCC)	Growth	Recovery on XLD Agar (Colour of colony)	RGI
S.serotype Typhimurium (23564)	Luxuriant	Red colonies with black center	More than 70%
Salmonella abony	Luxuriant	Red colonies with	More than 70%

NCTC (6017)		black center	
Salmonella serotype typhi *NCTC* (780)	Luxuriant	Red colonies with black center	More than 70%
Salmonella enterica (13076)	Luxuriant	Red colonies with black center	More than 70%
Escherichia coli (25922)	Fair	yellow	More than 70%
Staphylococcus aureus (6538)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. For pre-enrichment, aseptically add the test specimen to Buffered Peptone Water (AM10211/50211) and incubate at 37°C for 18-24 hours.
2. Inoculate 0.1 ml of the pre-enrichment Buffered peptone water culture in to the 10 ml of Modified Rappaport Vassiliadis and incubate at 30-350C for 18-24 hours.C for 24 to 48 hours.
3. Subculture the broth by streaking on to plates of X.L.D. Agar (AM1112 / 5112) and Brilliant Green Agar, Modified (AM1018 / 5018). Incubate at 30-35°C for 18-48 hours.

Interpretation of Results

Examine cultured plates for typical Salmonella colonies.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Modified Teepol Broth (Twin Pack) ISO AM506931

Use

Modified Teepol Broth (Twin Pack) ISO is used for selective isolation and identification of enteric lactose fermenting bacteria.

Summary

Modified Teepol Broth is formulated as described by Burman (11.3), where he substituted Teepol in place of bile salts in the formulation of Membrane Enrichment Teepol Broth. The use of Teepol in place of bile salts was previously recommended by Jameeson and Emberley (47.1). Burman (11.4) showed that if a preliminary incubation is carried out at a lower temperature resuscitation is not required. Non-chlorinated organisms benefit from 4 hour incubation at 30°C but chlorinated organisms require 6 hours incubation at 25°C.

Principle

The coliform and *Escherichia coli* count are made on separate volumes of water. The water samples are filtered through membrane filter and this filter is placed face upwards on an absorbent pad saturated with Modified Teepol Broth. The yellow colonies formed are further identified.

Presumptive coliform organisms: Yellow colonies from membranes incubated at 35°C, when subcultured in Lactose Peptone Water produce gas at 35°C after 43 hours.

Presumptive *Escherichia coli* : Yellow colonies from membrane at 44°C produce gas and indole after 24 hours.

Formula*

Ingredients in grams per liter

Part A :	
Peptic digest of animal tissue	40.00
Yeast extract	6.00
Lactose	30.00
Phenol red	0.2
Part B:	
Teepol	4.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 76.2 grams of Part A in 1000 ml distilled water containing 4 grams of Part B.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in tubes containing inverted Durham's tubes.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A: Pink coloured, homogeneous, free flowing powder.

Part B: Colourless viscous solution.

Prepared Appearance

Red coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 24-48 hours at .....

Organisms (ATCC)	Growth at 35°C	Growth at 44°C
Escherichia coli (25922)	+	+
Enterobacter aerogenes (13048)	+	–

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Modified Tergitol 7 Agar Base ISO

AM506932

Use

Modified Tergitol 7 Agar Base is used for selective isolation and enumeration of coliform organisms in water by membrane filteration method.

Summary

Tergitol 7 Agar is a selective and differential medium for the detection and enumeration of coliforms in water. Chapman, (16.2 & 16.3) modified his original formula of Tergitol 7 Agar by addition of Triphenyl Tetrazolium Chloride (TTC). It is now recommended by ISO Committee (46.4).

Principal

Tergitol 7 is a selective agent (89.3) which inhibits gram positive organisms and minimizes swarming of *Proteus* species enabling better coliform recovery. Lactose fermentation is observed by change in colour of bromothymol blue, the pH indicator. Triphenyl Tetrazolium Chloride (TTC) allows earlier recognition and identification of *Escherichia coli* and *Enterobacter aerogenes* in water and food (81.3). TTC is rapidly reduced by coliforms except *Escherichia coli* and *Enterobacter aerogenes* to insoluble fromazan which gives red colour to the colonies. The lactose fermenters show greenish yellow colonies with yellow zones while lactose non fermenters show red colonies surrounded by blue zones.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.00
Yeast extract	6.00
Meat extract	5.00
Lactose	20.00
Bromo thymol blue	0.05
Tergitol	0.10
Agar	16.00

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 57.15 in 1000 ml distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to 45-50°C.
5. Add 2.5 ml of 1 % 2,3,5 Triphenyl Tetrazolium Chloride (AS0271).
6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Green coloured, clear to slightly opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C

Organisms (ATCC)	Growth	Colour or colony*	Colour of colony**	RGI
Escherichia coli (25922)	Luxuriant	Yellow	Yellow with yellow zone	More than 70%
Enterobacter aerogenes	Luxuriant	Yellow	Greenish yellow	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant	Yellow	Greenish yellow	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Colurless	Red with bluish zone	More than 70%
Pseudomonas aeruginosa (27853)	Good	Colourless	Red with bluish zone	More than 70%
Proteus vulgaris (13315)	Good	Colourless	Red with bluish zone	More than 70%
Staphylococcus aureus (25923)	Inhibited	–	–	0%

Key: *= on plain medium

**= on medium with TTC

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Motility Test Medium

AM506933

Use

Motility Test Medium is recommended for detection of bacterial motility.

Summary

Bacterial motility is an important determinant in making a final species identification. In 1936, Tittsler and Sandholzer first introduced the use of semi solid agar for the detection of bacterial motility (90.2). Edward and Ewing formulated Motility Test medium (23.3).

Principle

Tryptose serves as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. In motility media agar concentrations is used higher than 0.3% to produce semi solid media. In semi solid media motility is detected

more easily. Motile organisms spread out from the line of inoculation, while non-motile organisms grow only along the stab line.

Formula*

Ingredients in grams per liter

Tryptose	10.0
Sodium chloride	5.0
Agar	5.0

Final pH (at 25°C) 7.2 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 20 gms of powder in 1000ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Dispense in test tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes. Allow tubed medium to cool in an upright position.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel form in tubes as butts.

Cultural Response

Cultural characteristics after 18- 48 hours at 35-37°C.

Organisms (ATCC)	Growth	Motility
Escherichia coli (25922)	Luxuriant	+
Enterobacter aerogenes (13048)	Luxuriant	+
Klebsiella pneumoniae (13883)	Luxuriant	-
Staphylococcus aureus (25923)	Luxuriant	-
Salmonella enteritidis (13076)	Luxuriant	+

Procedure

1. Tubes are inoculated by stabbing with a straight wire.
2. Incubate tubes for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Interpretation of Results

Motile organisms spread out from the line of inoculation. But the non-motile organisms grow only along the line of inoculation.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Motility Test Medium (Edwards and Ewing) BIS AM506934

Use

Motility Test Medium (Edwards and Ewing) BIS is used for testing motility of enteric bacteria in compliance with BIS specifications IS: 5887 (Part 1 and Part 5) 1976 reaffirmed, 1986.

Summary

Bacterial motility is an important determinant in making a final species identification. In 1936, Tittsler and Sandholzer first introduced the use of semi solid agar for the detection of bacterial motility. Edward and Ewing formulated Motility Test medium.

Principle

Peptone serves as a source of carbon, nitrogen, vitamins and minerals. Meat extract also serves as a good source of nutrition. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. In motility media agar concentrations is used higher than 0.3% to produce semi solid media. In semi solid media motility is detected more easily. Motile organisms spread out from the line of inoculation, while non-motile organisms grow only along the stab line.

Formula*

Ingredients in grams per liter

Peptone	10.0
Meat extract	3.0
Sodium chloride	5.0
Agar	4.0

Final pH (at 25°C) 7.5 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 22 gms of powder in 1000ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Dispense 8ml amounts in test tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Yellow coloured, clear gel form in tubes as butts.

Cultural Response

Cultural characteristics after 18- 24 hours at 35-37°C.

Organisms (ATCC)	Growth	Motility
Escherichia coli (25922)	Luxuriant	+
Enterobacter aerogenes (13048)	Luxuriant	+
S. serotype Enteritidis (13076)	Luxuriant	+

Klebsiella pneumoniae (13883)	Luxuriant	-
Staphylococcus aureus (25923)	Luxuriant	-

Procedure

1. Tubes are inoculated by stabbing with a straight wire.
2. Incubate tubes for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Interpretation of Results

Motile organisms spread out from the line of inoculation. But the non-motile organisms grow only along the line of inoculation.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MR-VP Medium AM1070/AM5070

Use

MR-VP Medium also known as Buffered Peptone Glucose Broth is used for the differentiation of *coli-aerogenes* group by means of the Methyl Red and Voges-Proskauer reactions.

Summary

Clark and Lubs found that the addition of methyl red to cultures of *E.coli* resulted in a red colour due to the high acidity produced during dextrose fermentation. Voges-Proskauer reported red colouration after the addition of potassium hydroxide to specific culture media with organisms in it. Thus, the investigators developed MR-VP Medium, which enables both tests to be performed in the same medium in different tubes. ISO has recommended this medium for the detection of *coli-aerogenes* group. MR-VP Medium is included in the Bacteriological Analytical Manual for food and cosmetics testing (113) and is also recommended by APHA for the examination of foods (20) and milk (39).

Principle

Methyl red positive organisms produce high levels of acid during fermentation of dextrose, overcoming the phosphate buffering system and produce a red colour on the addition of methyl red pH indicator.

In Voges-Proskauer test, the red colour produced by the addition of potassium hydroxide to cultures of certain microbial species is due to their ability to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose. The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl, which reacts with creatine to give a red colour, which is a positive VP test.

Formula*

Ingredients in grams per liter

Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0

Final pH (at 25°C) 6.9 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 17 gms of the powder in 1000 ml distilled water and mix well.
2. If necessary, heat to dissolve the medium.
3. Dispense 10 ml amounts in test tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 48 hours at 30°C.

Organisms (ATCC)	Growth	MR Test	VP Test
Enterobacter aerogenes (13048)	Luxuriant	- (yellow)	+ (red)
Escherichia coli (25922)	Luxuriant	+ (red)	- (no change)
Klebsiella pneumoniae (23357)	Luxuriant	-(yellow)	+(red)

Procedure

1. Using a light inoculum, inoculate tubes of MR-VP Medium with an 18-24 hour pure cultures.
2. Incubate tubes aerobically at 30°C for a minimum of 48 hours for Voges-Proskaeur test and preferably for 5 days for Methyl Red test.
3. Prepare the methyl red indicator by dissolving 0.1 gm of methyl red in 300 ml of 95% ethyl alcohol. Add sufficient water to make 500 ml.
4. After the appropriate incubation period, aseptically remove aliquots of the medium and conduct the tests.
   - Methyl Red Test- Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the result immediately.
   - Voges-Proskaeur Test- Add 15 drops of reagent A (5% w/v a-naphthol in absolute alcohol) and 5 drops of reagent B (40 % w/v Potassium hydroxide in distilled water) to 1 ml of broth culture. Shake well after the addition of each reagent to aerate the sample.

Interpretation of Results

1. Methyl Red Test
   - Positive test - red colour at the surface of the medium.
   - Negative test - yellow colour at the surface of the medium.

2. Voges-Proskauer Test
   - Positive test - development of a distinct red colour within 5 minutes.
   - Negative test - appearance of a yellow colour or copper like colour on the surface of the medium.
3. Certain species within *Enterobacteriaceae* genera may react differently or give variable results.

Precautions / Limitations

1. While adding the VP reagents to the medium, it is important that the a-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.
2. False positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.
3. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the *Enterobacteriaceae.*
4. A precipitate may form in the potassium hydroxide reagent solution. The precipitate has not been shown to reduce the effectiveness of the reagent.
5. Most members of the family *Enterobacteriaceae* give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both.
6. Incubation time for the methyl red test cannot be shortened by increasing the concentration of dextrose in the medium or by heavily inoculating the broth.
7. Incubate MR negative tests for more than 48 hours and test again.
8. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials, the use of media, low in these substances (MR-VP Medium) is recommended for this test.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MR-VP Medium BIS — AM10701/AM50701

Use

MR-VP Medium is used for the differentiation of *coli-aerogenes* group by means of the Methyl Red and Voges-Proskauer reactions in compliance with BIS.

Summary

Clark and Lubs (12.1) found that the addition of methyl red to cultures of *E.coli* resulted in a red colour due to the high acidity produced during dextrose fermentation. Voges-Proskauer (116.1) reported red colouration after the addition of potassium hydroxide to specific culture media with organisms in it. Thus, the investigators developed MR-VP Medium, which enables both tests to be performed in the same medium in different tubes. ISO has recommended this medium for the detection of *coli-aerogenes* group. MR-VP Medium is included in the Bacteriological Analytical Manual for food and cosmetics testing and is also recommended by APHA for the examination of foods and milk.

Principle

Methyl red positive organisms produce high levels of acid during fermentation of dextrose, overcoming the phosphate buffering system and produce a red colour on the addition of methyl red pH indicator. In Voges-Proskauer test, the red colour produced by the addition of potassium hydroxide to cultures of certain microbial species is due to their ability to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose (77.1). The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl, which reacts with creatine to give a red colour, which is a positive VP test.

Formula*

Ingredients in grams per liter

Peptone	5.0
Dextrose	5.0
Dipotassium hydrogen phosphate	5.0

Final pH (at 25°C) 7.5 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15 gms of the powder in 1000 ml distilled water and mix well.
2. If necessary, heat to dissolve the medium.
3. Dispense 10 ml amounts in test tubes.
4. Sterilize by autoclaving at 115°C (10lbs pressure) for 10 minutes.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

A Differential Dehydrated Culture Medium

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 48 hours at 30°C.

Organisms (ATCC)	Growth	MR Test	VP Test
Enterobacter aerogenes (13048)	Luxuriant	- (Yellow)	+ (Red)
Escherichia coli (25922)	Luxuriant	+ (Red)	- (No change)
Klebsiella pneumoniae (23357)	Luxuriant	-(Yellow)	+(Red)

Procedure

1. Using a light inoculum, inoculate tubes of MR-VP Medium with an 18-24 hour pure cultures.
2. Incubate tubes aerobically at 30°C for a minimum of 48 hours for Voges-Proskaeur test and preferably for 5 days for Methyl Red test.
3. Prepare the methyl red indicator by dissolving 0.1 gm of methyl red in 300 ml of 95% ethyl alcohol. Add sufficient water to make 500 ml.
4. After the appropriate incubation period, aseptically remove aliquots of the medium and conduct the tests.
   - Methyl Red Test- Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the result immediately.
   - Voges-Proskaeur Test- Add 15 drops of reagent A (5% w/v naphthol in absolute alcohol) and 5 drops of reagent B (40 % w/v Potassium hydroxide in distilled water) to 1 ml of broth culture. Shake well after the addition of each reagent to aerate the sample.

Interpretation of Results

1. Methyl Red Test
   - Positive test - red colour at the surface of the medium.
   - Negative test - yellow colour at the surface of the medium.
2. Voges-Proskauer Test
   - Positive test - development of a distinct red colour within 5 minutes.
   - Negative test - appearance of a yellow colour or copper like colour on the surface of the medium.
3. Certain species within enterobacteriaceae genera may react differently or give variable results.

Precautions / Limitations

1. While adding the VP reagents to the medium, it is important that the a-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.
2. False positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.
3. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the enterobacteriaceae.
4. A precipitate may form in the potassium hydroxide reagent solution. The precipitate has not been shown to reduce the effectiveness of the reagent.
5. Most members of the family enterobacteriaceae ive either a positive MR test or a positive VP test. However, certain organisms such as Hafnia alvei and Proteus mirabilis ay give a positive result for both.
6. Incubation time for the methyl red test cannot be shortened by increasing the concentration of dextrose in the medium or by heavily inoculating the broth.
7. Incubate MR negative tests for more than 48 hours and test again.
8. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials, the use of media, low in these substances (MR-VP

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MR-VP Medium ISO — AM10702/AM50702

Use

MR-VP Medium used for the differentiation of coli-aerogenes group by means of the Methyl Red and Voges-Proskauer reactions in compliance with ISO.

Summary

Clark and Lubs found that the addition of methyl red to cultures of *E.coli* resulted in a red colour due to the high acidity produced during dextrose fermentation. Voges-Proskauer reported red colouration after the addition of potassium hydroxide to specific culture media with organisms in it. Thus, the investigators developed MR-VP Medium, which enables both tests to be performed in the same medium in different tubes. ISO has recommended this medium for the detection of *coli-aerogenes* group. MR-VP Medium is included in the Bacteriological Analytical Manual for food and cosmetics testing and is also recommended by APHA for the examination of foods and milk .

Principle

Methyl red positive organisms produce high levels of acid during fermentation of dextrose, overcoming the phosphate buffering system and produce a red colour on the addition of methyl red pH indicator. In Voges-Proskauer test, the red colour produced by the addition of potassium hydroxide to cultures of certain microbial species is due to their ability to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose. The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl, which reacts with creatine to give a red colour, which is a positive VP test.

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	3.5
Peptic digest of animal tissue	3.5

Dextrose	5.0
Dipotassium phosphate	5.0

Final pH (at 25°C) 6.9 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 17 gms of the powder in 1000 ml distilled water and mix well.
2. If necessary, heat to dissolve the medium.
3. Dispense 10 ml amounts in test tubes.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 48 hours at 30°C.

Organisms (ATCC)	Growth	MR Test	VP Test
Enterobacter aerogenes (13048)	Luxuriant	- (Yellow)	+ (Red)
Escherichia coli (25922)	Luxuriant	+ Red)	- (No change)
Klebsiella pneumoniae (23357)	Luxuriant	-(Yellow)	+(Red)

Procedure

1. Using a light inoculum, inoculate tubes of MR-VP Medium with an 18-24 hour pure cultures.
2. Incubate tubes aerobically at 30°C for a minimum of 48 hours for Voges-Proskaeur test and preferably for 5 days for Methyl Red test.
3. Prepare the methyl red indicator by dissolving 0.1 gm of methyl red in 300 ml of 95% ethyl alcohol. Add sufficient water to make 500 ml.
4. After the appropriate incubation period, aseptically remove aliquots of the medium and conduct the tests.
   - Methyl Red Test- Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the result immediately.
   - Voges-Proskaeur Test- Add 15 drops of reagent A (5% w/v a-naphthol in absolute alcohol) and 5 drops of reagent B (40 % w/v Potassium hydroxide in distilled water) to 1 ml of broth culture. Shake well after the addition of each reagent to aerate the sample.

Interpretation of Results

1. Methyl Red Test

   Positive test - red colour at the surface of the medium.

   Negative test - yellow colour at the surface of the medium.
2. Voges-Proskauer Test

   Positive test - development of a distinct red colour within 5 minutes.

   Negative test - appearance of a yellow colour or copper like colour on the surface of the medium.
3. Certain species within Enterobacteriaceae genera may react differently or give variable results.

Precautions / Limitations

1. While adding the VP reagents to the medium, it is important that the a-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.
2. False positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.
3. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the Enterobacteriaceae.
4. A precipitate may form in the potassium hydroxide reagent solution. The precipitate has not been shown to reduce the effectiveness of the reagent.
5. Most members of the family Enterobacteriaceae give either a positive MR test or a positive VP test. However, certain organisms such as Hafnia alvei and Proteus mirabilis may give a positive result for both.
6. Incubation time for the methyl red test cannot be shortened by increasing the concentration of dextrose in the medium or by heavily inoculating the broth.
7. Incubate MR negative tests for more than 48 hours and test again.
8. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials, the use of media, low in these substances (MR-VP Medium) is recommended for this test.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Mueller Hinton Agar AM1071/AM5071

Use

Mueller Hinton Agar is used for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria by the Bauer-Kirby method.

Summary

Mueller Hinton Agar was originally developed for the cultivation of *Neisseria* (82). These organisms are now isolated on selective media. Since clinical

laboratories were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others (6) developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium. Subsequently, international collaborative study confirmed the value of Mueller Hinton Agar for this purpose due to its relatively good reproducibility, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium. Mueller Hinton Agar complies with the requirements of World Health Organization and is specified in the FDA's Bacteriological Analytical Manual for food testing (113).

For additional details refer to The National Committee for Clinical Laboratory Standards (NCCLS) which contains the performance standard for the Baeur-Kirby procedure (84). This procedure is recommended for testing rapidly growing aerobic or facultative anaerobic bacterial pathogens, such as staphylococci, members of the *Enterobacteriaceae*, aerobic gram-negative rods, e.g. *Pseudomonas* species and *Acinetobacter* species, enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e. *H.influenza, N.gonorrhoeae, S.pneumoniae* and other streptococci. The NCCLS Document M2, Performance for Antimicrobial Disc Susceptibility Tests, recommends Mueller Hinton Agar supplemented with 5% defibrinated sheep blood for fastidious organisms.

Principle

Casein acid hydrolysate and beef infusion supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of starch during autoclaving provides a small amount of dextrose, which is a source of energy.

Formula*

Ingredients in grams per liter

Casein Acid Hydrolysate	17.5
Beef, Infusion from	300.0
Starch	1.5
Agar	17.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38 gms of the powder in 1000 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely. DO NOT OVER HEAT.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well before pouring.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Luxuriant	More than 70%
Neisseria gonorrhoeae (49226)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus faecalis (19433)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Standard Method

1. Gram staining is done before starting susceptibility testing to confirm the culture purity and to determine appropriate battery of tests.
2. 3-5 well-isolated colonies should be selected and transferred to 4-5 ml of a suitable broth using an inoculating needle.
3. Incubate the broth (usually 2-6 hours) at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland's barium sulphate standard. This results in a suspension containing approximately 1 - 2 X 10^8 CFU/ml.
4. Adjust the turbidity to the barium sulphate standard. For the diluents use sterile broth or sterile saline. The turbidity of the standard and the test inoculums should be compared by holding both tubes in front of a white background with finely divided lines or by use of a photometric device.
5. Within 15 minutes of adjusting the turbidity of the inoculum, immerse a sterile cotton swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streaking to obtain even inoculation. Swab the rim of the agar bed too.
7. The lid may be left ajar for 3-5 minutes and the plate held at room temperature for not more than 15 minutes to allow the surface moisture to be absorbed before applying the antibiotic discs.
8. Apply discs by means of an antimicrobial dics dispenser, aseptically, at least 24 mm apart. Preferably, deposit Penicillin and Cephalosporin discs not more than 10 mm from the edge of the Petri dish, and their centers at least 30 mm apart. Avoid placing such discs adjacent to one another. Tap the discs with a sterile needle or forceps after placing them on the agar for complete contact with the medium surface.
9. Within 15 minutes of applying the discs, invert the plates and incubate at 37°C. With non-fastidious organisms the plates should not be incubated

under an increased concentration of carbon dioxide.

10. Examine plates after 16-18 hours of incubation. A full 24 hours is recommended for *S.aureus* with oxacillin to detect methicillin-resistant *S.aureus* (MRSA) and for *Enterococcus* species when tested with vancomycin to detect vancomycin resistant strains. Growth within the apparent zone of inhibition is indicative of resistance.

Interpretation of Results

1. A confluent "lawn" of growth should be obtained. Too light inoculum gives isolated colonies and the test should be repeated. Measure the diameter of the zones of complete inhibition, including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the inverted plate over a black, non-reflecting background, and illuminated from above. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies, which can be detected with difficulty near the edge of the obvious zone of inhibition. The zone diameters measured around the discs should be compared with those in the NCCLS Document M100 (M2).
2. *S.aureus* when tested with oxacillin discs is an exception, as are enterococci when tested with vancomycin. In these cases, transmitted light should be used to detect a haze of growth around the disc, which is shown, by "occult resistant" MRSA strains or vancomycin-resistant enterococci. With *Proteus* species, if the zone of inhibition is distinct enough to measure, disregard any swarming inside the zone. With trimethoprim and sulphonamides, antagonistics in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
3. The results obtained with specific organisms may be reported as resistant, intermediate or susceptible.

   NOTE: Informational supplements to NCCLS Document M2, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations.

Precautions / Limitations

1. Unsupplemented Mueller Hinton Agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *S.pneumoniae*.
2. Numerous factors can affect the result: inoculum size, rate of growth, media formulation, pH, length of incubation, disc content, drug diffusion rates, and measurement of endpoints. Hence, strict adherence to protocol is required to ensure reliable results.
3. Mueller Hinton Agar deeper than 4 mm may cause false resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.
4. pH outside the range of 7.3 ± 0.2 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity. The opposite effects are possible if the pH is too high.
5. The following technical and human errors may occur which compromise on reliability and accuracy and must be avoided:-
   - Improper disc storage.
   - Inoculum not properly adjusted (too light or too heavy).
   - Incubation temperature deviating from 35-37°C.
   - Use of an increased CO_2 atmosphere.
   - Reading plates before or after the full 16-18 hours of incubation.
   - Transcribing errors.
   - Reading error while measuring zone diameter.
   - Deterioration of the McFarland Turbidity Standard.
   - Contamination or mutation in the control strain.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Mueller Hinton Broth — AM1072/AM5072

Use

Mueller Hinton Broth is used to determine the antimicrobial susceptibility of bacteria by the tube dilution method.

Summary

Mueller Hinton Broth is used for determining the Minimal Inhibitory Concentration (MIC) of antimicrobials for aerobic bacteria. The tube dilution test involves exposing bacteria to decreasing concentrations of antimicrobial agents in liquid medium, usually by serial dilution. The mixture, consisting of microorganisms, nutrient medium and antimicrobial agent, is incubated at 35°C for 16-20 hours. The lowest concentration of antimicrobial agent at which no visible growth occurs is defined as the Minimal Inhibitory Concentration (MIC).

The quantitative disc diffusion antimicrobial susceptibility procedure has been standardized. The rationale for an MIC susceptibility test rather than the disc diffusion test is that it gives quantitative information. It provides a relationship

between the amount of antimicrobial agent required to inhibit the growth of an organism *in vitro* and the achievable concentration in the blood, urine, cerebrospinal fluid or bile, under various dosage conditions. It has been suggested that in the treatment of systemic infections the drug dosage should yield a peak concentration at the site of infection that is two to four times greater than the MIC value, while for urinary tract infections, a peak urine concentration of 10-20 times the MIC value should be achieved. However, effective antimicrobial therapy also depends on many other factors.

Principle

Casein acid hydrolysate and beef infusion supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of starch during autoclaving provides a small amount of dextrose, which is a source of energy.

Formula*

Ingredients in grams per liter

Beef, Infusion from	300.0
Casein Acid Hydrolysate	17.5
Starch	1.5

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired test tubes as per requirements.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Neisseria gonorrhoeae (49226)	Luxuriant
Pseudomonas aeruginosa (27853)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant
Streptococcus faecalis (19433)	Luxuriant

Procedure

1. Inoculate as per recognized practices for the cultivation of microorganisms.
2. Consult appropriate references for procedures for broth dilution antimicrobial susceptibility testing.

Interpretation of Results

1. The minimum inhibitory concentration (MIC) of an antimicrobial agent for a specific organism is the lowest concentration, which will inhibit the growth of the organism.
2. Growth is indicated by turbidity or sediment.
3. Some microorganisms when tested against trimethoprim / sulphamethoxazole or sulphonamides alone do not always give clear-cut end points.
4. In the case of doubling dilutions of trimethoprim / sulphamethoxazole, there may be a "trailing" of growth. Such a pattern typically shows an obvious reduction in the amount of growth and, then, either small pellets (usually less than 1 mm in diameter) in the rest of the wells, or an obvious reduction in the amount of growth and then a slight but detectable graduation in the size of the pellets. In these cases, the MIC end point should be identified as the lowest concentration of antimicrobial agent beyond which there is no further reduction in the size of the pellet or the amount of turbidity.
5. An organism may be susceptible, intermediate or resistant for a given antimicrobial agent depending on the MIC value.
6. The interpretive standards for MIC values with various drugs may be found in NCCLS document M100 (M7) obtained from the manufacturer.

Precautions / Limitations

1. Muller Hinton Broth should be inoculated within15 minutes after preparing the inoculum suspension.
2. Inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation environment, drug diffusion rate and measurement of end points can affect results, hence care should be taken to follow procedures accurately to ensure reliable results.
3. Some strains may be encountered that fail to grow or grow poorly in this broth.
4. A pH outside the range of 7.4 ± 0.2 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excess activity. The opposite effects are possible if the pH is too high.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

MUG Lauryl Sulphate Broth AM50721

Use

MUG Lauryl Sulphate Broth is recommended for detection of coliform organisms in water, food by fluorogenic method.

Summary

Lauryl Sulphate Broth was formulated by Mallmann and Darby (78) and is recommended by APHA for the detection and enumeration of coliform organisms in foods, water and wastewater (103.2). MUG is added in Lauryl sulphate Broth as the fluorogenic compound which permits the rapid detection of *Escherichia coli* when observed under UV light where further confirmation is not required (93.1). MUG detects anaerogenic strains which may not be detected in the conventional procedure. Feng and Hartman (30.1) used MUG- containing medium for studying β-glucuronidase activity and found *Escherichia coli* has 96-1000C activity, *Salmonella* species with 17% and *Shigella* species 40% activity and other genera were negative. For weakly positive strains incubation should be carried out overnight. Robison reported that no false negative results and about 5% false positive results.

Principle

Casein enzymic hydrolysate provides nutrients while lactose act as energy source. Sodium Lauryl sulphate inhibits many organisms other than coliforms. 4-methylumbelliferyl-β-D-glucuronide is hydrolyzed by an enzyme β-glucuronidase possessed by organisms to yield 4-methylumbelliferone, a fluorescent end product.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	20.0
Lactose	5.0
Sodium chloride	5.0
Dipotassium phosphate	2.75
Monopotassium phosphate	2.75
Sodium lauryl sulphate	0.10
4- Methylumbelliferyl β –D- glucuronide (MUG)	0.05

Final pH (at 25°C) 6.8±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 35.65 gms of powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense into tubes with inverted Durham's tube.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics after 4-24 hours at 35°C.

Organisms (ATCC)	Growth	Fluorescence
Enterobacter aerogenes (13048)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	+

Key: +=Fluorescence under UV light at 366nm.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Muller Kauffmann Tetrathionate Broth Base (Novobiocin Broth) AM50722

Use

Muller Kauffmann Tetrathionate Broth Base (Novobiocin Broth) is used for selective and differential isolation of *Salmonella* species.

Summary

Muller (82.4) recommended Tetrathionate Broth as a selective medium for the recovery of *Salmonella* and demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Kauffmann (51 & 52) modified this formula to include Oxbile and Brilliant Green for their selective properties. Using modified Muller's broth, Kauffmann increased the number of rapid screening of *Salmonella* in food (22.3).

The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products and from poultry and poultry products (46.1.3). Muller Kauffmann Tetrathionate Broth Base conforms with ISO Standards.

Principle

Enzymatic Digest of Casein and Meat Peptone provide nitrogen, carbon, vitamins, and amino acids. Sodium Chloride maintains the osmotic balance of the medium. Calcium Carbonate neutralizes and absorbs toxic metabolites. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms. Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Ox Bile and Brilliant Green are additional selective agents used to suppress coliform

bacteria and inhibit Gram-positive organisms. The addition of Novobiocin was described by Jeffries11 and use to suppress the growth of Proteus species

Formula*

Ingredients in grams per liter

Enzymatic Digest of Casein	8.6
Meat Peptone	4.3
Sodium Chloride	2.6
Calcium Carbonate	38.7
Sodium Thiosulfate, anhydrous	30.5
Ox Bile	4.78
Brilliant Green	0.0096

Final pH (at 25°C) 8.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 89.5 gms in 1000 ml distilled water and bring to a boil.
2. Cool to less than 45°C before adding supplements.
3. Supplement with 40 mg per liter of Novobiocin.
4. Immediately before use aseptically add 20 mL of an iodine-iodide solution prepared by dissolving 25 g of potassium iodide in 10 mL sterile water, adding 20 g of iodine and then diluting to 100 mL with sterile water.
5. Mix well and dispense into sterile containers.

Quality Control

Dehydrated Appearance

White to cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Milky pale green to slightly yellow-green and opaque solution.

Cultural Response

Culture characteristics after 18-24hours at 35-37°C. After incubation in Muller Kauffmann Tetrathionate Broth Base (With Novobiocin), organisms were subcultured to XLD Agar (AM1112/AM5112), incubated at 35 -37°C 2 for 18 - 24 hours.

Microorganisms(ATCC)	Recovery on XLD Agar	
	Growth	Colour of colony
Salmonella typhimurium (14028)	Good	Red colonies with black center
Salmonella enteritidis (13076)	Good	Red colonies with black center
Escherichia coli (25922)	None to poor	Yellow
Pseudomonas aeruginosa (27853)	None to poor	Yellow
Enterococcus faecalis (29212)	Inhibited	-
Staphylococcus aureus (25923)	Inhibited	-

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Mycoplasma Agar Base (PPLO Agar) AM1073/AM5073

Use

Mycoplasma Agar Base (PPLO Agar) when supplemented with nutritive enrichments is used for isolation and cultivation of *Mycoplasma* species.

Summary

Mycoplasma was first recognized from a case of pleuropneumonia in a cow. The organism was designated "pleuropneumonia-like organism," or PPLO. Although some species are normal human respiratory tract flora, *M.pneumoniae* is a major cause of respiratory disease (primary atypical pneumoniae, sometimes called "walking pneumonia"). *M.hominis, M.genitalium* and *Ureaplasma urealyticum* are important pathogens of the human genital tract. Morton, Smith and Leverman (81) formulated Mycoplasma Agar. It was used in the study of the growth requirements as well as for the identification and cultivation of *Mycoplasma.*

Principle

Peptone and beef heart infusion provides nitrogen, vitamins, amino acids and carbon. Sodium chloride maintains the osmotic balance. *Mycoplasma* cultivation requires all ingredients of medium and supplement to be free from even small amounts of toxic substances. Many mycoplasmas require serum and antibiotics for their good growth as well as to prevent the growth of contaminating microorganisms. Crystal violet and potassium tellurite inhibits many gram-negative and gram-positive bacteria. Most *Mycoplasma* species are aerobic or facultatively anaerobic while some are microaerophilic. Anaerobic saprophytic *mycoplasmas* grows best at 22-25°C while pathogenic strains grow at 35°C. Mycoplasmas do not grow without serum, but when grown in the agar medium it shows typical colony morphology, and forms subsurface colonies.

Formula*

Ingredients in grams per liter

Beef Heart, Infusion from	250.0
Peptone	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25°C) 7.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36 gms of the powder in 700 ml distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45°C and aseptically add 300 ml of Horse Serum (AS015) or 10 vials

of Mycoplasma Enrichment Supplement (AS019).

5. Mix well before dispensing.
6. 25% ascitic fluid can be used instead of Horse Serum.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48 hours at 35°C with 10% carbon dioxide.

Organisms (ATCC)	Growth	RGI
Mycoplasma gallinarium (19708)	Good to luxuriant	More than 70%
Mycoplasma pneumoniae (15531)	Good to luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Inoculate the surface of the plates containing the medium by adding drops of liquid inoculum or by swab inoculation technique.
2. Incubate the plates at 35°C for up to 21 days in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M.buccale, M.faucium, M.orale,* or *M.salivarium* is suspected.

Interpretation of Results

1. The colonies on Mycoplasma Agar Base are round with a dense center and a less dense periphery, giving a "fried egg" appearance.
2. Vacuoles and large bodies are characteristic of mycoplasma species and are seen in the periphery.
3. The colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

Precautions / Limitations

1. Thallium acetate is toxic, proper care should be taken while handling the Mycoplasma Enrichment Supplement.
2. Some mycoplasmas may be inhibited by thallium acetate.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nitrate Broth BIS AM10731/AM50731

Nitrate Broth ISO

Use

Nitrate Broth is used for the detection of nitrate reduction by bacteria. It is also recommended for the enumeration of Bacillus cereus in compliance with BIS specification IS: 5887 (Part 4), 1976 and compliance with ISO specification ISO/DIS 7932, 1993.

Summary

Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate. In presence of nitrate reductase enzyme nitrate reduces to nitrite, which can be tested for by an appropriate colorimetric reagent (77.2).

Principle

Nitrate Broth is recommended for the detection of nitrate reduction. Peptone and beef extract provide the essential nutrient for growth of bacteria while potassium nitrate is the source of nitrate.

Formula*

Ingredients in grams per liter

Peptone	5.0
Beef extract	3.0
Potassium nitrate	1.0

Final pH (at 25°C) 7.0 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 9 gms in 100 ml distilled water. Soak for 5 minutes.
2. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured clear solution.

Cultural Response

Culture characteristics after 18-24hours at 35°C.

Organisms (ATCC)	Growth	Nitrate Reduction
Bacillus cereus (6633)	Good	+
Enterobacter aerogenes (13048)	Good	+
Escherichia coli (25922)	Luxuriant	+
Salmonella typhimurium (14028)	Luxuriant	+

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nitrofurantoin Broth Base AM50733

Use

Nitrofurantoin Broth Base is used for the selective enrichment and cultivation of *Pseudomonas* species from food.

Principle

Casein enzymic hydrolysate and peptic digest of animal tissue provides the essential nutrients especially nitrogenous sources. Nitrofurantoin, (17.2) [(5-nitrofurfurylidene)amino] hydantoin, is a synthetic antibacterial agent which is effective against most common gram-negative and grampositive urinary tract pathogenic bacteria (16.4).

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	7.5
Casein enzymic hydrolysate	7.5
Sodium chloride	5.0

Final pH (at 25°C) 7.2 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 20 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to room temperature and aseptically add 50 ml sterile 0.2% Nitrofurantoin solution prepared by dissolving 1 gm Nitrofurantoin in 500 ml polyethylene glycol 30.
5. Mix well and dispense in tubes or flasks as desired.

Quality Control

Dehydrated Appearance

Cream to yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Fluorescent yellow coloured with added Nitrofurantoin, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Inhibited
Pseudomonas aeruginosa (27853)	Good-luxuriant
Staphylococcus aureus (25923)	Inhibited

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar AM1074/AM5074

Nutrient Broth AM1077/AM5077

Use

Nutrient Agar and Nutrient Broth are used as general-purpose culture media for the cultivation of bacteria, which may also be used as enrichment media by incorporating 10% v/v sterile blood or other biological fluids.

Summary

Nutrient Agar is a basic culture medium used to subculture organisms for maintenance purpose or to check the purity of sub-cultures from isolation plates prior to biochemical or serological testing. It is used for the cultivation and enumeration of organisms in water, sewage, faeces and other materials, which are not particularly fastidious. Nutrient Agar is suitable for teaching purpose and maintenance of cultures, where a prolonged survival of organisms at an ambient temperature is required without risk of the overgrowth that can occur with a more nutritious medium.

Nutrient Broth is a basic non-selective culture medium used for the routine cultivation of microorganisms. Nutrient Agar and Nutrient Broth can be used for the cultivation of more exacting bacteria by incorporating biological fluids like horse or sheep blood, serum, ascitic fluid, egg yolk, etc. Nutrient Agar is included in the Bacteriological Analytical Manual for food testing (113).

Principle

Peptone and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptone is the principle source of organic nitrogen, particularly amino acids and long chained peptides.

Formula*

Ingredients in grams per liter	Nutrient Agar	Nutrient Broth
Peptone	5.0	5.0
Sodium Chloride	5.0	5.0
Beef Extract	1.5	1.5
Yeast Extract	1.5	1.5
Agar	15.0	-
Final pH (at 25°C)	7.4 ± 0.2	7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water and mix thoroughly.
   Nutrient Agar - 28 gms
   Nutrient Broth - 13 gms
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Nutrient Agar - Light amber coloured, clear to slightly opalescent gel.
Nutrient Broth - Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth on Nutrient Agar and in Nutrient Broth	RGI
Escherichia coli (25922)	Good to luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	More than 70%
Streptococcus pyogenes (19615)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

For Nutrient Agar

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 35-37°C for 18-24 hours and 42-48 hours, if required.

For Nutrient Broth

1. Inoculate tubes of medium with the test samples.
2. Incubate for 18-24 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

Nutrient Agar

1. Examine plates for growth.
2. Growth from tubes inoculated with pure cultures can be used for biochemical and serological testing.

Nutrient Broth

1. Growth is seen as turbidity in the medium.
2. Aliquots of the medium can be used for sub-culturing onto a solid medium for isolation and identification of pure cultures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar IP — AM10741/AM50741

Use

Nutrient Agar is used as general purpose culture media for the cultivation of bacteria in compliance with IP, which may also be used as enrichment media by incorporating 10% v/v sterile blood or other biological fluid.

Summary

Nutrient Agar is a basic culture medium used to subculture organisms for maintenance purpose or to check the purity of sub-cultures from isolation plates prior to biochemical or serological testing. It is used for the cultivation and enumeration of organisms in water, sewage, faeces and other materials, which are not particularly fastidious. Nutrient Agar is suitable for teaching purpose and maintenance of cultures, where a prolonged survival of organisms at an ambient temperature is required without risk of the overgrowth that can occur with a more nutritious medium. Nutrient Agar can be used for the cultivation of more exacting bacteria by incorporating biological fluid like horse or sheep blood, serum, ascitic fluid, egg yolk, etc.

Principle

Peptone and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptone is the principle source of organic nitrogen, particularly amino acids and long chained peptides.

Formula*

Ingredients in grams per liter	
Peptone	10.00
Beef extract	10.00

Sodium chloride	5.00
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 40 gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Pseudomonas aeruginosa (27853)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	More than 70%
Escherichia coli (25922)	Good to luxuriant	More than 70%
Streptococcus pyogenes (19615)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 35-37°C for 18-24 hours and 42-48 hours, if required.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar 1.5% ISO — AM50742

Use

Nutrient Agar 1.5% ISO is a general purpose culture medium for the cultivation of fastidious bacteria after enrichment by incorporating 10% v/v sterile blood or other biological fluids, in compliance with ISO specification ISO/DIS 13720:1995.

Summary

Nutrient Agar is a basic culture medium used to subculture organisms for maintenance purpose or to check the purity of sub-cultures from isolation plates prior to biochemical or serological testing. It is used for the cultivation and enumeration of organisms in water, sewage, faeces and other materials, which are not particularly fastidious. Nutrient Agar is suitable for teaching purpose and maintenance of cultures, where a prolonged survival of organisms at an ambient temperature is required without risk of the overgrowth that can occur with a more nutritious medium. Nutrient Agar is recommended by APHA for cultivation and maintenance of nonfastidious microorganisms. Recently ISO Commended it with a slight modification (AM50742) for sub cultivation of Pseudomonas species isolated from meat and meat products.

Principle

Peptic digest of animal tissue and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptic digest of animal tissue is the principle source of organic nitrogen, particularly amino acids and long chained peptides.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.00
Beef extract	3.00
Sodium chloride	5.00
Agar	15.00

Final pH (at 25°C) 7.0± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 28gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Creamish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear gel forms in petri plates. With the addition of blood, cherry red coloured, opaque gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	RGI
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 35-37°C for 18-24 hours and 42-48 hours, if required.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar pH 6.8 AM1075/AM5075

Use

Nutrient Agar pH 6.8 is used for the cultivation of a wide variety of bacteria and for the enumeration of microorganisms in water, sewage, faeces and other materials.

Summary

Nutrient Agar pH 6.8 is a basic culture media used to subculture organisms for maintenance purposes or to check the purity of sub-cultures prior to biochemical or serological tests from water, dairy, etc.

Principle

Peptone and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptone	5.0
Beef Extract	3.0
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2
* Formula adjusted to suit performance parameters

Directions

1. Suspend 23 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. If required, enrich with 5-10% v/v sterile defibrinated blood.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	RGI
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 37°C for 18-24 hours and 42-48 hours, if required.

Interpretation of Results

1. Examine plates for growth.
2. Growth from tubes inoculated with pure cultures can be used for biochemical and serological testing.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar pH 7.0 AM50751

Use

Nutrient agars are used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, faeces and other materials.

Summary

Nutrient agar is a basic culture medium used for maintenance or to check purity of subcultures prior to biochemical or serological tests from water, dairy etc.

Principle

Peptone and beef extract provide water – soluble substance including carbohydrates, vitamins, organic nitrogen compound and salts. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.00
Beef extract	3.00
Agar	15.00

Final pH (at 25°C) 7.0±0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 23 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. If desired, the medium can be enriched with 5-10% v/v sterile defibrinated blood.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder

Prepared Appearance

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 18-24 hours at 37°C.

Organisms (ATCC)	Growth	RGI
Enetrococcus faecalis (29212)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Salmonella serotype Enteritidis (1307 6)	Luxuriant	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Agar with 1% Peptone AM1076/AM5076
Nutrient Broth with 1% Peptone AM1078/AM5078

Use

Nutrient Agar with 1% Peptone and Nutrient Broth with 1% Peptone are general-purpose media used for the examination of water and dairy products.

Summary

Nutrient Agar with 1% Peptone is a basic culture medium used to subculture organisms for maintenance purpose or to check the purity of sub-cultures from isolated plates prior to biochemical or serological testing and is also used for the cultivation and enumeration of organisms in water, sewage, faeces and other materials which are not particularly fastidious.

Nutrient Broth with 1% Peptone is a basic non-selective medium used for the routine cultivation of microorganisms. Nutrient Agar with 1% Peptone and Nutrient Broth with 1% Peptone can be enriched by the addition of 10% v/v sterile blood or other biological fluids for the cultivation of more fastidious organisms. Nutrient Agar with 1% Peptone and Nutrient Broth with 1% Peptone are used for the examination of water, wastewater and dairy products.

Principle

Peptone and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter	Nutrient Agar with 1% Peptone	Nutrient Broth with 1% Peptone
Peptone	10.0	10.0
Beef Extract	5.0	10.0
Sodium Chloride	5.0	5.0
Agar	15.0	-
Final pH (at 25°C)	7.4 ± 0.2	7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Nutrient Agar with 1% Peptone - 35 gms

   Nutrient Broth with 1% Peptone - 25 gms
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Nutrient Agar with 1% Peptone: Basal medium - Light yellow coloured, clear to slightly opalescent gel.

With the addition of blood - Cherry red coloured, opaque gel.

Nutrient Broth with 1% Peptone - Light yellow coloured clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth without Blood	Growth with Blood	Haemolysis
Streptococcus pneumoniae (6303)	Good	Luxuriant	Alpha
Streptococcus pyogenes (19615)	Good	Luxuriant	Beta
Staphylococcus aureus (25923)	Luxuriant	Luxuriant	Beta

Procedure

For Nutrient Agar with 1% Peptone

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 35-37°C for 18-24 hours and 42-48 hours, if required.

For Nutrient Broth with 1% Peptone

1. Inoculate medium with the test samples.
2. Incubate for 18-24 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

Nutrient Agar with 1% Peptone

1. Examine plates for growth.
2. Growth from tubes inoculated with pure cultures can be used for biochemical and serological testing.

Nutrient Broth with 1% Peptone

1. Growth is seen as turbidity in the medium.
2. Aliquots of the medium can be used for sub-culturing onto a solid media for isolation and identification of pure cultures.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Nutrient Broth IP — AM10781/AM50781

Use

Nutrient Broth is used as general purpose culture media for the cultivation of bacteria in compliance with IP, which may also be used as enrichment media by incorporating 10% v/v sterile blood or other biological fluid.

Summary

Nutrient Broth is a basic non- selective culture medium used for the routine cultivation of microorganisms. Nutrient Broth can be used for the cultivation of more exacting bacteria by incorporating biological fluid like horse or sheep blood, serum, ascitic fluid, egg yolk, etc.

Principle

Peptone and beef extract provide water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptone is the principle source of organic nitrogen, particularly amino acids and long chained peptides.

Formula*

Ingredients in grams per liter

Peptone	10.00
Beef extract	10.00
Sodium chloride	5.00

Final pH (at 25°C) 7.3 ± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 25gms powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 115°C (15 lbs pressure) for 30minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms(ATCC)	Growth
Pseudomonas aeruginosa (27853)	Good to luxuriant
Staphylococcus aureus (25923)	Good to luxuriant
Escherichia coli (25922)	Good to luxuriant
Streptococcus pyogenes (19615)	Good to luxuriant

Procedure

1. Use standard procedures like streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small agar surface and streak for isolation with a sterile loop.
3. Incubate plates at 35-37°C for 18-24 hours and 42-48 hours, if required.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Oxytetracyclin Glucose Yeast Extract Agar Base AM507811

Use

Oxytetra Glucose Yeast Agar Base (OGYE Agar Base) is recommended for isolation and enumeration of yeasts and moulds from foods.

Summary

Acidified agar may be used for enumerating yeasts and molds in foods and diary products. But Acidic media are not completely suitable for counting yeasts and moulds in foods since yeast cells, stressed by heat do not tolerate the acidic conditions necessary to inhibit bacterial contamination. Yeast and mould growth is often limited by the presence of acid-tolerant bacterial flora. Therefore it is evident that more active media and different selective agents are needed in order to deal with various kinds of foodstuffs, incubation conditions and types of microorganisms to be studied.

Mossel et al., described Oxytetra Glucose Yeast Agar Base (OGYE Agar Base) for the selective isolation and enumeration of yeast and moulds in foods (81.5 & 81.6).

Principle

OGYE Agar Base contains yeast extract to supply B-complex vitamins which stimulate bacterial growth. Dextrose is the energy source. Oxytetracycline inhibits the growth of bacteria.

Formula*

Ingredients in grams per liter

Yeast extract	5.00
Glucose	20.00
Agar	12.00

Final pH (at 25°C) 7.0±0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 18.5 grams in 500 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to 50°C and aseptically add reconstituted contents of one vial of Oxytetra Selective Supplement (AS0202).
5. Mix well and pour into sterile Petri plates.

Quality Control

Dehydrated Appearance

Cream to light yellow homogeneous free flowing powder

Prepared Appearance

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 2-5 days at 25-30°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Good - luxuriant	More than 70%
Candida albicans (10231)	Good – luxuriant	More than 70%
Escherichia coli (25922	inhibited	0%
Saccharomyces cerevisiae (9763)	Good – luxuriant	More than 70%
Saccharomyces uvarum (9080)	Good – luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Orange Serum Agar AM50782

Use

Orange Serum Agar is used for cultivation and enumeration of microorganisms associated with the spoilage of citrus products, cultivation of *Lactobacilli,* other aciduric organisms and pathogenic fungi.

Summary

Orange Serum Agar was originally developed by Murdock *et al.,*(82.2) and Hays (45.3) for examining citrus concentrates. Hays and Riester (45.4) further used this medium for studying spoilage of orange juice. Dehydrated agar media containing orange serum was reported by Stevens (103.3) and these media are recommended by APHA (103.4).

Principle

Casein enzymic hydrolysate and yeast extract provide essential nitrogenous nutrients while dextrose serves as the fermentable carbohydrates and energy sources. Orange Serum gives an optimal environment for the recovery of acid tolerant microorganisms from citrus fruit products.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Dextrose	4.00
Dipotassium phosphate	2.50
Orange serum (solid from 200ml)	9.00
Agar	17.00

Final pH (at 25°C) 5.5± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 45.5gms powder in 1000 ml distilled water and mix thoroughly.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes. AVOID OVER HEATING

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium to dark amber coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 40-48 hours at 37°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Good to luxuriant	More than 70%
Lactobacillus fermentum (9338)	Good to luxuriant	More than 70%
Lactobacillus acidophilus (4356)	Good to luxuriant	More than 70%
Leuconostoc mesenteroides (12291)	Good to luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Orange Serum Broth AM50783

Use

Orange Serum Broth is used for cultivation of microorganisms associated with the spoilage of citrus products, cultivation of *Lactobacilli,* other aciduric organisms and pathogenic fungi.

Summary

Murdock and Brokaw employed Orange Serum Broth for studies of sanitary control of the processing of citrus concentrates. Hays and Riester recommended Orange serum Broth, pH 5.5 which is accepted as a control medium is most productive for the growth of spoilage organisms.

Principle

Casein enzymic hydrolysate and yeast extract provide essential nitrogenous nutrients while dextrose serves as the fermentable carbohydrates and energy sources.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Dextrose	4.00
Dipotassium phosphate	2.50
Orange serum (Solids from 200 ml)	9.00

Final pH (at 25°C) 5.5± 0.2

*Formula adjusted to suit performance parameters

Directions

1. Suspend 28.5 gms powder in 1000 ml distilled water and mix thoroughly.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes. AVOID OVER HEATING

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium to dark amber coloured, clear to slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 40-48 hours at 37°C.

Organisms (ATCC)	Growth
Aspergillus niger (16404)	Good to luxuriant
Lactobacillus fermentum (9338)	Good to luxuriant
Lactobacillus acidophilus (4356)	Good to luxuriant
Leuconostoc mesenteroides (12291)	Good to luxuriant
Saccharomyces cerevisiae (9763)	Good to luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

PA Broth AM507831

Use

PA Broth is used for the detection of presence or absence of coliform bacteria from water samples.

Summary

The coliform group of bacteria is the principle indicator of suitability of water for domestic, industrial or other uses. Presence-absence procedures can be effectively used to monitor water from treatment plants and distribution systems. The presence-absence test for the coliform group is a simple modification of the multiple tube procedure. Simplification, by use of one large test portion (100 ml) in a single culture bottle to obtain qualitative information on the presence or absence of coliforms, is justified on the theory that no coliforms should be present in 100 ml of a drinking water sample.

Principle

The medium contains nutrients required for the growth of coliforms. Lactose is the fermentable carbohydrate. Phosphates provide buffering action while sodium lauryl sulphate inhibits organisms other than coliforms. Bromocresol purple is the pH indicator, which turns yellow at acidic pH.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Tryptose	9.83
Beef extract	3.00
Lactose	7.46
Sodium chloride	2.46
Dipotassium phosphate	1.35
Monopotassium phosphate	1.35
Sodium lauryl sulphate	0.05
Bromo cresol purple	0.0085
Final pH (at 25°C) 6.8 ± 0.2	

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30.5 gms in 1000 ml distilled water.
2. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 12 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured with green tinge, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics observed after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium
Escherichia coli (25922	Good-Luxuriant	Yellow
Enterobacter aerogenes (13048)	Good-Luxuriant	Light yellow
Klebsiella pneumoniae (13883)	Good-Luxuriant	Yellow
S. serotype Typhimurium (14028)	Good-Luxuriant	No change (purple)
Enterococcus faecalis (29212)	Inhibited	-

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

PE-2 Medium

AM507832

Use

PE-2 Medium is used for detection and cultivation of mesophilic anaerobic spore-formers in specimens collected from food processing plants.

Summary

PE-2 Medium is prepared as per the formulation described by Folinazzo and Troy (31.1.2) and recommended by APHA (20) for detection and cultivation of mesophilic anaerobic spore-formers in specimens from food processing plants. These organisms mainly include the genus Clostridium.

Clostridial species are highly heat resistant and are able to grow in the absence of oxygen. Clostridial growth range covers the temperature of the normal storage of canned and other processed foods including refrigerated storage of cured meats and hence these anaerobes are important in the spoilage of low-acid foods packed in hermetically sealed containers.

Principle

Peptic digest of animal tissue and yeast extract provide nitrogenous compounds, vitamin B complex and trace ingredients required for the growth of clostridia. Addition of untreated alaska seed peas creates anaerobic conditions in the medium.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.00
Yeast extract	3.00
Bromocresol purple	0.04

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23.04 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense 18-20 ml aliquots into 18 x 150 mm screw capped test tubes.
4. Add 8-10 untreated Alaska seed peas and let the tubes stand for 1 hour to effect hydration.
5. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured without green tinge, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured, clear to slightly opalescent solution over alaska seeds.

Cultural Response

Cultural characteristics observed after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Clostridium sporogenes (11437)	Luxuriant
Clostridium botulinum (25763)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pantothenate Assay Medium

AM10784

Use

Pantothenate Assay Medium is recommended for the microbiological assay of Pantothenic acid or its salts using *Lactobacillus plantarum.*

Summary

Pantothenate Culture Agar is formulated according to United States Pharmacopoeia (USP) (111.1) and is recommended for culturing *Lactobacillus plantarum* ATCC 8014 used in the microbiological assay of pantothenate. *Lactobacillus plantarum* requires pantothenate for the growth but the medium lacks pantothenate. The growth of the organism will occur if the materials being assayed contain pantothenate.

Principle

Casein acid hydrolysate supplies the nitrogen and other essential elements for the growth of the bacteria. Dextrose is the source of energy. Sodium acetate inhibits the growth of some organisms including Gram negative bacteria and moulds.

Formula*

Ingredients in grams per liter

Casein acid hydrolysate	10.0
Dextrose	40.0
Sodium acetate	20.0
L- Cystine	0.40
DL- Tryptophan	0.20
Adenine sulphate	0.02
Guanine hydrochloride	0.02
Uracil	0.02
Thiamine hydrochloride	0.0002
Riboflavin (Vitamin B2)	0.0004
Niacin	0.001
Pyridoxin	0.0008
p- Amino benzoic acid	0.0002
Biotin	0.0000008
Monopotassium phosphate	1.0

Dipotassium phosphate	1.0
Magnesium sulphate	0.40
Sodium chloride	0.02
Ferrous sulphate	0.02
Manganese sulphate	0.02

Final pH (at 25ºC) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 73.12 gms of the powder in 1000 ml distilled water.
2. Boil to dissolve the medium completely. Mix well.
3. Dispense in 5ml amounts to assay tube in increasing amounts of the standard or unknown and total volume 10ml per tube is adjusted by addition of distilled water.
4. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 10 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution which may have a slight precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth
Lactobacillus plantarum (8014)	Good to luxuriant

Storage

Store at 2-8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pantothenate Medium AOAC AM10785

Use

Pantothenate Medium AOAC is used for determining the concentration of pantothenic acid or its salts using *Lactobacillus platarum* ATCC8014.

Summary

Pantothenate Medium AOAC is prepared for use in the microbiological assay of pantothenic acid and pantothenate according to the procedures of Calcium Pantothenate Assay in the USP and Pantothenate Acid Assay in the Official Methods of Analysis of AOAC International (AOAC). *Lactobacillus plantarum* ATCC 8014 is the test organism used in this assay (85.1).

Principle

Pantothenate Medium AOAC is a pantothenic acid/pantothenatefree dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *Lactobacillus plantarum* ATCC 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula*

Ingredients in grams per liter

Dextrose	40.0 g
Sodium acetate	20.0 g
Casein acid hydrolysate	10.0 g
Dipotassium phosphate	1.0 g
Monopotassium phosphate	1.0 g
L-Cystine	0.4 g
L-Tryptophan	0.2 g
Magnesium sulfate	0.4 g
Sodium chloride	20.0 mg
Ferrous sulfate	20.0 mg
Manganese sulfate	20.0 mg
Adenine sulfate	20.0 mg
Guanine hydrochloride	20.0 mg
Uracil	20.0 mg
Riboflavin	400.0 µg
Thiamine hydrochloride	200.0 µg
Biotin	0.8 µg
p-Aminobenzoic acid	200.0 µg
Niacin	1.0 mg
Pyridoxine hydrochloride	800.0 µg

Final pH (at 25ºC) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 73 g of the powder in 1000 mL of purified water.
2. Boil to dissolve the medium completely. Mix well.
3. Dispense in 5ml amounts to assay tube in increasing amounts of the standard or unknown and total volume 10ml per tube is adjusted by addition of distilled water.

4. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 10 minutes.

Quality Control

Dehydrated Appearance

White to very light beige, homogeneous, tendency to clump.

Prepared Appearance

(Single strength) light amber, clear, may have a very slight precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth
Lactobacillus plantarum (8014)	Good to luxuriant

Storage

Store below 2-8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pantothenate Culture Agar AM10786

Use

Pantothenate Culture Agar is used for culturing Lactobacillus plantarum ATCC 8014

Summary

Pantothenate Culture Agar is formulated according to United States Pharmacopoeia (USP) and is recommended for culturing Lactobacillus plantarum ATCC 8014 used in the microbiological assay of pantothenate. Lactobacillus plantarum requires pantothenate for the growth but the medium lacks pantothenate. The growth of the organism will occur if the materials being assayed contain pantothenate.

Principle

Yeast Extract supplies the nitrogen and other essential elements for the growth of the bacteria. Dextrose is the source of energy. Sodium acetate inhibits the growth of some organisms including Gram negative bacteria and moulds.

Formula*

Ingredients in grams per liter

Yeast extract	20.0
Dextrose	5.0
Sodium acetate	5.0
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45 gms of powder in 1000ml distilled water and mix thoroughly.
2. Boil with the frequent agitation to dissolve the powder completely.
3. Distribute in tubes and sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to very slightly opalescent gel forms in tubes as butts.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Lactobacillus plantarum (8014)	Good-luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pantothenate Inoculum Broth AM10787

Use

Pantothenate Inoculum Broth is used in preparation of inoculum for pantothenate assay.

Summary

Pantothenate Inoculum Broth is recommended for the preparation of inoculum for pantothenate assay. Kulp and White have formulated the Pantothenate Inoculum Broth (63).

Principle

Peptonized milk is a good source of carbon and nitrogenous compound. Yeast extract provides vitamin B complex, nitrogenous complex and trace elements. Dextrose is the source of energy. Tomato juice makes the environment favorable for acidophilic bacteria like Lactobacillus, inhibiting other microorganisms. Polysorbate 80 provides fatty acids for the growth of bacteria.

Formula*

Ingredients in grams per liter

Peptonized milk	15.0
Yeast extract	5.0
Dextrose	10.0

Monopotassium phosphate	2.0
Tomato juice (100ml)	5.0
Polysorbate 80	1.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38 gms of powder in 1000ml distilled water and mix thoroughly.
2. Boil with the frequent agitation to dissolve the powder completely.
3. Distribute in tubes and sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Lactobacillus casei (9595)	Luxuriant
Lactobacillus leichmannii (4797)	Luxuriant
Lactobacillus plantarum (8014)	Luxuriant

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Peptone Water BIS — AM10791/AM50791

Use

Peptone Water BIS is used for cultivating non-fastidious organisms and a base for carbohydrate fermentation media in compliance with BIS specification IS: 5887 (Part 1) : 1976.

Summary

Peptone Water BIS is used for biochemical tests such as determining carbohydrate fermentation pattern, which help in differentiation of genera and species. With the pH adjusted to 8.4 it is suitable for the cultivation and enrichment of Vibrio cholerae. Adding Andrade indicator and the test carbohydrate to detect the fermentation reactions may modify Peptone Water BIS.

Principle

Peptone provides the essential nutrients while sodium chloride maintains the osmotic equilibrium. Andrade's indicator acts as a pH indicator, which shows a colour change of the medium from yellow to pink in the presence of an acid.

Formula*

Ingredients in grams per liter

Peptone	10.0
Sodium chloride	5.0
Andrade's Indicator	10 ml

Final pH (at 25°C) 7.2± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 25 gms of powder in 900 ml distilled water.
2. Mix thoroughly.
3. Add 10 ml Andrade indicator & make the pH upto 7.5.
4. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
5. Dispense in tubes with or without Durham's tubes as desired and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.
6. Aseptically add sterile carbohydrate solution to yield a 1% final concentration (10 gm of requisite sugar in 90 ml of water and steam for 30 minutes or sterilize by filtration)
7. Dispense in tubes with or without Durham's tubes as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Salmonella serotype Typhimurium (23564)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant

Procedure

1. Andrade indicator is a solution of acid fuchsin titrated with sodium hydroxide until the colour changes from pink to yellow.
2. When the indicator is added to Peptone Water it is colourless to slightly pink

at pH 7.2; it becomes pink at acid pH levels and yellow at alkaline pH levels.

3. Filter sterilized sugar solutions are added to the base medium after sterilization.
4. Inoculate tubes with test organism.
5. Incubate at 35-37°C for 18-48 hours
6. Observe for colour change.

Interpretation of Results

1. Acid is produced when carbohydrates are fermented which is indicated by a pink colour in the medium and gas production is detected by formation of gas bubbles in the Durham's tubes.

Precautions / Limitations

1. A pure culture in Peptone Water is a convenient inoculum.
2. Each tube of Peptone Water with carbohydrate should be correctly coded for the contained carbohydrate.
3. Peptone Water with Andrade indicator is reddish- pink when hot; and should return to a colourless or slightly pink colour when cooled to room temperature.
4. Some sugar solutions may affect the pH of Peptone Water, which must be checked and corrected.
5. It may be required to make subcultures to ensure purity of the inoculum since mixed or contaminated cultures may give false reactions.
6. Andrade indicator may fade on prolonged storage; do not use beyond expiry period.
7. It is advisable to maintain cultures of organisms, which have known positive and negative reactions in each sugar. Using fresh sub-cultures, test each batch of sugar media with the appropriate organisms.
8. Vibrio species should not be incubated longer than 18-20 hours as it may lead to development of suppressed forms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Peptone Water AM1079/AM5079

Peptone Water with Phenol Red AM1080/AM5080

Use

Peptone Water and Peptone Water with Phenol Red are non-selective media used for cultivating non-fastidious organisms and as a base for carbohydrate fermentation media.

Summary

Peptone Water is used for biochemical tests such as determining carbohydrate fermentation pattern, which help in differentiation of genera and species. With the pH adjusted to 8.4 it is suitable for the cultivation and enrichment of *Vibrio cholerae*. Peptone Water may be modified by adding Andrade indicator and the test carbohydrate to detect the fermentation reactions.

Principle

Peptone provides the essential nutrients while sodium chloride maintains the osmotic equilibrium. Phenol red is the pH indicator, which shows a colour change of the medium from red to yellow in the presence of an acid.

Formula*

Ingredients in grams per liter	Peptone Water	Peptone Water with Phenol Red
Peptone	10.0	10.0
Sodium Chloride	5.0	5.0
Phenol Red	-	0.02
Final pH (at 25°C)	7.2 ± 0.2	6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Peptone Water - 15 gms

   Peptone Water with Phenol Red - 15.02 gms
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Dispense in tubes with or without Durham's tubes as desired.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Aseptically add sterile carbohydrate solution to yield a 1% final concentration. Rotate the tubes thoroughly to distribute the carbohydrate.

Quality Control

Dehydrated Appearance

Peptone Water - Light yellow coloured, homogeneous, free flowing powder.

Peptone Water with Phenol Red - Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Peptone Water - Light yellow coloured, clear solution without any precipitate.

Peptone Water with Phenol Red - Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Peptone Water

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Salmonella serotype Typhimurium (23564)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant

Peptone Water with Phenol Red

Organisms (ATCC)	Growth	Sacrose Acid	L(+) Rhamnose Acid	Salicin Acid
Yersinia enterocolitica (27729)	Luxuriant	+	+*	-

Key:

* = Moderate

Procedure

For Peptone Water

1. Andrade indicator is a solution of acid fuchsin titrated with sodium hydroxide until the colour changes from pink to yellow.
2. When the indicator is added to Peptone Water it is colourless to slightly pink at pH 7.2; it becomes pink at acid pH levels and yellow at alkaline pH levels.
3. Filter sterilized sugar solutions are added to the base medium after sterilization.
4. Inoculate tubes with test organism.
5. Incubate at 35-37°C for 18-48 hours.
6. Observe for colour change.

For Peptone Water with Phenol Red

1. Inoculate tubes with test organism.
2. Incubate at 35-37°C for 18-48 hours.
3. Observe for colour change.

Interpretation of Results

Peptone Water

1. Acid is produced when carbohydrates are fermented which is indicated by a pink colour in the medium and gas production is detected by formation of gas bubbles in the Durham's tubes.

Peptone Water with Phenol Red

1. Acid is produced when carbohydrates are fermented which is indicated by a yellow colour in the medium and gas production is detected by formation of gas bubbles in the Durham's tubes.

Precautions / Limitations

1. A pure culture in Peptone Water is a convenient inoculum.
2. Each tube of Peptone Water with carbohydrate should be correctly coded for the contained carbohydrate.
3. Peptone Water with Andrade indicator is reddish- pink when hot; and should return to a colourless or slightly pink colour when cooled to room temperature.
4. Some sugar solutions may affect the pH of Peptone Water, which must be checked and corrected.
5. It may be required to make subcultures to ensure purity of the inoculum since mixed or contaminated cultures may give false reactions.
6. Andrade indicator may fade on prolonged storage; do not use beyond expiry period.
7. It is advisable to maintain cultures of organisms, which have known positive and negative reactions in each sugar. Using fresh sub-cultures, test each batch of sugar media with the appropriate organisms.
8. *Vibrio* species should not be incubated longer than 18-20 hours as it may lead to development of suppressed forms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Peptone Water with Phenol Red ISO — AM50801

Use

Peptone Water with Phenol Red medium for studying fermentation ability of Yersinia enterocolitica, In compliance with ISO specification ISO/DIS 10273:1994.

Summary

Peptone Water is used for biochemical tests such as determining carbohydrate fermentation pattern, which help in differentiation of genera and species. With the pH adjusted to 8.4 it is suitable for the cultivation and enrichment of Vibrio cholerae. Peptone Water may be modified by adding Andrade indicator and the test carbohydrate to detect the fermentation reactions.

Principle

Peptone provides the essential nutrients while sodium chloride maintains the osmotic equilibrium. Phenol red is the pH indicator, which shows a colour change

of the medium from red to yellow in the presence of an acid.

Formula*

Ingredients in grams per liter

Peptone	10.0
Sodium chloride	5.0
Phenol red	0.02

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15.02 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Dispense in tubes with or without Durham's tubes as desired.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Aseptically add sterile carbohydrate solution to yield a 1% final concentration. Rotate the tubes thoroughly to distribute the carbohydrate.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Peptone Water

Organisms (ATCC)	Growth	Sacrose Acid	L (+) Rhamnose Acid	Salicin Acid
Yersinia enterocolitica (27729)	Luxuriant	+	+*	-

Key: * = Moderate

Procedure

1. Inoculate tubes with test organism.
2. Incubate at 35-37°C for 18-48 hours.
3. Observe for colour change.

Interpretation of Results

Acid is produced when carbohydrates are fermented which is indicated by a yellow colour in the medium and gas production is detected by formation of gas bubbles in the Durham's tubes.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pfizer Selective Enterococcus Agar AM508011

Use

Pfizer Selective Enterococcus Agar is used for selective isolation and cultivation of *Enterococci.*

Summary

Pfizer Selective Enterococcus Agar is used for the selective isolation and cultivation of *Enterococci.* This medium is formulated as per Isenberg, Goldberg and Sampson (46.1.1) by reducing the concentration of bile salts and sodium azide from the original formulation. The importance of esculin hydrolysis in differentiating *Enterococci* and *streptococci* was first reported by Rochaix as streptococci do not exhibit esculin hydrolysis (94).

Principle

Casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract provide nutrients like nitrogenous compounds, carbon, sulphur, vitamin B complex and trace ingredients for the growth of *Enterococci.* Esculin, a glycoside, is hydrolyzed by *Enterococci* to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a dark brown to black coloured complex.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	17.00
Peptic digest of animal tissue	3.00
Yeast extract	5.00
Bile salts (ox gall)	10.00
Sodium chloride	5.00
Sodium citrate	1.00
Esculin	1.00
Ferric ammonium citrate	0.50
Sodium azide	0.25
Agar	15.00

Final pH (at 25°C) 7.1±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 57.75 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Mix well and pour into Petri plates

Warning : Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Quality Control

Dehydrated Appearance

Light yellow to pale green homogeneous free flowing powder

Prepared Appearance

Light amber coloured clear to slightly opalescent gel with a bluish tinge forms in Petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	Esculin hydrolysis	RGI
Enterobacter aerogenes (13048)	Inhibited	-	0%
Escherichia coli (25922)	Inhibited	-	0%
Staphylococcus aureus (25923)	Fair-good	Negative reaction	More than 70%
Enterococcus faecalis (29212)	Good-luxuriant	Positive reaction, blackening around the colony	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Dextrose Broth AM50802

Use

Phenol Red Dextrose Broth media is used for dextrose fermentation studies of microorganisms.

Summary

Phenol Red Dextrose Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Dextrose	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate. Petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism	Growth	Acid	Gas
Citrobacter ferundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	+	+
S. serotype Typhimurium (14028)	Luxuriant	+	+
S. serotype Typhi (6539)	Luxuriant	+	-
Serratia marcescens (8100)	Luxuriant	+	+
Shigella flexneri (12022)	Luxuriant	+	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Lactose Broth AM50803

Use

Phenol Red Lactose Broth media is used for lactose fermentation studies of microorganisms.

Summary

Phenol Red Lactose Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Dextrose	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism	Growth	Acid	Gas
Citrobacter ferundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	-	-
S. serotype Typhimurium (14028)	Luxuriant	-	-
S. serotype Typhi (6539)	Luxuriant	-	-
Serratia marcescens (8100)	Luxuriant	-	-
Shigella flexneri (12022)	Luxuriant	-	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Lactose Broth ISO AM50804

Use

Phenol Red Lactose Broth media is used for lactose fermentation studies. It is recommended by ISO Committee under the specification ISO 9308-1:1990.

Summary

Phenol Red Lactose Broth media is formulated by as recommended by ISO committee for studying lactose fermentation by coliforms which is an important differentiating characteristics for the members of *Enterobacteriaceae.*

Principle

Peptic digest of animal tissue provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.00
Sodium chloride	5.00
Lactose	10.00
Phenol red	0.018

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organism	Growth	Acid	Gas
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	-	-
S. serotype Typhimurium (14028)	Luxuriant	-	-
Shigella flexneri (12022)	Luxuriant	-	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Maltose Broth AM50805

Use

Phenol Red Maltose Broth media is used for maltose fermentation studies of microorganisms.

Summary

Phenol Red Maltose Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone
10.00

Beef extract
1.00

Sodium chloride
5.00

Maltose
5.00

Phenol red
0.018

Final pH (at 25^{0}C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organism	Growth	Acid	Gas
Citrobacter freundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	+	+
S. serotype Typhimurium (14028)	Luxuriant	+	+
S. serotype Typhi (6539)	Luxuriant	+	-
Serratia marcescens (8100)	Luxuriant	+	-
Shigella flexneri (12022)	Luxuriant	+	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Mannitol Broth AM50806

Use

Phenol Red Mannitol Broth media is used for mannitol fermentation studies of microorganisms.

Summary

Phenol Red Mannitol Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Mannitol	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism	Growth	Acid	Gas
Citrobacter freundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	-	-
S. serotype Typhimurium (14028)	Luxuriant	+	+
S. serotype Typhi (6539)	Luxuriant	+	-
Serratia marcescens (8100)	Luxuriant	+	-
Shigella flexneri (12022)	Luxuriant	+	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Sorbitol Broth AM50807

Use

Phenol Red Sorbitol Broth media is used for sorbitol fermentation studies of microorganisms.

Summary

Phenol Red Sorbitol Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Sorbitol	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.

3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	Acid	Gas
Citrobacter freundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	-	-
S. serotype Typhimurium (14028)	Luxuriant	+	+
S. serotype Typhi (6539)	Luxuriant	+	-
Serratia marcescens (8100)	Luxuriant	+	-
Shigella flexneri (12022)	Luxuriant	+	-

Key: += Positive reaction yellow colour

-= Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Sucrose Broth AM50808

Use

Phenol Red Sucrose Broth media is used for sucrose fermentation studies of microorganisms.

Summary

Phenol Red Sucrose Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Sorbitol	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	Acid	Gas
Citrobacter freundii (8090)	Luxuriant	[+]	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	-	-
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	+	+
S. serotype Typhimurium (14028)	Luxuriant	-	-
S. serotype Typhi (6539)	Luxuriant	-	-
Serratia marcescens (8100)	Luxuriant	+	+
Shigella flexneri (12022)	Luxuriant	-	-

Key: + = Positive reaction yellow colour

[+] = Weak/slight

- = Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Phenol Red Xylose Broth AM50809

Use

Phenol Red xylose Broth media is used for xylose fermentation studies of microorganisms.

Summary

Phenol Red Xylose Broth media are recommended (77.1, 31.1 & 28.1) for determination of fermentation reactions in differentiation of microorganisms. Ability of an organism to ferment specific carbohydrate added in a basal medium, results in the production of acid and gas which helps in the differentiation between genera and species.

Principle

Proteose peptone and beef extract provide nitrogenous nutrients to the organisms. Phenol red is the pH indicator which turns yellow at acidic pH. Sodium chloride maintains osmotic equilibrium. Gas formation is seen in Durham's tubes.

Formula*

Ingredients in grams per liter

Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Xylose	5.00
Phenol red	0.018

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 21 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely.
4. Dispense in tubes containing inverted Durham's tubes.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	Acid	Gas
Citrobacter freundii (8090)	Luxuriant	+	+
Enterobacter aerogenes (13048)	Luxuriant	+	+
Escherichia coli (25922)	Luxuriant	+	+
Klebsiella pneumoniae (13883)	Luxuriant	+	+
Proteus vulgaris (13315)	Luxuriant	+	[+]
S. serotype Typhimurium (14028)	Luxuriant	+	+
S. serotype Typhi (6539)	Luxuriant	+	-
Serratia marcescens (8100)	Luxuriant	-	-
Shigella flexneri (12022)	Luxuriant	-	-

Key: + = Positive reaction yellow colour

[+] = Weak/slight

- = Negative reaction, no colour change or red.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Phenylalanine Agar

Use

Phenylalanine Agar is used for differentiation of *Proteus* and *Providencia* from other Enterobacteriaceae on the basis of deamination of phenylalanine.

Summary

Phenylalanine Agar is a modification of the medium originally formulated by Ewing et al. Proteus, Providencia and *Morganella* species are capable to deaminate phenylalanine and form phenylpyruvic acid by enzymatic action (23).

Principle

Yeast extract provides the essential nutrients for the growth of the microorganisms. Sodium chloride maintains the osmotic balance. DL-Phenylalanine is the substrate for the deaminase enzyme which converts phenylalanine to phenylpyruvic acid. And addition of few drops of ferric chloride gives green colour, indicating a positive test result.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Sodium chloride	5.0
DL- Phenylalanine	2.0
Disodium phosphate	1.0
Agar	15.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 26 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.

4. Dispense in tubes and sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Allow the medium to cool in slanting position.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, slightly opalescent gel forms in tubes and slants.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	Phenylalanine deaminase	RGI
Enterobacter aerogenes (13048)	Luxuriant	+	More than 70%
Escherichia coli (25922)	Luxuriant	+	More than 70%
Proteus vulgaris (13315)	Luxuriant	+	More than 70%
Proteus mirabilis (25933)	Luxuriant	+	More than 70%
Providencia alcalifaciens (12013)	Luxuriant	-	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key:

+ = Green colouration after addition of 10% ferric chloride

Procedure

Tubes are inoculated with heavy inoculum and incubated aerobically at 35±2°C for 18-24 hours.

Following the incubation period, add few drops of the 10% aqueous ferric chloride reagent.

Interpretation of Results

Appearance of green colour is an indication of positive test result.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pikovskaya's Agar AM108092/AM508092

Use

Pikovskaya's Agar is used for the detection of phosphate solubilizing microorganisms.

Summary

Phosphorus is one of major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation (42.2). Phosphate dissolving soil microorganisms play part in correcting phosphorus balance of crop plants. Many fungi and bacteria are potential solubilizers of bound phosphates so they are used in phosphate dissolving culture preparations (84.4).

Principle

Dextrose serves as energy source. Yeast extract supplies the nitrogen for the support of bacterial growth. Calcium phosphate is the source of phosphorus. Various salts support the growth of the microorganism.

Formula*

Ingredients in grams per liter

Yeast extract	0.50
Dextrose	10.00
Calcium phosphate	5.00
Ammonium sulphate	0.50
Potassium chloride	0.20
Magnesium sulphate	0.10
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 31.3 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Off white coloured, homogeneous, free flowing powder.

Prepared Appearance

White with flocculant precipitate, opaque gel forms in petri plates.

Cultural Response

i) Cultural characteristics after 5 days at R.T.

ii)Cultural characteristics after 48 hours at 35-37°C.

Organism (ATCC)	Growth	Phenylalanine solubilization	RGI
i) *a-- Aspergillus niger* (16404)	Luxuriant	+	More than 70%
b-- Penicillin notatum	Luxuriant	+	More than 70%
ii) *a-- Pseudomonas aeruginosa* (27853)	Luxuriant	+	More than 70%
b-- Bacillus subtilis (6633)	Good	(+)	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key: + = clear zone surrounding the colony

(+) = moderate clear zone surrounding the colony

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pikovskaya's Broth AM508093

Use

Pikovskaya's Broth is used for the detection of phosphate solubilizing microorganisms.

Summary

Phosphorus is one of major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation. Phosphate dissolving soil microorganisms play part in correcting phosphorus balance of crop plants. Many fungi and bacteria are potential solubilizers of bound phosphates so they are used in phosphate dissolving culture preparations (84.4).

Principle

Dextrose serves as energy source. Yeast extract supplies the nitrogen for the support of bacterial growth. Calcium phosphate is the source of phosphorus. Various salts support the growth of the microorganism.

Formula*

Ingredients in grams per liter

Yeast extract	0.50
Dextrose	10.00
Calcium phosphate	5.00
Ammonium sulphate	0.50
Potassium chloride	0.20
Magnesium sulphate	0.10
Manganese sulphate	0.0001
Ferrous sulphate	0.0001

Final pH (at 25°C) 6.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 16.3 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Off white coloured, homogeneous, free flowing powder.

Prepared Appearance

White with flocculant precipitate, opaque solutions in tubes.

Cultural Response

i) Cultural characteristics after 5 days at R.T.

ii) Cultural characteristics after 48 hours at 35-37°C.

Organism (ATCC) **Growth**

i) a-- *Aspergillus niger(16404)*	Luxuriant
b-- *Penicillin notatum*	Luxuriant
ii) a-- *Pseudomonas aeruginosa (27853)*	Luxuriant
b-- *Bacillus subtilis (6633)*	Good

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Plate Count Agar (Standard Methods Agar) AM1081/AM5081

Use

Plate Count Agar (Standard Methods Agar) is used for obtaining microbial plate counts from milk and dairy products, foods, water and other materials of sanitary importance.

Summary

Plate Count Agar is formulated as described by Buchbinder *et al* (10). It is equivalent to the medium recommended by APHA for the plate count of microorganisms in milk (39) and other dairy products and may also be used to

determine sanitary quality of foods (20), water (36) and other materials. This medium is suitable for obtaining bacterial counts of sterile rooms. It is included in the Bacteriological Analytical Manual for food and cosmetics testing (113).

Principle

Tryptone provides nitrogenous substances and other amino acids. Yeast extract provides B complex vitamins while dextrose is the energy source.

Formula*

Ingredients in grams per liter

Tryptone	5.0
Yeast Extract	2.5
Dextrose	1.0
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Lactobacillus casei (9595)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. APHA recommends pour plate technique.
2. Samples are diluted and appropriate dilutions are pipetted into sterile petri plates.
3. Sterile molten medium is added followed by gentle mixing to distribute the sample throughout the agar.
4. Incubate plates for 48 hours at 32°C (dairy products) or 35°C (for foods) in an aerobic atmosphere.
5. For the enumeration of microorganisms with other temperature requirements, plates may also be incubated for 7-10 days at 5-7°C, for 3-5 days at 20°C, for 2-3 days at 45°C, or for 48 hours at 55°C.

Interpretation of Results

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. After autoclaving, do not heat the medium longer than 3 hours at 45-50°C.
2. Sterile solidified medium can be remelted only once.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Plate Count Agar BIS — AM10811/AM50811

Use

Plate Count Agar is used for obtaining microbial plate counts from milk and dairy products, foods, water and other materials of sanitary importance, in compliance with BIS.

Summary

Plate Count Agar is formulated as described by Buchbinder *et al.*, (10) It is equivalent to the medium recommended by APHA for the plate count of microorganisms in milk and other dairy products and may also be used to determine sanitary quality of foods, water and other materials. This medium is suitable for obtaining bacterial counts of sterile rooms. It is included in the Bacteriological Analytical Manual for food and cosmetics testing.

Principle

Tryptone provides nitrogenous substances and other amino acids. Yeast extract provides B complex vitamins while dextrose is the energy source.

Formula*

Ingredients in grams per liter

Tryptone	5.0
Dehydrated yeast extract	2.5
Anhydrous glucose	1.0
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured clear to slightly opalescent gel..

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Lactobacillus casei (9595)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. APHA recommends pour plate technique.
2. Samples are diluted and appropriate dilutions are pipetted into sterile petri plates.
3. Sterile molten medium is added followed by gentle mixing to distribute the sample throughout the agar.
4. Incubate plates for 48 hours at 32°C (dairy products) or 35°C (for foods) in an aerobic atmosphere.
5. For the enumeration of microorganisms with other temperature requirements, plates may also be incubated for 7-10 days at 5- 7°C, for 3 5 days at 20°C, for 2-3 days at 45°C, or for 48 hours at 55°C.

Interpretation of Results

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. After autoclaving, do not heat the medium longer than 3 hours at 45-50°C.
2. Sterile solidified medium can be remelted only once.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

PNY Medium

AM10692711/AM50692711

Use

PNY Medium is used for cultivation and isolation of *Lactobacillus* species.

Summary

Lactobacillus is a genus of Gram positive facultative anaerobic or microaerophilic bacteria. Some *Lactobacillus* species are used industrially for the production of yogurt, cheese, sauerkraut, pickles, beer, wine and other fermented foods. PNY Medium is recommended for cultivation and isolation of *Lactobacillus* species.

Principle

Peptic digest of animal tissue and yeast extract provide nitrogen compounds, carbon compounds and nutrients. Dextrose is the source of energy. Different salts support the growth of the bacteria.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Yeast extract	5.0
Dextrose	5.0
Monopotassium phosphate	0.5
Dipotassium phosphate	0.5
Magnesium sulphate	0.25
Ferrous sulphate	0.01
Manganese sulphate	0.01
Sodium chloride	0.01
Copper sulphate	0.001
Cobalt sulphate	0.001
Zinc sulphate	0.001
Agar	15.0

Final pH (at 25°C) 6.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 31.28 gms powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT **OVERHEAT.**
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Warning: Cobalt sulphate is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Cream to yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear gel formed in petriplates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organism (ATCC)	Growth	RGI
Lactobacillus casei (9595)	Luxuriant	More than 70%
Lactobacillus leichmannii (4797)	Luxuriant	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results:

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Potato Dextrose Agar (Harmonized) AMH5082

Use

Potato Dextrose Agar Harmonized is used for the cultivation and enumeration of yeasts and moulds from dairy and other food products.importance.

Summary

Potato Dextrose Agar is recommended by the USP and IP for use in the performance of Microbial Limit Tests and by APHA. This medium is included in the Bacteriological Analytical Manual for food and cosmetics testing and is also used for stimulation of sporulation, maintenance of stock cultures of certain dermatophytes and in differentiating atypical varieties of dermatophytes on the basis of pigment production.

Principle

Potato infusion and dextrose provide nutrients for luxuriant growth of fungi. Acidifying the medium by lowering the pH to 3.5 with sterile tartaric acid inhibits bacterial growth.

Formula*

Ingredients in grams per liter

Potato, Infusion from	200.0
Dextrose	20.0
Agar	15.0

Final pH (at 25°C)) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 39 gms of the powder in 1000 ml distilled water. Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well before dispensing.
5. When pH 3.5 is required, cool the base to 45°C and aseptically add an appropriate amount of sterile 10% tartaric acid (approximately 1ml in 100 ml of medium) to each liter of the medium and mix well.
6. Do not reheat the medium after addition of acid.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 4-5 days at 20-25°C.

Organisms (ATCC)	Growth	Ascospore Formation	RGI
Aspergillus niger (16404)	Luxuriant	-	More than 70%
Candida albicans (10231)	Luxuriant	-	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	+	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Isolate using spread plate or pour plate technique.
3. Incubate plates aerobically for 48 hours at 37°C.

For the determination of yeasts and mould counts

1. Adjust the pH to approximately 3.5 with sterile tartaric acid and use in pour plate technique.
2. Incubate the plates at 20-25°C in an inverted position with increased humidity.

For isolation of specimens from potentially contaminated specimens

1. Isolate using the streak plate method to get isolated colonies.
2. A selective medium should be inoculated along with this nonselective medium.
3. When used as a non-selective medium, do not add the acid.
4. Alternatively, a general-purpose mycological medium such as Malt Extract Agar can be used.

For isolating fungi causing systemic mycosis

1. Inoculate two sets of media, with one set incubated at 25-30°C and the other set at 35-37°C.

Interpretation of Results

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. If duplicate plates were set up, express the average for the two plates in terms of number of micro-organisms per gram or ml of sample.
3. Examine all cultures at least weekly for fungal growth and preserve for at least 4-6 weeks before being reported as negative.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Potato Dextrose Agar USP AM10821/AM50821

Use

Potato Dextrose Agar USP is used for the cultivation and enumeration of yeasts and moulds from dairy and other food products in compliance with USP.

Summary

Potato Dextrose Agar is recommended by the USP and IP for use in the performance of Microbial Limit Tests and by APHA. This medium is included in the Bacteriological Analytical Manual for food and cosmetics testing and is also used for stimulation of sporulation, maintenance of stock cultures of certain dermatophytes and in differentiating atypical varieties of dermatophytes on the basis of pigment production.

Principle

Potato infusion and dextrose provide nutrients for luxuriant growth of fungi. Acidifying the medium by lowering the pH to 3.5 with sterile tartaric acid inhibits bacterial growth.

Formula*

Ingredients in grams per liter

Potato, Infusion from	300.0
Glucose	20.0
Agar	15.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41 gms of the powder in 1000 ml distilled water. Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well before dispensing.
5. When pH 3.5 is required, cool the base to 45°C and aseptically add an appropriate amount of sterile 10% tartaric acid (approximately 1ml in 100 ml of medium) to each liter of the medium and mix well.
6. Do not reheat the medium after addition of acid.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 4-5 days at 20-25°C.

Organisms (ATCC)	Growth	Ascospore Formation	RGI
Aspergillus niger (16404)	Luxuriant	-	More than 70%
Candida albicans (10231)	Luxuriant	-	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	+	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Isolate using spread plate or pour plate technique.
3. Incubate plates aerobically for 48 hours at 37°C.

For the determination of yeasts and mould counts

1. Adjust the pH to approximately 3.5 with sterile tartaric acid and use in pour plate technique.
2. Incubate the plates at 25-30°C in an inverted position with increased humidity.

For isolation of specimens from potentially contaminated specimens

1. Isolate using the streak plate method to get isolated colonies.
2. A selective medium should be inoculated along with this nonselective medium.
3. When used as a non-selective medium, do not add the acid.
4. Alternatively, a general-purpose mycological medium such as Malt Extract Agar can be used. For isolating fungi causing systemic mycosis

1. Inoculate two sets of media, with one set incubated at 25-30°C and the other set at 35-37°C.

Interpretation of Results

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. If duplicate plates were set up, express the average for the two plates in terms of number of micro-organisms per gram or ml of sample.
3. Examine all cultures at least weekly for fungal growth and preserve for at least 4- 6 weeks before being reported as negative.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Potato Dextrose Agar AM1082/AM5082

Potato Dextrose Broth AM1083/AM5083

Use

Potato Dextrose Agar and Potato Dextrose Broth are used for the cultivation and enumeration of yeasts and moulds from dairy and other food products.

Summary

Potato Dextrose Agar is recommended by the USP (114) and IP (46) for use in the performance of Microbial Limit Tests and by APHA (20). This medium is included in the Bacteriological Analytical Manual for food and cosmetics testing (113) and is also used for stimulation of sporulation, maintenance of stock cultures of certain dermatophytes and in differentiating atypical varieties of dermatophytes on the basis of pigment production. Potato Dextrose Broth is a general-purpose medium used for the cultivation of yeasts and moulds.

Principle

Potato infusion and dextrose provide nutrients for luxuriant growth of fungi. Acidifying the medium by lowering the pH to 3.5 with sterile tartaric acid inhibits bacterial growth.

Formula*

Ingredients in grams per liter	Potato Dextrose Agar	Potato Dextrose Broth
Potato, Infusion from	200.0	200.0
Dextrose	20.0	20.0
Agar	15.0	-
Final pH (at 25°C)	5.6 ± 0.2	5.1 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Potato Dextrose Agar - 39 gms

   Potato Dextrose Broth - 24 gms

   Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Mix well before dispensing.
5. When pH 3.5 is required, cool the base to 45°C and aseptically add an appropriate amount of sterile 10% tartaric acid (approximately 1 ml in 100 ml of medium) to each liter of the medium and mix well.
6. Do not reheat the medium after addition of acid.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Potato Dextrose Agar - Light amber coloured, clear to slightly opalescent gel.

Potato Dextrose Broth - Light amber coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 4-5 days at 22-25°C.

Organisms (ATCC)	Growth	Ascospore Formation	RGI
Aspergillus niger (16404)	Luxuriant	-	More than 70%
Candida albicans (10231)	Luxuriant	-	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	+	More than 70%

Procedure

For Potato Dextrose Agar

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Isolate using spread plate or pour plate technique.
3. Incubate plates aerobically for 48 hours at 37°C.

For the determination of yeasts and mould counts

1. Adjust the pH to approximately 3.5 with sterile tartaric acid and use in pour plate technique.
2. Incubate the plates at 25-30°C in an inverted position with increased humidity.

For isolation of specimens from potentially contaminated specimens

1. Isolate using the streak plate method to get isolated colonies.
2. A selective medium should be inoculated along with this non-selective medium.
3. When used as a non-selective medium, do not add the acid.
4. Alternatively, a general-purpose mycological medium such as Malt Extract Agar can be used.

For isolating fungi causing systemic mycosis

1. Inoculate two sets of media, with one set incubated at 25-30^{0}C and the other set at 35-37^{0}C.

For Potato Dextrose Broth

1. Inoculate the broth tubes.
2. Incubate for 40-48 hours at 22-25^{0}C.

Interpretation of Results

Potato Dextrose Agar

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. If duplicate plates were set up, express the average for the two plates in terms of number of micro-organisms per gram or ml of sample.
3. Examine all cultures at least weekly for fungal growth and preserve for at least 4-6 weeks before being reported as negative.

Potato Dextrose Broth

1. Observe cultures for surface growth and pellicle formation.

Precautions / Limitations

1. Heating the medium after acidification hydrolyzes the agar and may destroy the gelling properties.
2. This medium is not a primary isolation medium. Direct inoculation of specimens will give wrong results.
3. For proper identification of yeasts and moulds, microscopic examination and evaluation of morphological structures may be required.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Preston Agar Base — AM10831/AM50831

Use

Preston Agar Base with added supplements is recommended for selective isolation of thermotolerant Campylobacter species especially *Campylobacter jejuni* and *Campylobacter coli*.

Summary

Campylobacter species can cause mild to severe diarrhea with loose watery stools often followed by bloody diarrhea. These pathogens are highly infective and transmitted by contaminated food or water.

Preston Agar Base is based on the formulation described by Bolton and Robertson (1). This formula with the addition of Preston Selective Supplement is used to isolate *Campylobacter* species from human, animal and environmental specimens.

Principle

Peptic digest of animal tissue and beef extract provide nutritional sources of nitrogen, vitamins, minerals and amino acids required for growth. Sodium chloride maintains the osmotic equilibrium. Agar is the solidification agent.

Formula*

Ingredients in grams per liter

Peptic Digest of animal Tissue	10.0
Beef Extract	10.0
Sodium Chloride	5.0
Agar	12.0

Final pH (at 25^0 C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.5 grams of the powder in 470 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121^0 C (15 lbs pressure) for 15 minutes.
4. Cool to 45-50^0 C.
5. Aseptically add 25 ml sterile, lysed horse blood and reconstituted contents of 1 vial of Preston Selective Supplement (Campylobacter Selective Supplement IV, Modified) (AS0231).
6. Mix well and pour into sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured clear to slightly opalescent gel. On addition of sterile Horse Blood, chocolate brown coloured opaque gel.

Cultural Response

Cultural response at 37^0 C after 18 - 48 hours incubation on 5% Horse Blood plates in an atmosphere consisting of approximately 5-6% oxygen, 3-10% carbon dioxide and 84-85% nitrogen.

Organisms (ATCC)	Growth	RGI
Campylobacter jejuni (29428)	Luxuriant	More than 70%
Campylobacter coli (33559)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Staphylococcus aureus (25923)	Inhibited	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. Streak the isolated specimen directly onto the surface of the medium.
2. Incubate at 37°C in an atmosphere consisting of approximately 5-6% oxygen, 3-10% carbon dioxide and 84-85% nitrogen for 24-48 hours.
3. Observe for growth.

Interpretation of Results

1. Campylobacter colonies are round to irregular with smooth edges. They may exhibit translucent, white colonies to spreading, flat, transparent growth. Some strains may appear tan or slightly pink.
2. Normal enteric flora is completely to markedly inhibited.

Limitations

Some strains of Campylobacter may grow poorly or may be inhibited due to variation in nutritional requirements.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Agar Base AM1084/AM5084

Use

Pseudomonas Agar Base with added supplements is used for the selective isolation of *Pseudomonas* species.

Summary

Pseudomonas Agar Base is a modification of King's A medium (55) in which magnesium chloride and potassium sulphate are incorporated to enhance pigment production and is recommended by USP (114) and IP (46) for detecting pyocyanin, a water soluble pigment produced by *Pseudomonas* species.

Principle

Pancreatic digest of gelatin and tryptone provides amino acids and other essential nutrients. Magnesium chloride and potassium sulphate enhance pigment production. The peptic digest of gelatin is low in phosphorus to minimize the inhibitory action on pyocyannin production. This medium enhances the formation of pyocyanin but inhibits the formation of fluorescein pigment. The fluorescein pigment diffuses from the colonies of *Pseudomonas* into the agar to give a blue colouration. Some *Pseudomonas* species produce small amounts of fluorescein resulting in a blue-green colouration. Addition of CFC Supplement (AS009) makes the medium selective for *Pseudomonas* species. Mead and Adam's showed that reducing the cetrimide content to 10 microgram per ml allowed the growth of all pigmented and non-pigmented psychrophilic *Pseudomonas* species. Addition of cephaloridine (50 microgram/ ml) and fucidin (10 microgram/ml) makes the medium more specific for isolating *Pseudomonas* species from chilled foods and processing plants. Goto and Enomoto formulated Cetrinix Supplement (AS008) for the selective isolation of *Pseudomonas aeruginosa*. Cetrinix Supplement (AS008) suppresses *Klebsiella, Proteus* and *Providencia* species. Considerable importance is given to detection of *Burkholderia cepacia* (*formerly P. cepacia*) in water systems, particularly where the water is to be used in the preparation of pharmaceuticals and cosmetics. The organism is resistant to many commonly used disinfectants. It has emerged as an important opportunistic pathogen in urinary, abdominal, respiratory and other infections and could be isolated from mixed cultures of cystic fibrosis patients.

Formula*

Ingredients in grams per liter

Pancreatic Digest of Gelatin	16.0
Potassium Sulphate	10.0
Tryptone	10.0
Magnesium Chloride	1.4
Agar	11.0

Final pH (at 25°C) 7.1 ± 0.2
* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.2 gms of the powder in 500 ml distilled water containing 5 ml glycerol and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Cool to 45°C and aseptically add sterile rehydrated contents of 1 vial each either Cetrinix Supplement (AS008) or CFC Supplement (AS009) as desired.
5. Mix well and pour into sterile petri plates.

Note: Do not keep the molten agar for longer than 4 hours.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics at 24-48 hours at 25°C or 35°C.

Organisms (ATCC)	Growth with Cetrinix Supplement (35°C)	Growth with CFC Supplement (25°C)
Proteus vulgaris (13315)	-	-
Pseudomonas aeruginosa (27853)	Luxuriant	-
Pseudomonas cepacia (10661)	-	Luxuriant
Staphylococcus aureus (25923)	-	-

Procedure

For food, water, and environmental samples.

1. Prepare Pseudomonas Agar Base and add CFC supplement as directed.
2. Pour plates under aseptic conditions and dry the surface.
3. Prepare food samples by diluting 1 in 5 or 1 in 10 with 1% (w/v) sterile peptone water and homogenize in a blender.
4. Pipette 0.5 ml or 1 ml of the homogenate onto the plate and spread evenly over the surface with a sterile glass spreader. Inoculate water and swab samples directly on the surface of the medium.
5. Incubate at 25°C and examine after 24 and 48 hours under both, white and ultraviolet light.

Interpretation of Results

1. The presence of blue-green pigmentation, or fluorescence is a presumptive evidence of *Pseudomonas* species.

Precautions / Limitations

1. Growth on Pseudomonas Agar Base with CFC supplement is usually limited to *Pseudomonas* species, but some members of *Enterobacteriaceae* may also grow.
2. Freshly prepared medium should be used as much as possible and should not be remelted. Molten agar should not be kept for more than 4 hours.
3. If swarming of *Proteus* species is a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days.
4. Chilled foods can carry a wide range of *Pseudomonas* and the colonies on Pseudomonas Agar Base with CFC supplement, incubated at lower temperatures, may include *P.fluorescens, P.putida* as well as *P.aeruginosa*. *Aeromonas* species if present will also appear as pink / brown colonies, particularly from fish products.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label. l.

Pseudomonas Agar (for Fluorescein) AM108411/AM508411

Use

Pseudomonas Agar for Fluorescein is used for the detection of fluorescein production by *Pseudomonas* species.

Summary

Pseudomonas species may produce water-soluble pigments in culture media. This property is sometimes used as a characteristic for the taxonomic classification of different species of *Pseudomonas* (33.1). Most strains of *P. aeruginosa* produce pyocyanin(blue) or pyoverdin (Yellow) or both, as well as pyorubrin (red), pyomelanin (Brown), or various combinations of these pigments (49.3). *P. aeruginosa* and other Pseudomonas isolated from humans often produce water soluble fluorescent pigments; pyoverdin is also one of these. Fluorescent pigment-producing strains fluoresce in under short-wave ultraviolet light. the fluorescence of pseudomonas is best observed at 254nm (19.3). *Pseudomonas* Agar (for fluorescein) is a modification of formulation described by King et al., This medium is recommended by USP for use in Microbial Limit Tests.

Principle

Pancreatic digest of casein and proteose peptone provide nutrients, carbon, sulphur and trace elements for growth. Equal proportion of enzymetic hydrolysate of casein and pancreatic digest of animal tissue is helpful for fluorescin production by pseudomonas. Dipotassium phosphate serve as the buffer. Magnesium sulphate enhance fluorescin production. Glycerin as an energy source also increases fluorescin production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Enzymetic hydrolysate of Casein	10.0
Proteose peptone	10.0
Dipotassium phosphate	1.5
Magnesium sulphate	1.5
Agar	15.0

Final pH (at 25°C) 7.0± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 38 gms of powder in 990 ml distilled water.
2. Add10 ml of glycerin and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (27853)	Good	Greenish yellow	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Inoculate and incubate plates aerobically at 35-37°C for 18-48 hours.
3. Examine for growth and fluorescein production.

Interpretation of Results

1. Examine growth under short wavelength UV light (254nm) for fluorescin.
2. Presence of fluorescin is appear with a greenish yellow fluorescent pigment in the colonies and surrounding medium.

Limitations

Some strains of Campylobacter may grow poorly or may be inhibited due to variation in nutritional requirements.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Agar (For Fluorescein) IP AM108412/AM508412

Use

Pseudomonas Agar for Fluorescein is used for the detection of fluorescein production by Pseudomonas species in compliance with IP.

Summary

Pseudomonas species may produce water-soluble pigments in culture media. This property is sometimes used as a characteristic for the taxonomic classification of different species of Pseudomonas. Most strains of P. aeruginosa produce pyocyanin(blue) or pyoverdin (Yellow) or both, as well as pyorubrin (red), pyomelanin (Brown), or various combinations of these pigments.

P. aeruginosa and other Pseudomonas isolated from humans often produce water soluble fluorescent pigments; pyoverdin is also one of these. Fluorescent pigment-producing strains fluoresce in under short-wave ultraviolet light. the fluorescence of pseudomonas is best observed at 254nm. Pseudomonas Agar (for fluorescein) is a modification of formulation described by King et al., This medium is recommended by IP for use in Microbial Limit Tests.

Principle

Pancreatic digest of casein and peptic digest of animal tissue provide nutrients, carbon, sulphur and trace elements for growth. Pancreatic digest of animal tissue is helpful for fluorescin production by pseudomonas. Dipotassium phosphate serve as the buffer. Magnesium sulphate enhance fluorescin production. Glycerin as an energy source also increases fluorescin production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Pancreatic Digest of Casein	10.0
Peptic Digest of animal tissue	10.0
Anhydrous Dibasic Potassium Phosphate	1.5
Magnesium Sulphate ($MgSO_{47}H_2O$)	1.5
Agar	15.0

Final pH (at 25°C) 7.2± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 38 gms of powder in 990 ml distilled water.
2. Add10 ml of glycerin and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (27853)	Good	Greenish yellow	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Inoculate and incubate plates aerobically at 35-37°C for 18-48 hours.
3. Examine for growth and fluorescein production.

Interpretation of Results

1. Examine growth under short wavelength UV light (254nm) for fluorescin.

2. Presence of fluorescin is appear with a greenish yellow fluorescent pigment in the colonies and surrounding medium.

Limitations

Some strains of Campylobacter may grow poorly or may be inhibited due to variation in nutritional requirements.

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Agar (For Fluorescein) USP AM108413/AM508413

Use

Pseudomonas Agar for Fluorescein is used for the detection of fluorescein production by *Pseudomonas* species in compliance with USP.

Summary

Pseudomonas species may produce water-soluble pigments in culture media. This property is sometimes used as a characteristic for the taxonomic classification of different species of *Pseudomonas.* Most strains of *P. aeruginosa* produce pyocyanin(blue) or pyoverdin (Yellow) or both, as well as pyorubrin (red), pyomelanin (Brown), or various combinations of these pigments.

P. aeruginosa and other Pseudomonas isolated from humans often produce water soluble fluorescent pigments; pyoverdin is also one of these. Fluorescent pigment-producing strains fluoresce in under short-wave ultraviolet light. the fluorescence of pseudomonas is best observed at 254nm. *Pseudomonas* Agar (for fluorescein) is a modification of formulation described by King et al, This medium is recommended by USP for use in Microbial Limit Tests.

Principle

Pancreatic digest of casein and proteose peptone provide nutrients, carbon, sulphur and trace elements for growth. Equal proportion of enzymetic hydrolysate of casein and pancreatic digest of animal tissue is helpful for fluorescin production by pseudomonas. Dipotassium phosphate serve as the buffer. Magnesium sulphate enhance fluorescin production. Glycerin as an energy source also increases fluorescin production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Pancreatic Digest of Casein	10.0
Peptic Digest of animal tissue	10.0
Anhydrous Dibasic Potassium Phosphate	1.5
Magnesium Sulphate (MgSO47H2O)	1.5
Agar	15.0

Final pH (at 25°C) 7.2± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 38 gms of powder in 990 ml distilled water.
2. Add10 ml of glycerin and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (27853)	Good	Greenish yellow	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Inoculate and incubate plates aerobically at 35-37°C for 18-48 hours.
3. Examine for growth and fluorescein production.

Interpretation of Results

1. Examine growth under short wavelength UV light (254nm) for fluorescin.
2. Presence of fluorescin is appear with a greenish yellow fluorescent pigment in the colonies and surrounding medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Agar (for Pyocyanin) AM108414/AM508414

Use

Pseudomonas Agar (for Pyocyanin) is used for the detection of Pseudomonas species using pyocyanin pigment production.

Summary

Pseudomonas species may produce water-soluble pigments in culture media. This property is sometimes used as a characteristic for the taxonomic classification of

different species of Pseudomonas. Most strains of *P. aeruginosa* produce pyocyanin(blue) or pyoverdin (Yellow) or both, as well as pyorubrin(red), pyomelanin(Brown), or various combinations of these pigments. *Pseudomonas* Agar (for Pyocyanin) is a modification of formulation described by King et al., This medium is recommended by USP and IP for use in Microbial Limit Tests.

Principle

Peptic digest of animal tissue provide nutrients, amino acids and trace elements for growth. Magnesium chloride and Potassium sulfate enhance pyocyanin production. Glycerin as an energy source also increases pyocyanin production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptic Digest of Animal Tissue	20.0
Anhydrous Magnesium Chloride	1.4
Anhydrous Potassium Sulfate	10.0
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 46.4 gms of powder in 990 ml distilled water.
2. Add10 ml of glycerin and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (27853)	Good	Blue to blue -green	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Inoculate and incubate plates aerobically at 35-37°C for 18-48 hours.
3. Examine for growth and pigment production.

Interpretation of Results

1. Presence of pyocyanin is appear with a blue to blue green pigment in the colonies and surrounding medium.
2. Confirm the presence of pyocyanin by adding several drops of chloroform and observe for a blue colour in chloroform.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Agar (for Pyocyanin) IP AM108415/AM508415

Pseudomonas Agar (for Pyocyanin) USP AM108416/AM508416

Use

Pseudomonas Agar (for Pyocyanin) is used for the detection of *Pseudomonas* species using pyocyanin pigment production.

Summary

Pseudomonas species may produce water-soluble pigments in culture media. This property is sometimes used as a characteristic for the taxonomic classification of different species of *Pseudomonas*. Most strains of *P. aeruginosa* produce pyocyanin(blue) or pyoverdin (Yellow) or both, as well as pyorubrin(red), pyomelanin(Brown), or various combinations of these pigments. *Pseudomonas* Agar (for Pyocyanin) is a modification of formulation described by King et al. This medium is recommended by USP and IP for use in Microbial Limit Tests.

Principle

Peptic digest of gelatin provide nutrients, amino acids and trace elements for growth. Magnesium chloride and Potassium sulfate enhance pyocyanin production. Glycerin as an energy source also increases pyocyanin production. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Pancreatic Digest of gelatin	20.0
Anhydrous Magnesium Chloride	1.4
Anhydrous Potassium Sulfate	10.0
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 46.4 gms of powder in 990 ml distilled water.
2. Add10 ml of glycerin and mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (27853)	Good blue -green	Blue to	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Obtain a pure culture of the organism to be tested.
2. Inoculate and incubate plates aerobically at 35-37°C for 18-48 hours.
3. Examine for growth and pigment production.

Interpretation of Results

1. Presence of pyocyanin is appear with a blue to blue green pigment in the colonies and surrounding medium.
2. Confirm the presence of pyocyanin by adding several drops of chloroform and observe for a blue colour in chloroform.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Isolation Agar AM108417/AM508417

Use

Pseudomonas Isolation Agar Base is recommended for selective isolation and identification of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

Summary

Pseudomonas aeruginosa is a classic opportunist pathogen and causes nosocomial infection. Pseudomonas Isolation Agar is formulated according to a slight modification of the medium A formulation of King, Ward and Raney (55). *P*seudomonas Isolation Agar incorporates triclosan, a potent broad-spectrum antimicrobial that makes the medium selective for *Pseudomonas aeruginosa.*

Principle

Peptic digest of animal tissue provides the carbon and nitrogen. Magnesium chloride and potassium sulfate support the bacterial growth. Triclosan is an antimicrobial agent selectively inhibits gram positive and gram negative bacteria other than *Pseudomonas* species.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.0
Magnesium chloride	1.40
Potassium sulphate	10.0
Triclosan(Irgasan)	0.025
Agar	13.6
Final pH (at 25°C) 7.0 ± 0.2	

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.03 gms of the powder in 1000ml distilled water. Containing 20 ml of glycerol.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, slightly opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium	RGI
Pseudomonas aeruginosa (10145)	Luxuriant	green	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	blue – blue-green	More than 70%
Proteus mirabilis (25933)	Inhibited	-	0%
Escherichia coli (25922)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

Inoculate the medium using streak plate method to obtain isolated colonies. Incubate for 18-48 hours at 35 ± 2°C.

Interpretation of Results

Examine the plates for the presence of Pseudomonas aeruginosa colonies. Pseudomonas aeruginosa colonies may be greenish after incubation for 18 hours and turn blue-green as incubation continues up to 24-48hours, with diffusion of the pigment into the medium.

Limitations

Some strains of Pseudomonas aeruginosa may fail to produce pyocyanin.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Pseudomonas Asparagine Broth AM108418/AM508418

Use

Pseudomonas Asparagine Broth used for presumptive determination of *Pseudomonas* aeruginosa from water samples.

Summary

Pseudomonas Asparagine Broth is formulated as recommended by APHA (36.1) for presumptive detection of *Pseudomonas* aeruginosa from recreational or natural waters. Recreational water like from swimming pool is a body of water in a holding structure. Microorganisms of concern are those causing infection of ear, skin and upper respiratory tract etc. *Pseudomonas* aeruginosa is one of those organisms which account for a large percentage of swimming pool associated illness.

Principle

This medium is a relatively simple medium containg an amino acid DL-Asparagine and two salts like dipotassium phosphate and magnesium sulphate. Asparagine is the amino acid source while phosphate and sulphate provide the ions for the growth of pseudomonas aeruginosa. Dipotassium phosphate also helps in maintaining the buffering condition of the medium. This medium is only a presumptive medium for *Pseudomonas* aeruginosa, and further confirmatory tests are necessary for the identification.

Formula*

Ingredients in grams per liter

DL-Asparagine	3.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.50

Final pH (at 25°C) 7.0±0.2
* Formula adjusted to suit performance parameters

Directions

1. Suspend 4.5gms of the powder in 1000ml-distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Pour into adequate containers.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

White coloured, homogeneous, free flowing powder.

Prepared Appearance

Colourless clear solution with slight precipitate.

Cultural Response

Cultural characteristics after 20-24 hours at 35°C.

Organisms(ATCC)	Growth
Pseudomonas aeruginosa (27853)	Luxuriant

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Purple Broth Base AM508419

Use

Purple Broth Base is recommended for the preparation of carbohydrate media used in fermentation studies for the culture identification of pure culture of enteric and other microorganisms.

Summary

Purple media is originally formulated by Vera (115.4). These media are recommended by FDA (2.1) for fermentation studies of sugars.

Principle

Peptone special supply the essential nutrients especially nitrogenous to the growing organisms. Sodium chloride maintains the osmotic balance to the medium. Bromo cresol purple is the pH indicator which turns yellow at acidic pH.

Gas production is evident by its collection in Durham's tube. The acid produced during the fermentation of carbohydrate causes bromo cresol purple, the pH indicator to turn yellow.

Formula*

Ingredients in grams per liter

Peptone, special	10.00
Sodium chloride	5.00
Bromo cresol purple	0.02

Final pH (at 25°C) 6.8±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15.02 gms of the powder in 1000ml-distilled water.
2. Add 5-10 grams of the carbohydrate to be tested.
3. Boil to dissolve the powder completely.
4. Dispense in tubes as desired and sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Alternatively sterilize the basal medium prepared using 900 ml distilled water and add 100 ml separately sterilized 5-10% solution of the desired carbohydrate to it.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Purple coloured clear solution form in tubes.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Without Carbohydrate		With 1% Carbohydrate	
		Acid	Gas	Acid	Gas
Neisseria meningitidis (13090)	Good- luxuriant	–	–	+	–
Escherichia coli (25922)	Luxuriant	–	–	+	+
Staphylococcus aureus (25923)	Luxuriant	–	–	+	–
Listeria monocytogenes (19112)*	Luxuriant	–	–	+	–

Key: Acid + = Yellow colur

** = Fermentative metabolism*

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

R-2A Agar — AM10841/AM50841
R-2A Agar (Agar Medium S) EP — AM50842

Use

R-2A Agar is used for obtaining heterotrophic plate count from treated potable water.

Summary

Reasoner and Geldreich (90.3) developed R2A medium to check the bacterial count in treated potable water. They found that plate count agar does not permit the growth of many bacteria that may be present in treated potable water supplies. Results from parallel studies with spread, membrane filter, and pour plate procedures showed that R2A medium yielded significantly higher bacterial counts than did plate count agar.

Low nutritional content, longer incubation time, yielded higher counts and increased detection of heterotrophic bacteria. As a tool to monitor heterotrophic bacterial populations in water treatment processes and in treated distribution water, R2A spread or membrane filter plates incubated at 28°C for 5 to 7 days is recommended. These conditions provide adequate time for growth of slow-growing bacteria.

R2A is useful in heterotrophic plate count analyses and for subculture of bacteria isolated from potable water samples. It is used for the recovery of stressed and chlorine-tolerant bacteria from drinking water. It is recommended by APHA for enumeration of heterotrophic bacteria in water and wastewater (17.1 & 32.1).

Principle

Since media contains low concentration of nutrients, it allows the growth of slow growing bacteria without suppressed by fast growing bacteria. Yeast extract provides a source of trace elements and vitamins. Proteose peptone provides nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium pyruvate increases the recovery of stressed cells. Potassium phosphate is used to balance the pH and provide phosphate. Magnesium sulfate is a source of divalent cations and sulfate. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Casein hydrolysate	0.50

Proteose peptone	0.50
Yeast extract	0.50
Glucose	0.5
Soluble starch	0.5
Sodium pyruvate .	0.3
Dipotassium hydrogen phosphate	0.3
Magnesium sulfate, anhydrous	0.024
Agar	15.00

Final pH: 7.2 ± 0.2 at 25°C

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.12 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the medium to approximately 45-50^{0}C, pour in to sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber, slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 5-7 days at 35^{0}C.

Organisms	Growth	RGI
Candia albicans (10231)	Good to luxuriant	More than 70%
Enterococcus faecalis (29212)	Good to luxuriant	More than 70%
Escherichia coli (25922)	Good to luxuriant	More than 70%
S. serotype Enteritidis (13076)	Good to luxuriant	More than 70%
S. serotype Typhi	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

(6539)

Procedure

1. Water samples should be collected as described in Standard Methods for the-Examination of Water and Wastewater, Section 9060A. Initiate analysis as soon as possible after collection to minimize changes in bacterial population. The recommended maximum elapsed time between collection and analysis of samples is 8h (maximum transit time 6h, maximum processing time 2h).when analysis cannot begin within 8h, maintain sample at a emperature below 40C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24h.
2. Prepare test dilutions for heterotrophic plate count.
3. Plate the test sample and dilutions by the spread plate, pour plate or membrane filter method. Do not exceed 1 mL of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
4. Maintain proper humidity during prolonged incubation.

Interpretation of Results

Count colonies promptly on spread or pour plates showing 30-300 colonies per plate using the membrane filter method Compute bacterial count per milliliter by the following equation:

$$\text{CFU/mL} = \frac{\text{Colonies counted}}{\text{Actual volume of sample in dish, mL}}$$

Limitations

1. Fast growing bacteria may produce smaller size colonies on R2A agar than on nutritionally rich media.
2. Pour plates do not give satisfactory results.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

R-2A Agar(Agar Medium S) BP — AM50843

Use

R-2A Agar is used for obtaining heterotrophic plate count from treated potable water in compliance with BP.

Summary

Reasoner and Geldreich (90.3) developed R2A medium to check the bacterial count in treated potable water. They found that plate count agar does not permit the growth of many bacteria that may be present in treated potable water supplies. Results from parallel studies with spread, membrane filter, and pour plate procedures showed that R2A medium yielded significantly higher bacterial counts than did plate count agar.

Low nutritional content, longer incubation time, yielded higher counts and increased detection of heterotrophic bacteria. As a tool to monitor heterotrophic

bacterial populations in water treatment processes and in treated distribution water, R2A spread or membrane filter plates incubated at 28°C for 5 to 7 days is recommended. These conditions provide adequate time for growth of slow-growing bacteria.

R2A is useful in heterotrophic plate count analyses and for subculture of bacteria isolated from potable water samples. It is used for the recovery of stressed and chlorine-tolerant bacteria from drinking water. It is recommended by APHA for enumeration of heterotrophic bacteria in water and wastewater (17.1 & 32.1).

Principle

Since media contains low concentration of nutrients, it allows the growth of slow growing bacteria without suppressed by fast growing bacteria. Yeast extract provides a source of trace elements and vitamins. Tryptone and peptone provides nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium pyruvate increases the recovery of stressed cells. Potassium phosphate is used to balance the pH and provide phosphate. Magnesium sulfate is a source of divalent cations and sulfate. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Casein hydrolysate	0.50
Proteose peptone	0.50
Yeast extract	0.50
Glucose	0.5
Soluble starch	0.5
Sodium pyruvate .	0.3
Dipotassium hydrogen phosphate	0.3
Magnesium sulfate, anhydrous	0.024
Agar	15.00

Final pH: 7.2 ± 0.2 at 25°C

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.12 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the medium to approximately 45-50°C, pour in to sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 5-7 days at 35°C.

Organisms	Growth	RGI
Candia albicans (10231)	Good to luxuriant	More than 70%
Enterococcus faecalis (29212)	Good to luxuriant	More than 70%
Escherichia coli (25922)	Good to luxuriant	More than 70%
S. serotype Enteritidis (13076)	Good to luxuriant	More than 70%
S. serotype Typhi (6539)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Water samples should be collected as described in Standard Methods for the-Examination of Water and Wastewater, Section 9060A. Initiate analysis as soon as possible after collection to minimize changes in bacterial population. The recommended maximum elapsed time between collection and analysis of samples is 8h (maximum transit time 6h, maximum processing time 2h).when analysis cannot begin within 8h, maintain sample at a temperature below 40C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24h.
2. Prepare test dilutions for heterotrophic plate count.
3. Plate the test sample and dilutions by the spread plate, pour plate or membrane filter method. Do not exceed 1 mL of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
4. Maintain proper humidity during prolonged incubation.

Interpretation of Results

Count colonies promptly on spread or pour plates showing 30-300 colonies per plate using the membrane filter method Compute bacterial count per milliliter by the following equation:

$$\text{CFU/mL} = \frac{\text{Colonies counted}}{\text{Actual volume of sample in dish, mL}}$$

Limitations

1. Fast growing bacteria may produce smaller size colonies on R2A agar than on nutritionally rich media.
2. Pour plates do not give satisfactory results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Raka-Ray Agar, Base
(Lactic Acid Bacteria Selective Agar, Base)

AM10844

Use

Raka-Ray Agar, Base medium used for the isolation of lactic acid bacteria in beer and brewing processes.

Summary

Raka-Ray Agar, was developed of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes. The European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC) recommend using this Agar. Diverse members of the family of *Lactobacillaceae* are important spoilage organisms in the brewing process. This medium was optimized to meet the natural requirements of the *Lactobacillaceae*. The original Medium Raka-Ray Medium No. 3 was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci. Originally diverse combinations of stimulating agents were added to Universal Beer Agar to get an optimized media with improved colony size, colony numbers and incubation time. This was then the base for Raka-Ray Medium No. 3. Van Keer et al., found that Raka-Ray Medium No. 3 yielded the highest colony count of divers media and allowed the enumeration of the greatest number of strains. 30 strains of Lactobacillus have been taken from different origins and was incubated 48 hours under semi-anaerobic conditions.

Principle

Casein enzymic hydrolysate, liver concentrate, yeast extract provide carbon, nitrogen, amino acids, minerals, vitamins, trace elements and other essential nutrients for growth. Potassium aspartate and potassium glutamate are additional sources of amino acids. Maltose is to detect *lactobacilli*, which cannot utilise glucose as a carbon source and fructose is the carbon source of Lactobacillus fructivorans. Glucose is needed as the fermentative energy source for the pediococci. Sorbitan mono-oleate act as a stimulant for lactic acid bacteria in general. Also N-acetyl glucosamine, yeast extract and betaine hydrochloride are used as growth stimulating agents. Diammonium hydrogen-citrate and Monopotassium phosphate are the buffering agents while Magnesium and Manganese are important trace elements for Lactobacillaceae. The addition of 3 g/l of phenylethanol inhibits Gram-negative organisms, 5 mg/l amphotericin B and 7mg/l of cycloheximide to inhibit yeasts and moulds.

Formula*

Ingredients in grams per liter

Yeast extract	5.0
Casein enzymic hydrolysate	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Glucose	5.0
Betaine hydrochloride	2.0
Diammonium hydrogen-citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate	2.0
Manganese sulphate	0.66
Monopotassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0

Final pH: 5.4± 0.2 at 25°C

* Formula adjusted to suit performance parameters

Directions

1. Suspend 77.1 g in 1000 ml of distilled water.
2. Bring to the boil and dissolve the medium completely.
3. Distribute into tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes.
4. Cool to 50-55°C and add contents of 1 vial of lactic acid bacteria selective supplement (AS0151).
5. Mix well and pour into sterile petri dishes or dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C

Organisms	Growth	RGI
Leuconostoc mesenteroides (8293)	Luxuriant	More than 70%
Lactobacillus acidophilus (11506)	Luxuriant	More than 70%
Lactobacillus bulgaricus (11842)	Luxuriant	More than 70%
Lactobacillus casei (7469)	Luxuriant	More than 70%
Lactobacillus leichmannii (7830)	Luxuriant	More than 70%
Lactobacillus plantarum (8014)	Luxuriant	More than 70%
Lactobacillus fermentans (9338)	Luxuriant	More than 70%
Escherichia coli (25922)	None-Poor	0%
Sacch. cerevisae (9763)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Incubation:

An incubation period of 4 days is generally sufficient. The test strains needs no

longer than 24 hours incubation but slower growing organisms may require up to 7 days. Depending on the species of lactic acid bacteria a semi-anaerobic atmosphere may be recommendable.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Raka Ray No 3 Broth Base (Lactic Acid Bacteria Selective Broth Base) AM10845

Use

Raka Ray No 3 Broth Base is a selective medium for the isolation of lactic acid bacteria in beer and brewing processes.

Summary

Raka Ray No 3 Broth, was developed of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes. Diverse members of the family of Lactobacillaceae are important spoilage organisms in the brewing process. This medium was optimised to meet the natural requirements of the Lactobacillaceae. This formulation was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci. Originally diverse combinations of stimulating agents were added to Universal Beer Agar to get an optimized media with improved colony size, colony numbers and incubation time. This was then the base for Raka-Ray Broth No. 3. Van Keer et al., found that Raka-Ray Broth No. 3 yielded the highest colony count of divers media and allowed the enumeration of the greatest number of strains. 30 strains of Lactobacillus have been taken from different origins and was incubated 48 hours under semi-anaerobic conditions.

Principle

Casein peptone, yeast extract provide carbon, nitrogen, amino acids, minerals, vitamins, trace

elements and other essential nutrients for growth. Potassium aspartate and potassium glutamate are additional sources of amino acids. Maltose is to detect lactobacilli, which cannot utilise glucose as a carbon source and fructose is the carbon source of Lactobacillus fructivorans. N-acetyl glucosamine, liver concentrate yeast extract and betaine hydrochloride are used as growth stimulating agents. Diammonium citrate and potassium hydrogen phosphate are buffering agents while Magnesium and Manganese are important trace elements for Lactobacillaceae. Tween 80 acts as a fatty acid source. The addition of 3 g/l of phenylethanol inhibits Gramnegative organisms, 5 mg/l amphotericin B and 7mg/l of cycloheximide to inhibit yeasts and moulds.

Formula*

Ingredients in grams per liter

Yeast extract	5.0
Casein peptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	10.0
Betaine hydrochloride	2.0
Diammonium citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulfate heptahydrate	0.98
Manganese sulfate monohydrate	0.42
Dipotassium hydrogen phosphate	2.0
N-Acetylglucosamine	0.5

Final pH: 5.4 ± 0.2 at 37°C

* Formula adjusted to suit performance parameters

Directions

1. Dissolve 58.9 gms. in 1000 ml distilled water and add 2 ml Tween 80.
2. Bring to the boil and dissolve the medium completely.
3. Distribute into tubes and sterilize by autoclaving at 121°C for 15 minutes.
4. Cool to 50-55°C and add contents of 1vial of lactic acid bacteria selective supplement (AS0151).
5. Mix well and pour into sterile petri dishes or dispense as desired.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Brown-yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 18-24 hours at 27-30°C under anaerobic atmosphere.

Organisms	Growth
Escherichia coli (25922)	-/+
Lactobacillus brevis (367)	+++
Lactobacillus buchneri (11307)	+++

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rapid Accu Coliform Broth AM1084511/AM5084511

Use

Rapid Accu Coliform Broth is used for detection and confirmation of *Escherichia coli* and total coliforms on the basis of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrate.

Summary

Rapid Accu Coliform Broth is the modification of LMX Broth described by Manafi and Kneifel (78.3). Rapid Accu Coliform Broth is recommended for the simultaneous detection of total coliforms and Escherichia coli.

Principle

Peptone, special provides the essential nutrient for the growth of the microorganisms and it is rich in tryptophan, useful for the simultaneous indole production. Sorbitol is the source of carbon. Phosphate salts acts as buffering agent while sodium chloride maintains the osmotic balance. Sodium lauryl sulphate makes the medium selective by inhibiting the Gram - positive microorganisms. The fluorogenic substrate is split by enzyme β-D-glucuronidase, which is specifically found in *Escherichia coli*. The reaction is indicated by a blue fluorescence under UV light. The presence of total coliforms is indicated by a blue-green colour of the broth due to the cleavages of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of β-D-galactosidase. To confirm the presence of *Escherichia coli* in broth medium by indole reaction overlay the medium with Kovac's reagent. The layers turn red within 2 minutes in case of positive reaction.

Formula*

Ingredients in grams per liter

Chromogenic substrate	0.08
Dipotassium hydrogen phosphate	2.70
Fluorogenic substrate	0.05
IPTG (Isopropyl-b-D-thiogalactopyranoside)	0.10
Peptone, Special	5.0
Potassium dihydrogen phosphate	2.0
Sodium chloride	5.0
Sodium lauryl sulphate	0.10
Sorbitol	1.0

Final pH (at 25°C) 6.8± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 16gms of the powder in 1000ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Pour into adequate containers.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution forms in tubes.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms	Colour change in medium	Fluorescence*	Indole reaction
Enterobacter aerogenes (13048)	Blue-green	-	-
Escherichia coli (25922)	Blue-green	+	+

Key: + = Positive reaction

- = negative reaction

* = Fluorescence as under UV light.

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store below 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rappaport Vassiliadis Salmonella Enrichment Broth (Harmonized) AMH508453

Use

Rappaport Vassiliadis Salmonella Enrichment Broth is recommended for selective enrichment of *Salmonella* species under conditions of high osmotic pressure and low pH with modest nutritional requirements.

Summary

Rappaport et al., formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al., Rappaport Vassiliadis (90.1 & 115.3) *Salmonella* Enrichment Broth is a selective enrichment for *Salmonella* species. This medium

is selective for *Salmonella* species because they are typically resistant to malachite green, high osmotic pressure and low pH. *S. typhi* and *S. choleraesuis* are sensitive to malachite green and may be inhibited. This medium is also recommended by United States of Pharmacopeia.

Principle

Soya peptone provides the essential nutrients for the growth of the bacteria. Phosphate salts act as buffer to maintain the pH. Magnesium chloride maintains the high osmotic pressure and *Salmonella* generally survive at little high osmotic pressure. Malachite green inhibits other microorganisms other than *Salmonella.*

Formula*

Ingredients in grams per liter

Soya peptone	4.5
Magnesium chloride hexahydrate	29.0
Sodium chloride	8.0
Dipotassium phosphate	0.4
Potassium dihydrogen phosphate	0.6
Malachite green	0.036

Final pH (at 25ºC) 5.2±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 42.54 gms of the powder in 1000ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes as desired.
4. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Pale blue coloured, homogeneous, free flowing powder.

Prepared Appearance

Blue coloured clear solution without any precipitate

Cultural Response

Cultural characteristics observed after an incubation of 18-24 hours at 30-35°C and subcultured on XLD Agar, incubated at 30-35°C for 18-48 hours..

Organisms	Growth	Colour of colony	RGI
Salmonella typhimurium (23564)	Luxuriant	Red colonies with black center	More than 70%
Salmonella typhi (6539)	Luxuriant	Red colonies with black center	More than 70%
Escherichia coli (25922)	Fair	yellow	0%
Salmonella abony NCTC (6017)	Luxuriant	Red colonies with black center	More than70%
Salmonella enterica (13076)	Luxuriant	Red colonies with black center	More than 70%
Salmonella typhi NCTC (786)	Luxuriant	Red colonies with black center	More than 70%
Staphylococcus aureus (6538)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Reddy's Differential Agar, Modified (Lactic Streak Agar) AM50846

Use

Reddy's Differential Agar, Modified is recommended for qualitative and quantitative differentiation of lactic *Streptococci.*

Summary

Reddy's Differential Agar, Modified was originally described by Reddy et al., (91.1) and further modified by Mullan and Walker (82.3) and recommended by APHA (115.1) for the differentiation of lactic *Streptococci.* This modification gives faster results and there is no need of incubation in CO_2 enrichment environment.

Principle

Lactococcus lactis and its subspecies *cremoris* and *diacetylactis* are used as starter cultures in dairy products. They are differentiated on the basis of arginine hydrolysis and citrate utilization. Lactose fermenters produce acid and are seen as yellow colonies. *Lactococcus* lactis initially produces acid but later on turns to violet – purple subspecies *diacetylactis* produces a more intense purple colour than *Lactococcus lactis.*

Citrate utilization is seen as clear zone around the colony. For quantitative determination, decimal dilution of cultures are prepared and spread on agar plates. After incubation at 36 to 40 hours at 32°C yellow colonies of subspecies cremoris are counted. The plates are further incubated for 4 days and then total count is taken as well as colonies with clearing zones of subspecies *diacetylactis* are counted and subtracted from total count to get *Lactococcus lactis* population in the mixture.

Formula*

Ingredients in grams per liter

Part A: Sodium carboxymethyl cellulose	10.00
Calcium citrate	10.00
Part B: Peptic digest of animal tissue	5.00
Peptic digest of soyabean meal	5.00
Yeast extract	5.00
Beef extract	5.00
Lactose	1.50
L-Arginine Hydrochloride	1.50
Bromo cresol purple	0.002
Agar	15.00

Final pH: at 25°C6.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38 gms of Part B in 800 ml distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Suspended 20 grams of Part A in 200 ml distilled water.
4. Mix Part A and Part B.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 10 minutes.

Quality Control

Dehydrated Appearance

Part A: Yellow coloured, homogeneous, free flowing powder.

Part B: Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, opalescent gel having greenish tinge forms in petri plates.

Cultural Response

Cultural characteristics after 4 hours at 32°C.

Organisms	Growth	Colour of colony	Citrate Utilization	RGI
Lactococcus lactis (8000)	Good-luxuriant	Yellow	–	More than 70%
Lactococcus lactis (19527) *subsp. Cremoris*	Good-luxuriant	Purple	–	More than 70%
Lactococcus lactis subsp. diacetylactis	Good-luxuriant	Purple	+	More than 70%

Key: + = Positive, clearing zone around colony

– = Negative

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Reinforced Clostridial Agar AM1085/AM5085

Use

Reinforced *Clostridial* Agar is used for the cultivation and enumeration of clostridia and other anaerobes.

Summary

Reinforced Medium For Clostridia is formulated by Hirsch and Grinsted (43.2). It can be used to initiate growth from small inocula and to obtain the highest viable count of *Clostridia*. Barnes and Ingrams used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens* (5). It can be used in studies of spore forming anaerobes, especially *Clostridium butyrium* in cheese, for enumeration of *clostridia* in tube dilution counts or for preparation of plates for isolation (71.1). Other spore forming anaerobes, *streptococci* and *Lactobacilli* also grow in these media. These are enriched but non selective media.

Principle

Casein enzymic hydrolysate, yeast extract, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia*. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintain osmotic equilibrium. These media can be made selective by addition of 15-20 mg Polymyxin B per liter of media.

Formula*

Ingredients in grams per liter

Peptone	10.00
Yeast extract	3.00
Beef extract	10.00
Glucose monohydrate	5.00
Sodium chloride	5.00
Sodium acetate	3.00
Starch, soluble	1.00
Cysteine hydrochloride	0.50
Agar	0.50

Final pH: 6.8 ± 0.2 at 25°C*

Formula adjusted to suit performance parameters

Directions

1. Suspend 38 gms in 1000 ml distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, Clear to slightly opalescent solution in tubes.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Bacteroides Fragilis (23745)	Good to luxuriant
Bacteroides vulgatus (8482)	Good to luxuriant
Clostridium butyricum (9690)	Good to luxuriant
Clostridium perfringens (13124)	Good to luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Reinforced Clostridial Broth (Harmonized) AMH50851

Reinforced Clostridial Broth USP AM10851/AM50851

Reinforced Clostridial Broth (Medium P) EP AM50852

Reinforced Clostridial Broth (Medium P) BP AM50853

Use

Reinforced Clostridial Broth is used for the cultivation and enumeration of clostridia and other anaerobes.

Summary

Reinforced Clostridial Broth is based on the formulation of Hirsch and Grinsted (43.2). The medium supports growth of clostridia from small inocula and produces higher viable cell counts. Barne's *et al.*, used this medium to enumerate clostridia in food. Attenborough and Scarr used this medium in conjunction with membrane filters, for the count of *C.thermosaccharolyticum* in sugar. This is a non-selective enriched medium, which allows growth of various anaerobic and facultative bacteria when incubated anaerobically. Reinforced Clostridial Broth is recommended by APHA for the examination of milk . Reinforced Clostridial Broth EP/USP/BP is recommended for the cultivation and enumeration of clostridia and other anaerobes in compliance with EP/USP/BP.

Principle

Peptone and beef extract provide sources of nitrogen, carbon and other growth factors. Yeast extract provides B complex vitamins while dextrose is the carbohydrate source. In low concentrations, soluble starch detoxifies metabolic byproducts and Cysteine hydrochloride apart from being a nutrient acts as a reducing agent. Sodium acetate is the buffering agent while sodium chloride maintains the osmotic balance. This medium can be made selective by the addition of 15-20 mg of polymixin B per liter of medium.

Formula*

Ingredients in grams per liter

Beef extract	10.0
Peptone	10.0
Sodium chloride	5.0
Glucose monohydrate	5.0
Sodium acetate	3.0
Yeast extract	3.0
Soluble starch	1.0
Cysteine hydrochloride	0.5
Agar	0.5

Final pH (at 25°C) 6.8 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 38.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, free flowing, homogeneous powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent solution in tubes.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C, in an anaerobic atmosphere.

Organisms (ATCC)	Growth
Bacteroides fragilis (23745)	Good to luxuriant
Bacteroides vulgatus (8482)	Good to luxuriant
Clostridium perfringens (13124)	Good to luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Precautions / Limitations

1. This is a non-selective medium. Other spore forming anaerobes, streptococci and lactobacilli also grow on this medium.
2. Biosafety Level 2 practices, containment equipment and facilities are

recommended for activities with clinical specimens of human or animal origin containing C.botulinum, C.tetani or their toxins.

3. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of these organisms and for activities with a high potential for aerosol or droplet production.

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Ringer Salt Solution Powder AM108531

Use

Ringer Salt Solution Powder is a recommended isotonic diluent for food, milk and dairy products during microbiological examinations.

Summary

Ringer Salt Solution is a balanced salt solution contains an array of inorganic ionic species that are essential for critical functions when cells are removed from their in *vivo milieu.*

Principle

Ringer Salt Solution Powder is used as isotonic solution. Different types of salts maintain the osmotic balance and make the medium isotonic. These include sodium and potassium to regulate toxicity and permeability, calcium to maintain the integrity of cell membranes and internal structures, and bicarbonate to control the hydrogen ion concentration through their buffering effect.

Formula*

Ingredients in grams per liter

Sodium chloride	8.50
Potassium chloride	0.20
Calcium chloride	0.20
Sodium bicarbonate	0.01
Final pH (at 25°C) 7.0 ± 0.2	

Formula adjusted to suit performance parameters

Directions

1. Suspend 8.91 gms powder in 1000ml of distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

White coloured, homogeneous, free flowing powder.

Prepared Appearance

Colourless clear solution without any precipitate.

Cultural Response

Satisfactory results are obtained when used as a diluent during bacteriological examination of foods, dairy products as well as for serial dilutions of pure cultures of bacteria.

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rogosa SL Agar AM50854

Use

Rogosa SL Agar is used for selective solid medium for cultivation of oral and faecal *Lactobacilli.*

Summary

Rogosa SL media are the modification of the medium described by Rogosa *et al.* (95), and give excellent results when used in qualitative and quantitative studies of *Lactobacilli* in faeces, saline and in dairy products.

Principle

Tryptose, yeast extarct provides nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of *Lactobacilli*. Dextrose, arabinose, saccharose are the fermentable carbohydrates. Polysorbate 80 is the source of fatty acids. Ammonium citrate and sodium acetate inhibit moulds, *Streptococci* and many other organisms. Low pH of the medium selective for *Lactobacilli* inhibiting other bacterial flora.

Formula*

Ingredients in grams per liter

Tryptose	10.0
Yeast Extract	5.0
Dextrose	10.0
Arabinose	5.0
Saccharose	5.0
Sodium acetate	15.0
Ammonium citrate	2.0

Monopotassium phosphate	6.0
Magnesium sulphate	0.57
Manganese sulphate	0.12
Ferrous sulphate	0.03
Polysorbate 80	1.00
Agar	15.00

Final pH (at 25ºC) 5.4 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 75 gms of the powder in 1000 ml distilled water.
2. Boil to dissolve the medium completely.
3. Add 1.32 ml glacial acetic acid. Mix thoroughly. Heat to 90-100ºC for 2-3 minutes. Cool to 45ºC for direct inoculation. DO NOT AUTOCLAVE.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, powder containing soft lumphs.

Prepared Appearance:

Light yellow coloured slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C, in 5% CO_2 and 95% H_2.

Organisms (ATCC)	Growth	RGI
Lactobacillus casei (9595)	Good to luxuriant	More than 70%
Lactobacillus fermentum (9338)	Good to luxuriant	More than 70%
Lactobacillus leichmanni (4797)	Good to luxuriant	More than 70%
Lactobacillus plantarum (8014)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index.

Storage

Store at 2- 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rogosa SL Broth AM50855

Use

Rogosa SL Broth is used for selective medium for cultivation of oral and faecal *Lactobacilli.*

Summary

Rogosa SL media are the modification of the medium described by Rogosa *et al.* (95), and give excellent results when used in qualitative and quantitative studies of *Lactobacilli* in faeces, saline and in dairy products.

Principle

Casein enzyme hydrolysate, yeast extract provides nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of *Lactobacilli.* Dextrose, arabinose, saccharose are the fermentable carbohydrates. Polysorbate 80 is the source of fatty acids. Ammonium citrate and sodium acetate inhibit moulds, Streptococci and many other organisms. Low pH of the medium selective for *Lactobacilli* inhibiting other bacterial flora.

Formula*

Ingredients in grams per liter

Casein enzyme hydrolysate	10.0
Yeast extract	5.0
Dextrose	10.0
Arabinose	5.0
Saccharose	5.0
Sodium acetate	15.0
Ammonium citrate	2.0
Monopotassium phosphate	6.0
Magnesium sulphate	0.57
Manganese sulphate	0.12
Ferrous sulphate	0.03
Polysorbate 80	1.00

Final pH (at 25ºC) 5.4 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 60 gms of the powder in 1000 ml distilled water.
2. Boil to dissolve the medium completely.
3. Add 1.32 ml glacial acetic acid. Mix thoroughly. Heat to 90-100ºC for 2-3 minutes. Cool to 45ºC for direct inoculation. DO NOT AUTOCLAVE.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, powder containing soft lumphs.

Prepared Appearance:

Light yellow coloured slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C, in 5% CO_2 and 95% H_2.

Organisms (ATCC)	Growth
Lactobacillus casei (9595)	Good to luxuriant
Lactobacillus fermentum (9338)	Good to luxuriant
Lactobacillus leichmanni (4797)	Good to luxuriant
Lactobacillus plantarum (8014)	Good to luxuriant
Staphylococcus aureus (25923)	Inhibited

Storage

Store at 2- 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rose Bengal Agar Base AM50856

Use

Rose Bengal Agar Base is recommended for selective isolation and enumeration of yeasts and moulds from environmental materials and food stuffs.

Summary

Yeasts and moulds can cause various degrees of food decomposition. Rose-Bengal Chloramphenicol Agar is a selective medium for the enumeration of yeasts and moulds from a wide variety of foodstuffs. Rose Bengal Chloramphenicol Agar was formulated originally by Jarvis (47.2) and further modified by Overcast and Weakly (85.2). Rose-Bengal is taken up by mould and yeast colonies thereby assisting enumeration of small colonies.

Principle

Papaic digest of soyabean meal serves as an essential source of nutrients. Dextrose is the fermentable carbohydrate. Monopotassium phosphate acts as a buffering agents and magnesium is a trace element important for the growth of yeasts and moulds. Rose bengal dye suppresses the development of bacteria and reduces the excessive mycelial growth of moulds.

Formula*

Ingredients in grams per liter

Papaic digest of soyabean meal	5.0
Dextrose	10.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.50
Rose Bengal	0.05
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 31.55 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45°C and add 2 ml of rehydrated Chloramphenicol Selective Supplement (AS00911) for each 500ml of Rose Bengal Agar Base.
6. Mix thoroughly and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Deep pink coloured, clear to very slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C for fungi and 24-48 hrs at 35-37°C for bacteria.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Good	More than 70%
Candida albicans (10231)	Good	More than 70%
Escherichia coli (25922)	Inhibited	0%
Micrococcus luteus (10240)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Limitations

It is essential to store plates of media containing Rose-Bengal in the dark to prevent toxic photo-oxidation of the dye.

Colonies of bacteria on this medium may be mistaken for those of yeasts and thus should be examined microscopically to confirm their identity.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Rose Bengal Chloramphenicol Agar AM10857/AM50857

Use

Rose Bengal Chloramphenicol Agar is used for selective isolation and enumeration of yeasts and moulds from environmental materials and foodstuffs.

Summary

Yeasts and molds can cause various degrees of food decomposition (1.3). Rose-Bengal Chloramphenicol Agar is a selective medium for the enumeration of yeasts and moulds from a wide variety of foodstuffs. Rose Bengal Chloramphenicol Agar was formulated originally by Jarvis (47.2) and further modified by Overcast and Weakly (85.2). Rose-Bengal is taken up by mould and yeast colonies thereby assisting enumeration of small colonies.

Principle

Mycological peptone serves as an essential source of nutrients. Dextrose is the fermentable carbohydrate. Monopotassium phosphate acts as a buffering agents and magnesium is a trace element important for the growth of yeasts and moulds. Rose bengal dye suppresses the development of bacteria and reduces the excessive mycelial growth of moulds. Chloramphenicol has inhibitory action on gram-negative bacteria.

Formula*

Ingredients in grams per liter

Mycological peptone	5.0

Dextrose	10.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.50
Rose Bengal	0.05
Chloramphenicol	0.10
Agar	15.5
Final pH (at 25°C)	7.2 ± 0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend 32.15 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Do not overheat.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Deep pink coloured, clear to very slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C for fungi and 24-48 hrs at 35-37°C for bacteria..

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Cladosporium cladosporoides (45534)	Luxuriant	More than 70%
Mucor racemosus (42647)	Luxuriant	More than 70%
Penicillium notatum (10108)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Inhibited	0%
Escherichia coli (25922)	Inhibited	0%
Bacillus subtilis (6633)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Inoculate the agar plates directly using surface spreading technique with serial dilutions.
2. Incubate the plates at 25°C for 5 days.
3. Examine the results of the plates.

Interpretation of Results

The number of yeasts or moulds is calculated per 1 g or 1 ml of sample being tested by multiplying the number of colonies observed by the dilution factor.

Limitations

1. It is essential to store plates of media containing Rose-Bengal in the dark to prevent toxic photo- oxidation of the dye.
2. Colonies of bacteria on this medium may be mistaken for those of yeasts and thus should be examined microscopically to confirm their identity.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Chloramphenicol Agar AM1086/AM5086

Sabouraud Chloramphenicol Agar IP AM50861

Sabouraud Chloramphenicol Agar EP AM50862

Sabouraud Chloramphenicol Agar BP AM50863

Use

Sabouraud Chloramphenicol Agar is used for selective cultivation of yeasts and moulds.

Summary

Sabouraud Chloramphenicol Agar is Carliers modification (12) of the formulation described by Sabouraud (99) with the addition of chloramphenicol, for the cultivation of fungi, particularly those associated with skin infections. It is used for the isolation of pathogenic fungi from materials containing large number of saprophytic fungi or bacteria. It is also recommended by the IP/EP/BP in Microbial Limit Tests for performing total yeast and mould count.

Principle

Tryptone and peptone provide nitrogenous compounds, carbon and other growth factors. Dextrose is the carbohydrate source. The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes and slightly inhibitory to contaminating bacteria. Chloramphenicol inhibits a wide range of gram-positive and gram-negative bacteria making the medium selective for fungi (7.1).

Formula*

Ingredients in grams per liter	AM1086/AM5086 AM50861	AM50862	AM50863
Peptic digest of animal tissue	5.0	-	-

Pancreatic digest of casein	5.0	-	-
Peptone (meat+casein)	-	10.0	10.0
Glucose monohydrate	-	40.0	40.0
Dextrose	40.0	-	-
Chloramphenicol	0.05	0.05	0.05
Agar	15.0	15.0	5.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C for fungi and 24-48 hours at 35-370C for bacteria.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Lactobacillus casei (9595)	Inhibited	0%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculation.
2. Inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Once inoculated, the medium should be protected from light and incubated aerobically at 20-25°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Isolate using pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 20-25°C.

Note: After autoclaving, do not heat medium longer than 3 hours at 45-50°C. Sterile solid medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observing colony morphology, characteristic microscopic structures, rate of growth, etc.
2. Yeasts are identified by various biochemical tests. For spread plate and pour plate method
3. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. Some of the pathogenic fungi may produce infective spores, which can be easily dispersed in the laboratory. Examine such organisms only within a protective cabinet.
2. When used for selective isolation, antimicrobials like chloramphenicol and cycloheximide may inhibit some pathogenic fungi. However, the mycelial phase of Histoplama capsulatum, Paracoccidioides brasiliensis, Sporothrix schoenckii and Blastomyces dermatidis is not inhibited by these antibiotics when incubated at 20-25°C .
3. A non-selective and selective medium should be inoculated for isolation of fungi from potentially contaminated specimens.

Storage

Store below 8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Chloramphenicol Agar with Lecithin and Tween 80 — AM50864

Use

Sabouraud Chloramphenicol Agar with Lecithin and Tween 80 medium used as a selective cultivation of yeasts and moulds.

Summary

Sabouraud Chloramphenicol Agar is Carliers modification of the formulation described by Sabouraud with the addition of chloramphenicol, for the cultivation of fungi, particularly those associated with skin infections. It is used for the isolation of pathogenic fungi from materials containing large number of saprophytic fungi or bacteria.

Principle

Tryptone and peptone provide nitrogenous compounds, carbon and other growth factors. Dextrose is the carbohydrate source. The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes and slightly inhibitory to contaminating bacteria. Chloramphenicol inhibits a wide range of gram-positive and gram-negative bacteria making the medium selective for fungi. Tween 80 and lecithin act as neutralizers to inactivate the residual disinfectants where the samples are collected. Lecithin inactivates quaternary ammonium compounds whereas tween 80 neutralizes formalin, phenolic disinfectants, hexachlorophene etc.

Formula*

Ingredients in grams per liter

Tryptone	5.00
Peptone	5.00
Dextrose	40.00
Chloramphenicol	0.05
Agar	15.00
Lecithin	0.70
Tween 80	5.00

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 70.7 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C for fungi and 24-48 hours at 35-37°C for bacteria..

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Lactobacillus casei (9595)	Inhibited	0%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 2-8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Dextrose Agar (Harmonized) AMH5087

Sabouraud Dextrose Agar IP AM10871/AM50871

Sabouraud Dextrose Agar USP AM10872/AM50872

Sabouraud Dextrose Agar EP AM10873/AM50873

Use

Sabouraud Dextrose Agar is general-purpose media used for the cultivation of yeasts, moulds and aciduric bacteria.

Summary

Sabouraud Dextrose Agar is Carliers (12) modification of the formulation described by Sabouraud for the cultivation of fungi, particularly those associated with skin infections. It is used in qualitative procedures for cultivation of pathogenic and non-pathogenic fungi, particularly dermatophytes. Carlier showed that this medium gives reliable results with *Microsporum audouini, M.canis, Trichophyton mentagrophytes, T.flavum, T.rubrum* and *Candida* albicans. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud. Sabouraud Dextrose Agar is recommended by the USP and IP in Microbial Limit Tests for performing total yeast and mould count and is included in the Bacteriological Analytical Manual for food testing. It is also recommended by APHA for the examination of foods. Sabouraud Dextrose Agar can be made inhibitory to most pathogenic fungi and bacteria by the addition of antibiotics. Gentamycin is an amino glycoside that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-positive and gram-negative bacteria, cycloheximide is an antifungal agent that inhibits saprophytic fungi while allowing the growth of yeasts or dermatophytes. George et al aseptically added 0.5 gm cycloheximide, 20000 units penicillin and 40000 units streptomycin to each liter of autoclaved, cooled medium. *Cryptococcus neoformans, Aspergillus fumigatus* and *Allescheria boydii* were found to be sensitive to cycloheximide; Actinomyces bovis and Nocardia asteroids were sensitive to penicillin and streptomycin. Hantshke used colistin, novobiocin and

cycloheximide to isolate *Candida albicans*. Dolan used gentamycin, chloramphenicol and cycloheximide for the selective isolation of pathogenic fungi.

Principle

Mixture of peptic digest of animal tissue and pancreatic digest of casein provide nitrogenous compounds, carbon and other growth factors. Dextrose is the carbohydrate source. The low pH of approximately 5.6 is favourable for the growth of fungi, especially dermatophytes and is slightly inhibitory to contaminating bacteria. Various antibiotics can be added to this medium for bacterial inhibition as well as to make it selective for the isolation of pathogenic fungi from material containing large number of other fungi or bacteria. Sabouraud Dextrose Agar may also be used as the basis of Pagano- Levin medium for the isolation of *Candida albicans*. 0.1 gm of filter sterilized triphenyltetrazolium chloride is added to each liter of autoclaved molten medium cooled to 55°C. After incubation at 25°C for 3 days, *Candida albicans* colonies are unpigmented or pale pink while other *Candida* species and other fungi form deep pink or red colonies. Other tests should be performed for identification of *Candida albicans*.

Formula*

Ingredients in grams per liter

Mixture of peptic digest of animal tissue and pancreatic digest of casein(1:1)	10.0
Dextrose	40.0
Agar	15.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65 gms of the powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. Avoid overheating the agar as it could cause a softer medium.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C for fungi and 24-48 hours at 35-37°C for bacteria.

Organisms (ATCC)	Growth	RGI
AAspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant*	More than 70%
Lactobacillus casei (9595)	Luxuriant*	More than 70%

Key:

** = inhibited on media with low pH*

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculating.
2. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plates in an inverted position.
5. Once inoculated, the medium should be protected from light and incubated aerobically at 20-25°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Inoculate using the pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 20-25°C.

Note: After autoclaving, do not heat the medium longer than 3 hours at 45-50°C. Sterile solidified medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observing colony morphology, characteristic microscopic structures, rate of growth, etc. Yeasts are identified by various biochemical tests. Pour plate and spread plate method
2. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. Some of the pathogenic fungi may produce infective spores, which can be easily dispersed in the laboratory. Examine such organisms only within a protective cabinet.
2. When used for selective isolation, antimicrobials like chloramphenicol and cycloheximide may inhibit some pathogenic fungi. However, the mycelial phase of Histoplama capsulatum, Paracoccidioides brasiliensis, Sporothrix schoenckii and Blastomyces dermatidis is not inhibited by these antibiotics when incubated at 20-25°C.
3. A non-selective and selective medium should be inoculated for isolation of fungi from potentially contaminated specimens.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Dextrose Agar AM1087/AM5087

Sabouraud Dextrose Broth AM1088/AM5088

Use

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth are general-purpose media used for the cultivation of yeasts, moulds and aciduric bacteria.

Summary

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth are Carliers (12) modification of the formulation described by Sabouraud (99) for the cultivation of fungi, particularly those associated with skin infections. It is used in qualitative procedures for cultivation of pathogenic and non-pathogenic fungi, particularly dermatophytes. Carlier showed that this medium gives reliable results with *Microsporum audouini, M.canis, Trichophyton mentagrophytes, T.flavum, T.rubrum* and *Candida albicans*. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud. Sabouraud Dextrose Agar is recommended by the USP (114) and IP (46) in Microbial Limit Tests for performing total yeast and mould count and is included in the Bacteriological Analytical Manual for food testing (113). It is also recommended by APHA for the examination of foods (20).

Sabouraud Dextrose Broth is recommended in the Bacteriological Analytical Manual for cosmetics testing (113). Sabouraud Dextrose Agar and Sabouraud Dextrose Broth can be made inhibitory to most pathogenic fungi and bacteria by the addition of antibiotics. Gentamycin is an amino glycoside that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-positive and gram-negative bacteria, cycloheximide is an antifungal agent that inhibits saprophytic fungi while allowing the growth of yeasts or dermatophytes. George et al aseptically added 0.5 gm cycloheximide, 20000 units penicillin and 40000 units streptomycin to each litre of autoclaved, cooled medium. *Cryptococcus neoformans, Aspergillus fumigatus* and *Allescheria boydii* were found to be sensitive to cycloheximide; *Actinomyces bovis* and *Nocardia asteroids* were sensitive to penicillin and streptomycin. Hantshke used colistin, novobiocin and cycloheximide to isolate *Candida albicans*. Dolan used gentamycin, chloramphenicol and cycloheximide for the selective isolation of pathogenic fungi.

Principle

Tryptone and mycological peptone provide nitrogenous compounds, carbon and other growth factors. Dextrose is the carbohydrate source. The low pH of approximately 5.6 is favourable for the growth of fungi, especially dermatophytes and is slightly inhibitory to contaminating bacteria. Various antibiotics can be added to this medium for bacterial inhibition as well as to make it selective for the isolation of pathogenic fungi from material containing large number of other fungi or bacteria.

Sabouraud Dextrose Agar may also be used as the basis of Pagano-Levin medium for the isolation of *Candida albicans*. 0.1 gm of filter sterilized triphenyltetrazolium chloride is added to each litre of autoclaved molten medium cooled to 55°C. After incubation at 25°C for 3 days, *Candida albicans* colonies are unpigmented or pale pink while other Candida species and other fungi form deep pink or red colonies. Other tests should be performed for identification of *Candida albicans*.

Formula*

Ingredients in grams per liter	Sabouraud Dextrose Agar	Sabouraud Dextrose Broth
Peptone	5.0	-
Tryptone	5.0	10.0
Dextrose	40.0	20.0
Agar	15.0	-
Final pH (at 25°C)	5.6±0.2	5.6±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water and mix thoroughly.

   Sabouraud Dextrose Agar - 65 gms

   Sabouraud Dextrose Broth - 30 gms
2. Boil with frequent agitation to dissolve the powder completely. Avoid overheating the agar as it could cause a softer medium.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Sabouraud Dextrose Agar - Light amber coloured, clear to slightly opalescent gel.

Sabouraud Dextrose Broth - Light amber coloured clear solution, without any precipitate.

Cultural Response

Cultural characteristics after 48-72 hours at 30°C.

Organisms (ATCC)	Growth on Sabouraud Dextrose Agar and in Sabouraud Dextrose Broth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant*	More than 70%
Lactobacillus casei (9595)	Luxuriant*	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key:

* = inhibited on media with low pH

Procedure

For Sabouraud Dextrose Agar

1. Allow the agar surface to dry before inoculating.
2. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plates in an inverted position.
5. Once inoculated, the medium should be protected from light and incubated aerobically at 25-30^{0}C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Inoculate using the pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 25-30^{0}C.

   Note: After autoclaving, do not heat the medium longer than 3 hours at 45-50^{0}C. Sterile solidified medium can be remelted only once.

For Sabouraud Dextrose Broth

1. Inoculate the medium as soon as the specimen has been collected.
2. Incubate with caps loosened at 30^{0}C for 18-24 hours or up to 7 days.

Interpretation of Results

Sabouraud Dextrose Agar

1. Identification of fungi is done by observing colony morphology, characteristic microscopic structures, rate of growth, etc. Yeasts are identified by various biochemical tests.

Pour plate and spread plate method

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Sabouraud Dextrose Broth

1. Growth in the broth is indicated by the presence of turbidity compared to an uninoculated control.

Precautions / Limitations

1. Some of the pathogenic fungi may produce infective spores, which can be easily dispersed in the laboratory. Examine such organisms only within a protective cabinet.
2. When used for selective isolation, antimicrobials like chloramphenicol and cycloheximide may inhibit some pathogenic fungi. However, the mycelial phase of *Histoplama capsulatum, Paracoccidioides brasiliensis, Sporothrix schoenckii* and *Blastomyces dermatidis* is not inhibited by these antibiotics when incubated at 25-30^{0}C.
3. A non-selective and selective medium should be inoculated for isolation of fungi from potentially contaminated specimens.

Storage

Store below 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Dextrose Broth (Harmonized) AMH5088

Sabouraud Dextrose Broth USP AM50881

Sabouraud Dextrose Broth EP AM50882

Use

Sabouraud Dextrose Broth is general-purpose media used for the cultivation of yeasts, moulds and aciduric bacteria.

Summary

Sabouraud Dextrose Broth is Carliers (12) modification of the formulation described by Sabouraud for the cultivation of fungi, particularly those associated with skin infections. It is used in qualitative procedures for cultivation of pathogenic and non-pathogenic fungi, particularly dermatophytes. Carlier showed that this medium gives reliable results with *Microsporum audouini, M.canis, Trichophyton mentagrophytes, T.flavum, T.rubrum* and *Candida*

albicans. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud. Sabouraud Dextrose Broth is recommended in the Bacteriological Analytical Manual for cosmetics testing.

Principle

Mixture of peptic digest of animal tissue & pancreatic digest of casein provide nitrogenous compounds, carbon and other growth factors. Dextrose is the carbohydrate source. The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes and is slightly inhibitory to contaminating bacteria. Various antibiotics can be added to this medium for bacterial inhibition as well as to make it selective for the isolation of pathogenic fungi from material containing large number of other fungi or bacteria.

Formula*

Ingredients in grams per liter

Mixture of peptic digest of animal tissue & pancreatic digest of casein (1:1)	10.0
Dextrose	20.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30 gms of the powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. Avoid overheating the agar as it could cause a softer medium.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 2-5 days at 20-25°C for fungi and 18-24 hours at 35-37°C for bacteria.

Organisms (ATCC)	Growth	RGI
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant*	More than 70%
Lactobacillus casei (9595)	Luxuriant*	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Aspergillus niger (16404)	Luxuriant	More than 70%

Key:

* = inhibited on media with low pH

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculating.
2. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plates in an inverted position.
5. Once inoculated, the medium should be protected from light and incubated aerobically at 25-30°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Inoculate using the pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 25-30°C.

Note: After autoclaving, do not heat the medium longer than 3 hours at 45-50°C. Sterile solidified medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observing colony morphology, characteristic microscopic structures, rate of growth, etc. Yeasts are identified by various biochemical tests. Pour plate and spread plate method
2. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Precautions / Limitations

1. Some of the pathogenic fungi may produce infective spores, which can be easily dispersed in the laboratory. Examine such organisms only within a protective cabinet.
2. When used for selective isolation, antimicrobials like chloramphenicol and cycloheximide may inhibit some pathogenic fungi. However, the mycelial phase of Histoplama capsulatum, Paracoccidioides brasiliensis, Sporothrix schoenckii and Blastomyces dermatidis is not inhibited by these antibiotics when incubated at 25-30°C.
3. A non-selective and selective medium should be inoculated for isolation of fungi from potentially contaminated specimens.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Glucose Agar AM108821/AM508821

Use

Sabouraud Glucose Agar is used for selective cultivation of yeasts, moulds and aciduric bacteria.

Summary

Sabouraud Glucose Agar is Carliers (12) modification of the formulation described by Sabouraud (99) for the cultivation of fungi, particularly those associated with skin infections. It is used in qualitative procedure for the cultivation of pathogenic and non pathogenic fungi particularly dermatophytes. Carlier showed that this medium gives reliable results with *Microsporum audouini, M. canis, Trichophyton mentagrophytes, T. flavum, T. rubrum* and *Candida albicans*. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud.

Principle

Tryptone and mycological peptone provides nitrogenous compounds, carbon and other growth factors. Glucose is the carbohydrate source. The low pH of approximately 5.6 is favourable for the growth of fungi, especially dermatophytes and slightly inhibitory to contaminating bacteria. Various antibiotic can be added to this medium for bacterial inhibition as well as to make it selective for the isolation of pathogenic fungi from from material containing large number of other fungi or bacteria.

Sabouraud Glucose Agar may also be used as the basis of Pagano-Levin medium for the isolation of *Candida* albicans. 0.1 gm of filter sterilized triphenyltetrazolium chloride is added to each litre of autoclaved molten medium cooled to 55^{0}C. After incubation at 25^{0}C for 3 days, *Candida* albicans colonies are unpigmented or pale pink while other *Candida* species and other fungi from deep pink or red colonies. Other tests should be performed for identification of *Candida* albicans.

Formula*

Ingredients in grams per liter

Peptones	5.0
Tryptone	5.0
Glucose	40.0
Agar	15.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C for fungi and 35-37^{0}C for bacteria.

Organisms (ATCC)	Growth
Aspergillus niger (16404)	Luxuriant
Candida albicans (10231)	Luxuriant
Saccharomyces cerevisiae (9763)	Luxuriant
Escherichia coli (25922)	Luxuriant*
Lactobacillus casei (9595)	Luxuriant*
Staphylococcus aureus (6538)	Luxuriant
Pseudomonas aeruginosa (9027)	Luxuriant
Clostridium sporogenes (11437)	Luxuriant
Bacillus subtilis (6633)	Luxuriant

Key:

* = inhibited on media with low pH

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculation.
2. Inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Once inoculated, the medium should be protected from light and incubated aerobically at 25-30°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony-forming units per plate.
2. Isolate using pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 25-30^{0}C.

Note: After autoclaving, do not heat medium longer than 3 hours at 45-50^{0}C. Sterile solid medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observation of colony morphology, characteristic microscopic structures, rate of growth, etc.
2. Yeasts are identified by various biochemical tests.

For spread plate and pour plate method

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Glucose Agar with Antibiotics IP AM50883

Use

Sabouraud Glucose Agar with antibiotics IP is used for selective cultivation of yeasts and moulds in compliance with IP.

Summary

Sabouraud Glucose Agar with antibiotics is Carliers (12) modification of the formulation described by Sabouraud (99) with the addition of chloramphenicol, for the cultivation of fungi, particularly those associated with skin infections. It is used for the isolation of pathogenic fungi from materials containing large number of saprophytic fungi or bacteria.

Principle

Peptone(meat and casein) provides nitrogenous compounds, carbon and other growth factors. Glucose is the carbohydrate source. The low pH of approximately 5.6 is favourable for the growth of fungi, especially dermatophytes and slightly inhibitory to contaminating bacteria. Chloramphenicol inhibits a wide range of gram-positive and gram-negative bacteria making the medium selective for fungi.

Formula*

Ingredients in grams per liter

Peptones (meat and casein)	10.0
Glucose monohydrate	40.0
Chloramphenicol	0.05
Agar	15.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65.05 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at20-25°C for fungi and 24-48 hours at 35-37^{0}C for bacteria.

Organisms (ATCC)	Growth	RGI
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Aspergillus niger (16404)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculation.
2. Inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Once inoculated, the medium should be protected from light and incubated aerobically at 25-30°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30300 colony-forming units per plate.
2. Isolate using pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 25-30^{0}C.

Note: After autoclaving, do not heat medium longer than 3 hours at 45-50^{0}C. Sterile solid medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observation of colony morphology, characteristic microscopic structures, rate of growth, etc.
2. Yeasts are identified by various biochemical tests.

For spread plate and pour plate method

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Sabouraud Glucose Agar with Antibiotics (Agar Medium C) EP AM50884

Sabouraud Glucose Agar with Antibiotics (Agar Medium C) BP AM50885

Use

Sabouraud Glucose Agar with antibiotics (Agar Medium C) EP/BP is used for selective cultivation of yeasts and moulds in compliance with EP/BP.

Summary

Sabouraud Glucose Agar with antibiotics is Carliers (12) modification of the formulation described by Sabouraud (99) with the addition of chloramphenicol, for the cultivation of fungi, particularly those associated with skin infections. It is used for the isolation of pathogenic fungi from materials containing large number of saprophytic fungi or bacteria. It is also recommended by the EP/BP in Microbial Limit Tests to perform total yeast and mould count.

Principle

Peptone(meat and casein) provides nitrogenous compounds, carbon and other growth factors. Glucose is the carbohydrate source. The low pH of approximately 5.6 is favourable for the growth of fungi, especially dermatophytes and slightly inhibitory to contaminating bacteria. Chloramphenicol inhibits a wide range of gram-positive and gram-negative bacteria making the medium selective for fungi.

Formula*

Ingredients in grams per liter

Peptones (meat and casein)	10.0
D-Glucose monohydrate	40.0
Chloramphenicol	0.05
Agar	15.0

Final pH (at 25°C) 5.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 65.05 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C for fungi and 24-48 hours at 35-37°C for bacteria..

Organisms (ATCC)	Growth	RGI
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Lactobacillus casei (9595)	Inhibited	0%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Aspergillus niger (16404)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculation.
2. Inoculate and streak the specimen as soon as possible after collection.
3. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
4. Once inoculated, the medium should be protected from light and incubated aerobically at 20-25°C with increased humidity for four weeks or longer.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony-forming units per plate.
2. Isolate using pour plate or streak plate technique.
3. Incubate plates aerobically for 7 days at 20-25°C.

Note: After autoclaving, do not heat medium longer than 3 hours at 45-50°C. Sterile solid medium can be remelted only once.

Interpretation of Results

1. Identification of fungi is done by observation of colony morphology, characteristic microscopic structures, rate of growth, etc.
2. Yeasts are identified by various biochemical tests.

For spread plate and pour plate method

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Salt Meat Broth AM50886

Use

Salt Meat Broth is used as an enrichment medium for the isolation of *Staphylococci* from grossly contaminated specimens.

Summary

Staphylococci are tolerant of concentrations of sodium chloride that inhibit most other bacteria. Salt Meat Broth is a selective growth medium for *staphylococci*. It is used for the preliminary enrichment for the isolation of small numbers of the cocci from heavily contaminated materials (77.2). This medium can detect halophilic *Staphylococci* from contaminated samples such as faeces especially in case of food poisoning (77.3). The medium is also an excellent substrate for the cultivation of some of the halophilic micrococci associated with hides and raw salt supplies.

Principle

Salt Meat Broth is a selective medium for staphylococci due to the extra concentration of sodium chloride. Peptic digest of animal tissue, beef extract and ox heart tissue supply the essential nutrients and support the growth of the bacteria.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	10.0
Beef Extract	10.0
Neutral Ox-heart tissue	30.0
Sodium chloride	100.0

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15 gms in 100 ml distilled water. Soak for 5 minutes.
2. Warm slightly with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Dispense in tubes or adequate containers and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Culture characteristics after 18-48hours at 35-37°C.

Organisms (ATCC)	Growth
Staphylococcus aureus (25923)	Luxuriant
Escherichia coli (25922)	Inhibited
Proteus vulgaris (13315)	Inhibited

Procedure

1. For the isolation of staphylococci from samples of food, emulsify the specimen in peptone water (AM1079/5079) and inoculate a tube of Salt Meat Broth.
2. After 24-48 hours incubation at 35-37°C, subculture on Mannitol Salt Agar (AM 1069/5069).

Interpretation of Results

Examine the colonies on Mannitol Salt Agar and do further biochemical tests for the identification.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Salmonella Differential Agar (Twin Pack) (Equivalent to Rajhans™ Medium) AM50887

Use

Salmonella Differential Agar medium is recommended for identification and differentiation of *Salmonella* species from members of *Enterobacteriaceae*, especially *Proteus* species.

Summary

Salmonella Differential Agar medium is a slight modification of original formulation of Rambach (89.9) used for differentiation of *Salmonella* species from *Proteus* species and other enteric bacteria. Production of acid from propylene glycol is a novel characteristic of Salmonella species and is utilised in this medium. Many of the media such as SS Agar, XLD Agar recommended for the identification and differentiation of *salmonella* species (22.2) are based on lactose fermentation and hydrogen sulphide production.

Principle

Peptone special and yeast extract supports the luxuriant growth of bacteria while sodium deoxycholate inhibits gram-positive organisms rendering the medium selective for enteric microorganisms. The BC indicator turns pink in presence of acid produced from propylene glycol. Lactose fermentating ability is determined by using an indicator which can detect the presence of enzyme ß-galactosidase. Lactose fermenting (ß-galactosidase producing) bacteria yield blue violet coloured colony. *Salmonellae* produce acid from propylene glycol and combine

with the pH indicator to give typical pink red colonies. Other enteric gram-negative bacteria form colourless colonies. *Salmonellae Typhimurium* and *Salmonellae Enteritidis* produce pink to red colonies.

Formula*

Ingredients in grams per liter

Part A -	
Peptone, special	8.0
Yeast extract	2.0
Sodium deoxycholate	1.0
B. C. Indicator	2.0
Agar	12.0
Part B -	
Propylene glycol	10.0

Final pH (at 25°C) 7.3±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 10 grams of fluid Part B in 1000 ml distilled water.
2. Add 25 grams of Part A. Mix well and boil to dissolve the medium completely. DO NOT AUTOCLAVE.
3. Cool to 45 - 50°C. Mix well before pouring into sterile Petri plates.

Quality Control

Dehydrated Appearance

Part A : Light yellow to light pink homogeneous free flowing powder

Part B: Colourless viscous solution

Prepared Appearance

Light orange coloured, clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics after 24-48 days at 35-37°C.

Organisms	Growth	Colour of colony	RGI
Escherichia coli (25922)	Luxuriant	Blue-green	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant	Blue-violet	More than 70%
Proteus mirabilis (25933)	Luxuriant	Colourless	More than 70%
Salmonella Typhimurium (14028)	Luxuriant	Pink-red	More than 70%
Salmonella Enteritidis (13076)	Luxuriant	Pink-red	More than 70%
Salmonella Typhi (6539)	Luxuriant	Colourless	More than 70%
Shigella flexneri (12022)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Selenite F Broth AM1089/AM5089

Use

Selenite F Broth is used as an enrichment medium for the isolation of *Salmonella* species from faeces, urine, water, foods and other materials of sanitary importance.

Summary

Selenite F Broth is based on the formulation devised by Leifson (65), who showed that selenite was beneficial in the isolation of *Salmonella* species while inhibiting coliforms and certain other microbial species like faecal *streptococci,* present in faecal specimens. An enrichment medium is routinely employed to detect pathogens in faecal specimens since the pathogens are generally present in a very small number compared to the intestinal flora. This medium is useful in detecting *Salmonella* in the non-acute stages of illness when the organisms occur in faeces in low numbers and for epidemiological studies to enhance the detection of low numbers of organisms from asymptomatic or convalescent patients. Selenite F broth is used in the recovery of *Salmonella* with subcultures being made after 12-18 hours of incubation.

Principle

Tryptone provides nitrogenous substances and other amino acids. Lactose maintains the pH in the medium as selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of selenite and results in the overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation helps to maintain a neutral pH. Disodium phosphate buffers the medium to maintain the pH and also lessens the toxicity of selenite, thus increasing the capacity of the medium. Sodium selenite inhibits gram-positive bacteria and suppresses the growth of most gram-negative bacteria and enterococci other than *Salmonella.*

Formula*

Ingredients in grams per liter

Tryptone	5.0
Lactose	4.0
Disodium Phosphate Dodecahydrate	10.0
Sodium Hydrogen Selenite	4.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.

3. Boil with frequent agitation to dissolve the powder completely. Sterilize in boiling water bath or free flowing steam for 10 minutes. AVOID OVERHEATING. DO NOT AUTOCLAVE. Excessive heating is detrimental.
4. Discard the prepared medium if a large amount of selenite is reduced, which is indicated by a red precipitate at the bottom of the tube/bottle.

Warning: Sodium Hydrogen Selenite is very toxic, corrosive and causes teratogenicity. Handle with care. On contact with skin wash immediately with plenty of water.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to very slightly opalescent solution, may have a slight precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C when subcultured on MacConkey Agar (AM10590/AM50590).

Organisms (ATCC)	Recovery	Colour of Colony
Escherichia coli (25922)	Little to none	Pink with bile precipitate
Salmonella serotype Choleraesuis (12011)	Good to excellent	Colourless
Salmonella serotype Typhimurium (14028)	Good to excellent	Colourless

Procedure

1. For faeces and other solid materials, suspend 1-2 gms of the specimen in the broth (approximately 10-15% by volume) and emulsify if necessary.
2. Solid material is added to the normal strength broth.
3. Liquid samples are mixed with double strength medium in the ratio of 1:1.
4. Incubate for 12-24 hours at 35-37°C.
5. Make sub-cultures after 12-18 hours of incubation.

Interpretation of Results

1. After incubation, there must be an increase in the number of pathogens that the medium is designed to select for and enrich.
2. Subculture onto any combination of greater and lesser inhibitory, selective and differential media for *Enterobacteriaceae*. e.g. MacConkey Agar, XL Agar, etc to isolate pathogens for identification.

Precautions / Limitations

1. Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate at the bottom of the tube.
2. Do not incubate for longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation and coliforms may overgrow the pathogens.
3. Take subcultures from the upper third of the broth column which should be at least 5 cm in depth
4. Enrichment broths should not be used as the sole isolation medium.
5. Use in conjunction with selective and non-selective plating media to increase the chances of isolating pathogens, particularly when they may be present in small numbers.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Selenite F Broth IP (Twin Pack) AM10891/AM50891

Use

Selenite F Broth is used as an enrichment medium for the isolation of *Salmonella* species, in accordance with IP.

Summary

Selenite F Broth is based on the formulation devised by Leifson (68), who showed that selenite was beneficial in the isolation of *Salmonella* species while inhibiting coliforms and certain other microbial species like faecal streptococci, present in faecal specimens. An enrichment medium is routinely employed to detect pathogens in faecal specimens since the pathogens are generally present in a very small number compared to the intestinal flora. This medium is useful in detecting *Salmonella* in the non-acute stages of illness when the organisms occur in faeces in low numbers and for epidemiological

studies to enhance the detection of low numbers of organisms from asymptomatic or convalescent patients. Selenite F broth is used in the recovery of *Salmonella* with subcultures being made after 12-18 hours of incubation.

Principle

Peptone provides nitrogenous substances and other amino acids. Lactose maintains the pH in the medium as selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of selenite and results in the overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation helps to maintain a neutral pH. Disodium Hydrogen phosphate buffers the medium to maintain the pH and also lessens the toxicity of selenite, thus increasing the capacity of the medium. Sodium selenite inhibits gram-positive bacteria and suppresses the growth of most gram-negative bacteria and enterococci other than *Salmonella*.

Formula*

Ingredients in grams per liter	Part A	Part B
Peptone	5.0	-
Lactose	4.0	-
Disodium Hydrogen Phosphate	10.0	-
Sodium Hydrogen Selenite	-	4.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 19 gms of the Part A & 4 gm of the Part B powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. Sterilize by maintaining at 100°C for 30 minutes. AVOID OVERHEATING. DO NOT AUTOCLAVE. Excessive heating is detrimental.
4. Discard the prepared medium if a large amount of selenite is reduced, which is indicated by a red precipitate at the bottom of the tube/bottle.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to very slightly opalescent solution, may have a slight precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C when subcultured on MacConkey Agar (AM10590/ AM50590).

Organisms (ATCC)	Recovery	Colour of Colony
Escherichia coli (25922)	Little to none	Pink with bile precipitate
Salmonella serotype Choleraesuis (12011)	Good to excellent	Colourless
Salmonella serotype Typhimurium (14028)	Good to excellent	Colourless

Procedure

1. For faeces and other solid materials, suspend 1-2 gms of the specimen in the broth (approximately 10-15% by volume) and emulsify if necessary.
2. Solid material is added to the normal strength broth.
3. Liquid samples are mixed with double strength medium in the ratio of 1:1.
4. Incubate for 12-24 hours at 35-37°C.
5. Make sub-cultures after 12-18 hours of incubation.

Interpretation of Results

1. After incubation, there must be an increase in the number of pathogens that the medium is designed to select for and enrich.
2. Subculture onto any combination of greater and lesser inhibitory, selective and differential media for Enterobacteriaceae. e.g. MacConkey Agar, XLD Agar, etc to isolate pathogens for identification.

Precautions / Limitations

1. Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate at the bottom of the tube.
2. Do not incubate for longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation and coliforms may overgrow the pathogens.
3. Take subcultures from the upper third of the broth column which should be at least 5 cm in depth.

Warning: Sodium Hydrogen Selenite is very toxic, corrosive and causes teratogenicity. Handle with care. On contact with skin wash immediately with plenty of water.

4. Enrichment broths should not be used as the sole isolation medium.
5. Use in conjunction with selective and non-selective plating media to increase the chances of isolating pathogens, particularly when they may be present in small numbers.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

SIM Medium AM50892

Use

SIM Medium is used for differentiation of enteric bacteria on the basis of sulphide production, indole production and motility.

Summary

Tests for indole production and motility are very commonly used in the identification of microorganisms in the diagnostic microbiology laboratory. A motility-indole medium has been found to be helpful in the identification of the *Enterobacteriaceae,* and especially in the differentiation of *Klebsiella* from *Enterobacter* and *Serratia* (6.2).

In SIM medium these two tests have been combined with sulphide-production test. The production of hydrogen sulphide is a useful diagnostic test in the identification of enteric bacteria and is helpful in the differentiation between *Salmonella* and *Shigella*. Since these organisms are encountered very often in clinical material, the use of a there in one test can result in a substantial saving of materials and time.

Principle

Peptic digest of animal tissue serves as a source of carbon, nitrogen, vitamins and minerals. sodium thiosulphate and peptonized iron are indicators of hydrogen sulfide production. hydrogen sulphide reacts with peptonized iron to form black precipitate of ferrous sulphide. The use of only 0.30% agar in the medium results in the production of a semi-solid medium, ideal for the examination of motility. Non-motile organisms will grow along the line of inoculation only, whereas motile species will grow away from it.

Peptic digest of animal tissue is rich in tryptophan, which is attacked by certain microorganisms resulting in the production of indole, which is detected by the addition of Kovac's reagents following the incubation period.

Formula*

Ingredients in grams per liter

PPeptic digest of animal tissue	30.0
Beef extract	3.0
Peptonized Iron	0.20
Sodium thiosulphate	0.025
Agar	3.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 36.23 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Warm slightly with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Semisolid, Medium amber coloured, Slightly opalescent gel forms in tubes.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Motility	H_2S	INDOLE
Escherichia coli (25922)	Luxuriant	+	_	+
S. serotype Typhimurium (14028)	Luxuriant	+	+	_
Shigella flexneri (12022)	Luxuriant	_	_	_

Key: + = Positive reaction

H_2S + = Blackening of the medium

Procedure

1. Charge straight wire inoculation loop with test organism from a pure culture.
2. Inoculated once by inserting a straight wire to about one third of the depth of the medium in the center of the tube.
3. Incubate tubes with loosened caps for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Interpretation of Results

Observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H_2S production (blackening along the stab line).

For indole test add 0.2 ml of Kovac's Reagent to the tube and allow to stand for 10 minutes. A dark red colour in the reagent constitutes a positive indole test. No change in the original colour of the reagent constitutes a negative test.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Simmons Citrate Agar BIS — AM109011/AM509011

Use

Simmons Citrate Agar is used for the differentiation of gram-negative bacteria on the basis of citrate utilization in compliance with BIS.

Summary

Simmons Citrate Agar is recommended by APHA (20,39) and is used for the differentiation of *Enterobacteriaceae* and members of the *aerogenes* group on the basis of citrate utilization. Koser (61) developed a liquid medium containing ammonium salt as the only source of nitrogen and citrate as the only source of carbon to differentiate between Escherichia coli and *Enterobacter aerogenes* based on the IMVIC reactions. Simmons (103) later on, modified this medium with the addition of agar and bromothymol blue. Organisms capable of utilizing citrate grow well on this medium. Simmons Citrate Agar is included in the Bacteriological Analytical Manual for food and cosmetics analysis (113).

Principle

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively while bromothymol blue is the pH indicator. Organisms able to utilize the above compounds as sole source of nitrogen and carbon, grow on this medium and produce an alkaline reaction as indicated by the change in colour of bromothymol blue indicator from green (neutral) to blue (alkaline).

Formula*

Ingredients in grams per liter

Sodium chloride	5.0
Sodium citrate	2.0

Dipotassium phosphate	1.0
Ammonium dihydrogen phosphate	1.0
Magnesium sulphate	0.2
Bromothymol blue	0.08
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.1

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.28 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in tubes or as desired.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Cool in slanted position for use as slants.

A Differential Dehydrated Culture Medium

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium to dark green, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Medium	Citrate Utilization
Enterobacter aerogenes (13048)	Good to luxuriant	Blue	+
Escherichia coli (25922)	Inhibited	Green	-
Salmonella serotype Enteritidis (13076)	Good to luxuriant	Blue	+
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Blue	+
Shigella dysenteriae (13313)	Inhibited	Green	-
Klebsiella Pneumoneae (33495)	Good to luxuriant	Green to Light blue	-

Procedure

1. The medium may be used as slants or as a plate medium in petri plates.
2. Inoculate with growth from a pure culture using a light inoculum.
3. Incubate for 48 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

1. A positive reaction (citrate utilization) is indicated by growth with an intense blue colour in the slant. A negative reaction is evidenced by no growth to slight growth with no change in colour of the medium.
2. *E.coli* including different serotypes from epidemic infantile enteritis, as well as *Shigella, Yersinia and Edwardsiella* species do not grow on this medium. *Serratia* and the majority of *Enterobacter, Citrobacter, Klebsiella, Proteus* and *Providencia* species, except *Morganella morgani* and *Klebsiella rhinoscleromatis* utilize citrate and produce the characteristic blue colouration.
3. This medium may also be able to differentiate citrate positive *Salmonella enteritidis* and members of *Salmonella subgenus* from the citrate negative *S.typhi, S.paratyphi A, S.pulloram and S.gallinarum.*

Precautions / Limitations

1. Do not carry over any nutrients into the medium as it may lead to falsepositive results. Dilute the inoculum before inoculating the medium to avoid a carry over of other carbon sources. Use a light inoculum while streaking.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Simmons Citrate Agar — AM1090/AM5090

Use

Simmons Citrate Agar is used for the differentiation of gram-negative bacteria on the basis of citrate utilization.

Summary

Simmons Citrate Agar is recommended by APHA (20, 39) and is used for the differentiation of *Enterobacteriaceae* and members of the *aerogenes* group on the basis of citrate utilization. Koser (61) developed a liquid medium containing ammonium salt as the only source of nitrogen and citrate as the only source of carbon to differentiate between *Escherichia coli* and *Enterobacter aerogenes* based on the IMViC reactions. Simmons (103) later on, modified this medium with the addition of agar and bromothymol blue. Organisms capable of utilizing citrate grow well on this medium. Simmons Citrate Agar is included in the Bacteriological Analytical Manual for food and cosmetics analysis (113).

Principle

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively while bromothymol blue is the pH indicator. Organisms able to utilize the above compounds as sole source of nitrogen and carbon, grow on this medium and produce an alkaline reaction as indicated by the change in colour of bromothymol blue indicator from green (neutral) to blue (alkaline).

Formula*

Ingredients in grams per liter

Sodium Chloride	5.0
Sodium Citrate	2.0
Dipotassium Phosphate	1.0
Ammonium Dihydrogen Phosphate	1.0
Magnesium Sulphate	0.2
Bromothymol Blue	0.08
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.28 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in tubes or as desired.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Cool in slanted position for use as slants.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Medium to dark green, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Medium	Citrate Utilization
Enterobacter aerogenes (13048)	Good to luxuriant	Blue	+
Escherichia coli (25922)	Inhibited	Green	-
Salmonella serotype Enteritidis (13076)	Good to luxuriant	Blue	+
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Blue	+
Shigella dysenteriae (13313)	Inhibited	Green	-
Klebsiella Pneumoneae (33495)	Good to luxuriant	Green to light blue	

Procedure

1. The medium may be used as slants or as a plate medium in petri plates.
2. Inoculate with growth from a pure culture using a light inoculum.
3. Incubate for 48 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

1. A positive reaction (citrate utilization) is indicated by growth with an intense blue colour in the slant. A negative reaction is evidenced by no growth to slight growth with no change in colour of the medium.
2. *E.coli* including different serotypes from epidemic infantile enteritis, as well as *Shigella, Yersinia* and *Edwardsiella* species do not grow on this medium. *Serratia* and the majority of *Enterobacter, Citrobacter, Klebsiella, Proteus* and *Providencia* species, except *Morganella morgani* and *Klebsiella rhinoscleromatis* utilize citrate and produce the characteristic blue colouration.
3. This medium may also be able to differentiate citrate positive *Salmonella enteritidis* and members of *Salmonella* subgenus 2, 3 and 5 from the citrate negative *S.typhi, S.paratyphi A, S.pulloram* and *S.gallinarum.*

Precautions / Limitations

1. Do not carry over any nutrients into the medium as it may lead to false positive results. Dilute the inoculum before inoculating the medium to avoid a carry over of other carbon sources. Use a light inoculum while streaking.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Skim Milk Agar — AM10901/AM50901

Use

Skim Milk Agar is used for the cultivation and enumeration of microorganisms in milk and dairy products.

Summary

Skim Milk Agar is used for detecting proteolytic microorganisms that hydrolyse casein (milk protein) and cause coagulation (clot formation) in dairy products (105). Proteolytic microorganisms hydrolyse casein to form soluble nitrogenous indicated as clear zone surrounding the colonies (20, 39).

Principle

Skim milk powder is a source of casein. Tryptone and yeast extract provide the essential nutrients of nitrogen, carbon, sulphur, vitamin B complex and trace elements to the organisms. Dextrose is the fermentable carbohydrate. Agar is the solidification agent.

Formula*

Ingredients in grams per liter

Skim Milk Powder	28.0
Tryptone	5.0
Yeast Extract	2.5
Dextrose	1.0
Agar	15.0

Final pH (at 25° C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 51.5 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121^0 C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Off-white coloured opaque gel.

Cultural Response

Cultural response after 18 - 24 hours at 35 - 37^0 C.

Organisms (ATCC)	Growth	Proteolyitc Activity	RGI
Bacillus subtilis (6633)	Luxuriant	+	More than 70%
Escherichia coli (25922)	Luxuriant	_	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	+	More than 70%
Proteus mirabilis (25933)	Luxuriant	+	More than 70%
Enterococcus faecalis	Luxuriant	-	More than 70%
cescens (8100)	Luxuriant	+	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Standard operating techniques like streak plate method may be used for isolating organisms.

Interpretation of Results

1. Proteolyitc bacteria produce a clear zone surrounding the colonies.

Storage

Store below 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Soyabean Casein Digest Agar (Tryptone Soya Agar) AM1091/AM5091

Soyabean Casein Digest Broth (Tryptone Soya Broth) AM1092/AM5092

Use

Soyabean Casein Digest Agar and Soyabean Casein Digest Broth are general-purpose media used for the isolation and cultivation of a wide variety of fastidious and non-fastidious microorganisms.

Summary

Soyabean Casein Digest Agar (SCDA) is used for total aerobic microbial count and antimicrobial preservative-effective test. It is also used for testing bacterial contaminants in cosmetics and for a multitude of purpose including maintenance of stock cultures, plate counts, phage typing, colicin typing and as a base for media containing blood. Since this medium does not contain the X and V growth factors, it can be used for determining the requirements of these growth factors by isolates of *Haemophilus*.

Gunn et al used this medium for the study of haemolytic reactions after addition of 5% v/v blood. When Chocolate Agar is prepared, this medium supports good growth of *Neisseria* species and *Haemophilus influenzae.*

Soyabean Casein Digest Broth (SCDB) is widely used for the cultivation of microorganisms from environmental sources, supporting the growth of a wide variety of microorganisms including common aerobic, facultative and anaerobic bacteria and fungi. It is also used for preparing dilutions of organisms for colony counts and preparation of standard inocula for disc diffusion and dilution antimicrobial susceptibility testing as standardized by the National Committee for Clinical Laboratory Standards (NCCLS). This medium is used in sterility testing for the detection of contamination with low incidence fungi and aerobic bacteria and in the performance of microbial limit test. It is used in the coliphage detection procedure, a Methodology in Standard Methods for the Examination of Water and Wastewater.

Soyabean Casein Digest Agar and Broth are included in the Bacteriological Analytical Manual for food and cosmetics testing (113), in the Compendia of Methods for the examination of milk (39), water and wastewater (36) and foods (20) and is also specified in the USP (113) and IP (46).

Principle

The combination of tryptone and soya peptone makes the medium highly nutritious by supplying organic nitrogen, particularly amino acids and long chain peptides. Sodium chloride maintains the osmotic balance. Soyabean Casein Digest Agar and Soyabean Casein Digest Broth may be supplemented with blood to provide a more nutritious medium for fastidious organisms, or with antimicrobials to provide a selective medium for specific organisms out of a mixed flora sample. Since Soyabean Casein Digest Agar contains no added carbohydrate, it may be used with added blood to determine haemolysis.

When Soyabean Casein Digest Agar is supplemented with 0.7 gms lecithin and 5 gms polysorbate (Tween 80) per liter of medium, it can be used as microbial content test agar for testing quaternary ammonium compounds (collection of samples from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation).

Formula*

Ingredients in grams per liter	Soyabean Casein Digest Agar	Soyabean Casein Digest Broth
Tryptone	15.0	17.0
Soya Peptone	5.0	3.0
Sodium Chloride	5.0	5.0
Dextrose	-	2.5
Dipotassium Phosphate	-	2.5
Agar	15.0	-
Final pH (at 25°C)	7.3 ± 0.2	7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water and mix thoroughly.

   Soyabean Casein Digest Agar - 40.0 gms

   Soyabean Casein Digest Broth - 30.0 gms
2. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To prepare Blood Agar plates, add 5-10% sterile, defibrinated blood to the sterile agar cooled to 45-50°C.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Soyabean Casein Digest Agar - Basal Medium - Light yellow coloured, clear to slightly opalescent gel.

With the addition of blood - Cherry red coloured gel.

Soyabean Casein Digest Broth - Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth without blood on SCDA and in SCDM	Growth with blood on SCDA	Haemolysis on SCDA
Candida albicans (10231)	Luxuriant	Luxuriant	None
Escherichia coli (25922)	Luxuriant	Luxuriant	Beta
Neisseria meningitidis (13090)	Luxuriant	Luxuriant	None
Staphylococcus aureus (25923)	Luxuriant	Luxuriant	Beta
Streptococcus pyogenes (19615)	Good to luxuriant	Luxuriant	Beta

Procedure

For Soyabean Casein Digest Agar

1. Allow the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection.
2. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plates aerobically at 35-37°C for 18-24 hours.
5. Since many pathogens require CO_2 on primary isolation, plates may be incubated in an atmosphere containing 3-10% CO_2.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Inoculate using the pour plate or streak plate technique.
3. Incubate plates aerobically for 48 hours at 35°C.

   Note: After autoclaving, do not heat the medium longer than 3 hours at 45-50°C. Sterile solidified medium can be remelted only once.

For Soyabean Casein Digest Broth

Aerobic and anaerobic cultivation

1. Swab specimens may be inserted into the medium after inoculation of appropriate plated media.
2. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium.
3. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.
4. Incubate the containers with loosened caps at 35-37°C aerobically with or without supplementation with CO_2.
5. Containers intended for cultivation of anaerobes must be incubated under anaerobic conditions.
6. Examine the growth after 18-24 hours and 42-48 hours of incubation.

Blood culture

1. The superior growth promoting properties of Soyabean Casein Digest Broth makes it especially useful for the isolation of organisms from blood or other body fluids.
2. Anticoagulants such as 'liquoid' (Sodium polyanethyl sulphonate) or sodium citrate may be added to the broth prior to sterilization.
3. 5-10 ml of blood may be added to 50 ml of medium.

Selective culture media

1. Soyabean Casein Digest Broth is used in food bacteriology as the basal medium to which a variety of selective agents are added for selective enrichment of *Staphylococcus aureus* and *Escherichia coli* 0157.

Interpretation of Results

Soyabean Casein Digest Agar

1. Count the number of colonies and express as colony forming units (CFU) per gram or ml of sample, taking into account the applicable dilution factor.
2. Subculture colonies of interest so that positive identification can be made by means of other tests.

Soyabean Casein Digest Broth

1. Growth in broth medium is indicated by the presence of turbidity compared to an un-inoculated control.
2. Broth cultures should be held at least for a week before discarding as negative.

Precautions / Limitations

1. Haemolytic reactions of streptococci on this medium can vary according to the origin of the blood.
2. The medium designed for sheep blood shows significant differences when used with horse blood and vice versa.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Soyabean Casein Digest Agar (Harmonized) — AMH5091

Soyabean Casein Digest Agar (Casein Soyabean Digest Agar) IP — AM10911/AM50911

Soyabean Casein Digest Agar Medium USP — AM10912/AM50912

Soyabean Casein Digest Agar (Agar Medium B)(Casein Soyabean Digest Agar) EP — AM10913/AM50913

Soyabean Casein Digest Agar (Agar Medium B) (Casein Soyabean Digest Agar) BP — AM10914/AM50914

Use

Soyabean Casein Digest Agar is general-purpose media used for the isolation and cultivation of a wide

variety of fastidious and non-fastidious microorganisms.

Summary

Soyabean Casein Digest Agar (SCDA) is used for total aerobic microbial count and antimicrobial preservative-effective test. It is also used for testing bacterial contaminants in cosmetics and for a multitude of purpose including maintenance of stock cultures, plate counts, phage typing, colicin typing and as a base for media containing blood. Since this medium does not contain the X and V growth factors, it can be used for determining the requirements of these growth factors by isolates of *Haemophilus*. Gunn *et al.*, used this medium for the study of haemolytic reactions after addition of 5% v/v blood. When Chocolate Agar is prepared, this medium supports good growth of *Neisseria* species and *Haemophilus influenzae*. Soyabean Casein Digest Agar is included in the Bacteriological Analytical Manual for food and cosmetics testing (113), in the Compendia of Methods for the examination of milk (39), water and wastewater (36) and foods (20).

Principle

The combination of Pancreatic Digest of casein and Papic Digest of soyabean meal makes the medium highly nutritious by supplying organic nitrogen, particularly amino acids and long chain peptides. Sodium chloride maintains the osmotic balance. In Soyabean Casein Digest Medium Dextrose is an energy source and Dipotassium phosphate acts as a buffer to control pH. Soyabean Casein Digest Agar may be supplemented with blood to provide a more nutritious medium for fastidious organisms, or with antimicrobials to provide a selective medium for specific organisms out of a mixed flora sample. Since Soyabean Casein Digest Agar contains no added carbohydrate, it may be used with added blood to determine haemolysis. When Soyabean Casein Digest Agar is supplemented with 0.7 gms lecithin and 5 gms polysorbate (Tween 80) per liter of medium, it can be used as microbial content test agar for testing quaternary ammonium compounds (collection of samples from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation).

Formula*

Ingredients in grams per liter

Pancreatic digest of casein	15.0
Papic digest of soyabean meal	5.0
Sodium chloride	5.0
Agar	15.0

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.0 gms of the powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To prepare Blood Agar plates, add 5-10% sterile, defibrinated blood to the sterile agar cooled to 45-50°C.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Basal Medium - Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C for bacteria and for fungi 2-5 days at 20-25°C.

Organisms(ATCC)	Growth	RGI
Candida albicans (10231)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Staphylococcus aureus (6538)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Good to luxuriant	More than 70%
Clostridium sporogenes (11437)	Luxuriant	More than 70%
Bacillus subtilis (6633)	Luxuriant	More than 70%
Aspergillus niger (16404)	Luxuriant	More than 70%
Streptococcus pneumonia (6303)	Luxuriant	More than 70%
Pseudomonas aeruginosa (9027)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Allow the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection.
2. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface.
3. Streak for isolation with a sterile loop.
4. Incubate plates aerobically at 35-37°C for 18-24 hours.
5. Since many pathogens require CO_2 on primary isolation, plates may 2 be incubated in an atmosphere containing 3-10% CO_2.

For Quantitative test

1. Prepare decimal dilutions of the sample in a sterile diluent to obtain 30-300 colony forming units per plate.
2. Inoculate using the pour plate or streak plate technique.
3. Incubate plates aerobically for 48 hours at 35°C.

Note: After autoclaving, do not heat the medium longer than 3 hours at 45-50°C. Sterile solidified medium can be remelted only once.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Soyabean Casein Digest Broth (Harmonized) — AMH5092

Soyabean Casein Digest Medium IP — AM10921/AM50921

Soyabean Casein Digest Medium EP — AM10923/AM50923

Soyabean Casein Digest Medium BP — AM10924/AM50924

Soyabean Casein Digest Medium USP — AM10922/AM50922

Use

Soyabean Casein Digest Medium is general-purpose media used for the isolation and cultivation of a wide variety of fastidious and non-fastidious microorganisms.

Summary

Soyabean Casein Digest Medium (SCDM) is widely used for the cultivation of microorganisms from environmental sources, supporting the growth of a wide variety of microorganisms including common aerobic, facultative and anaerobic bacteria and fungi. It is also used for preparing dilutions of organisms for colony counts and preparation of standard inocula for disc diffusion and dilution antimicrobial susceptibility testing as standardized by the National Committee for Clinical Laboratory Standards (NCCLS). This medium is used in sterility testing for the detection of contamination with low incidence fungi and aerobic bacteria and in the performance of microbial limit test. It is used in the coliphage detection procedure, a Methodology in Standard Methods for the Examination of Water and Wastewater. Soyabean Casein Digest Agar and Medium are included in the Bacteriological Analytical Manual for food and cosmetics testing, (113), in the Compendia of Methods for the examination of milk (39), water and wastewater (36) and foods (20).

Principle

The combination of Pancreatic Digest of casein and Papic Digest of soyabean meal makes the medium highly nutritious by supplying organic nitrogen, particularly amino acids and long chain peptides. Sodium chloride maintains the osmotic balance. In Soyabean Casein Digest Medium Dextrose is an energy source and Dipotassium phosphate acts as a buffer to control pH. Soyabean Casein Digest Agar and Soyabean Casein Digest Medium may be supplemented with blood to provide a more nutritious medium for fastidious organisms, or with antimicrobials to provide a selective medium for specific organisms out of a mixed flora sample. Since Soyabean Casein Digest Agar contains no added carbohydrate, it may be used with added blood to determine haemolysis. When Soyabean Casein Digest Agar is supplemented with 0.7 gms lecithin and 5 gms polysorbate (Tween 80) per liter of medium, it can be used as microbial content test agar for testing quaternary ammonium compounds (collection of samples from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation).

Formula*

Ingredients in grams per liter

	SCDM IP	SCDM USP	SCDM EP	SCDM BP	SCDM Harmonized
Pancreatic digest of casein	17.0	17.0	17.0	17.0	17.0
Papic digest of soyabean Meal	3.0	3.0	3.0	3.0	3.0
Sodium chloride	5.0	5.0	5.0	5.0	5.0
Dibasic potassium phosphate	–	2.5	–	–	–
Dipotassium hydrogen phosphate	2.5	–	2.5	2.5	2.5
Dextrose	–	–	–	–	–
Dextrose monohydrate	2.5	2.5	–	–	–
Glucose monohydrate	–	–	2.5	2.5	2.5

Final pH (at 25_0C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30.0 gms of the powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To prepare Blood Agar plates, add 5-10% sterile, defibrinated blood to the sterile agar cooled to 45-50°C.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 48-72 hours at 30-35°C for bacteria and 2-5 days at 20-25°C for fungi.

Organisms(ATCC)	Growth
Candida albicans (10231)	Luxuriant
Escherichia coli (25922)	Luxuriant
Neisseria meningitidis (13090)	Luxuriant
Staphylococcus aureus (6538)	Luxuriant
Streptococcus pyogenes (19615)	Good to luxuriant
Pseudomonas aeruginosa (9027)	Luxuriant
Bacillus subtilis (6633)	Luxuriant
Bacteroides vulgatus (8482)	Luxuriant
Clostridium sporogenes (11437)	Luxuriant
Aspergillus niger (16404)	Luxuriant
Mucococcus luteus (9341)	Luxuriant

Procedure

Aerobic and anaerobic cultivation

1. Swab specimens may be inserted into the medium after inoculation of appropriate plated media.
2. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium.
3. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.
4. Incubate the containers with loosened caps at 35-37°C aerobically with or without supplementation with CO_2.
5. Containers intended for cultivation of anaerobes must be incubated under anaerobic conditions.
6. Examine the growth after 18-24 hours and 42-48 hours of incubation.

Blood culture

1. The superior growth promoting properties of Soyabean Casein Digest Medium makes it especially useful for the isolation of organisms from blood or other body fluids.
2. Anticoagulants such as 'liquoid' (Sodium polyanethyl sulphonate) or sodium citrate may be added to the broth prior to sterilization.3. 5-10 ml of blood may be added to 50 ml of medium.

Selective culture media

1. Soyabean Casein Digest Medium is used in food bacteriology as the basal medium to which a variety of selective agents are added for selective enrichment of Staphylococcus aureus and Escherichia coli 0157.

Interpretation of Results

1. Growth in broth medium is indicated by the presence of turbidity compared to an un-inoculated control.
2. Broth cultures should be held at least for a week before discarding as negative.

Precautions / Limitations

1. Haemolytic reactions of streptococci on this medium can vary according to the origin of the blood.
2. The medium designed for sheep blood shows significant differences when used with horse blood and vice versa.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Soyabean Casein Digest Medium Sterile Powder (g irradiated) AM50925/AM50925-5K

Use

Soyabean Casein Digest Medium, sterile powder is used for the evaluation of sterility in manufacturing process.

Summary

Routine sampling for sterility testing is not sensitive enough to detect any low level contamination in sterile pharmaceutical formulations. Sample numbers are too small and only gross contamination is likely to be detected. Pharmaceutical manufactures therefore need other means of guaranteeing the quality of their product. This is why process stimulations (Media fill run) supported by environmental monitoring is must in pharmaceutical industry.

The new FDA guidelines pay particular attention to this aspect of aseptic processing and it is becoming an area requiring more work and focus to satisfy the regulators. The FDA guidelines have recommended using Soyabean Casein Digest Medium – a highly nutritious general-purpose medium that is ideal for microbiological media fill. It also recommended that in order to more closely mimic the process, the culture medium should be filtered into the process, just as would occur to liquid pharmaceutical product. Regular dehydrated culture media is usually supplied in non-sterile form, which carries a high bioburden and should not taken directly into a controlled area. Therefore g irradiated, sterile SCDM powder is use for media fills. Irradiation of media also assures the sterile medium is free from Mycoplasma.

Principle

The combination of Tryptone and Soya peptone makes the medium highly nutritious by supplying organic nitrogen, particularly amino acids and long chain peptides. Sodium Chloride maintains osmotic balance. This medium, which has sterilized by gamma irradiation, can be directly used for media-fill runs as recommended by FDA guidelines.

Formula*

Ingredients in grams per liter

Tryptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dextrose	2.5
Dipotassium phosphate	2.5

Final pH (at 25°C) 7.3 + 0.2

* Formula adjusted to suit performance parameters

Directions

1. For sterile liquid medium aseptically suspend the 30.0 gms medium in 1000 ml sterile distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. DO NOT AUTOCLAAVE OR OVERHEAT
4. Dispense aseptically in desired sterile containers.
5. Carry out all operations in aseptic condition.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 48-72 hours at 30-35°C for bacteria and 2-5 days at 20-25°C for fungi.

Organisms(ATCC)	Growth
Candida albicans (10231)	Luxuriant
Escherichia coli (25922)	Luxuriant
Neisseria meningitides (13090)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant
Streptococcus pyogenes (19615)	Good to luxuriant
Pseudomonas aeruginosa (9027)	Luxuriant
Aspergillus niger (16404)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Soyabean Casein Digest Medium with Lecithin & Tween 80 — AM50927

Use

Soyabean Casein Digest Medium with Lecithin & Tween 80 (Tryptose Soya Broth, Antibiotic Assay Medium No. 37) is used for the isolation and cultivation of a wide variety of microorganisms.

Summary

Soyabean Casein Digest Medium with Lecithin & Tween 80 (Tryptose Soya Agar) is a nutritious base and a variety of supplements are added to enhance the medium, including Lecithin and Tween 80. The Lecithin and Tween 80 inactivate some preservatives that may inhibit bacterial growth, reducing "Preservative carryover".

Principle

The combination of Tryptone and Soya peptone makes the medium highly nutritious by supplying organic nitrogen, particularly amino acids and long chain peptides. Sodium Chloride maintains osmotic balance. Dextrose is the energy source. Dipotassium phosphate act as a buffering system to control pH. Tween 80 and lecithin act as neutralizers to inactivate the residual disinfectants where the samples are collected. Lecithin inactivates quaternary ammonium compounds whereas tween 80 neutralizes formalin, phenolic disinfectants, hexachlorophene etc. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Tryptone	17.00
Soya peptone	3.00
Sodium chloride	5.00
Dextrose	2.50
Dipotassium phosphate	2.50
Lecithin	0.70
Tween 80	5.00

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35.7 gms of the powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance:

Light yellow coloured, clear solution.

Cultural Response

Cultural characteristics after 24-48 hours at 30°C.

Organisms(ATCC)	Growth
Listeria monocytogenes (19111)	Good to luxuriant
Listeria monocytogenes (19118)	Good to luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

SS Agar (Salmonella Shigella Agar) — AM1093/AM5093

Use

SS Agar is a differential and selective medium used for the isolation of *Salmonella* and some *Shigella* species from clinical and non-clinical specimens.

Summary

SS Agar is a modification of Deoxycholate Citrate Agar described by Leifson and is an example of media used in the plating of samples for the detection of enteric pathogens that contain bile salt mixtures. SS Agar is used in the performance of microbial limit tests and is recommended by APHA for the examination of foods (20).

Principle

Peptone and beef extract provide sources of carbon, nitrogen and other growth factors. Brilliant green, bile salt mixture, thiosulphate and citrates selectively inhibit gram-positive organisms and coliforms. Lactose is the fermentable carbohydrate and differentiation of enteric organisms is achieved based on lactose fermentation in the presence of neutral red. On fermentation of lactose by a few lactose fermenting normal intestinal flora, acid is produced, which is indicated by change in colour from yellow to red by the pH indicator neutral red and these organisms grow as red-pigmented colonies. Non-lactose fermenters grow as translucent colourless colonies. Sodium thiosulphate and ferric citrate enable the detection of H_2S production. Sodium thiosulphate is reduced by certain species to sulphide and H_2S gas in the presence of the enzyme thiosulphate reductase. Production of H_2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon the reaction of H_2S with ferric citrate, indicated as a black dot in the center of the colony. The high selectivity of SS Agar allows the use of large inocula directly from faeces, rectal swabs or other material suspected of containing the enteric pathogens.

Formula*

Ingredients in grams per liter

Lactose	10.0
Sodium Citrate	10.0

Bile Salts Mixture	8.5
Sodium Thiosulphate	8.5
Peptone	5.0
Beef Extract	5.0
Ferric Citrate	1.0
Neutral Red	0.025
Brilliant Green	0.00033
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 63 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. AVOID OVERHEATING. DO NOT AUTOCLAVE.
5. Cool the medium to approximately 45-50°C, pour into sterile petri plates.
6. Allow the plates to dry for about 2 hours with the covers partially removed under aseptic conditions.

Quality Control

Dehydrated Appearance

Pinkish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Poor to good	Cream pink	0%
Enterococcus faecalis (29212)	None to poor	Colourless	0%
Escherichia coli (25922)	Poor to good	Pink with bile precipitate	0%
Proteus mirabilis (25933)	Poor to good	Colourless may have black centre	0%
Salmonella serotype Enteritidis (13076)	Good	Colourless with black centre	More than 70%
Salmonella serotype Typhimurium (14028)	Good	Colourless with black centre	More than 70%
Shigella flexneri (12022)	Good	Colourless without black centre	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small area of the agar surface and streak for isolation with a sterile loop.
3. Incubate plates aerobically, protected from light, at 35-37°C for 18-24 hours.
4. If negative, incubate for an additional 24 hours.
5. Examine colony morphology.
6. **Note:** A non-selective medium should also be streaked to increase the chances of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.
7. In parallel with the SS Agar plate, inoculate a tube of Selenite Broth enrichment medium, incubate for 12 hours at 35°C, and subculture onto another SS Agar plate.

Interpretation of Results

Typical colonial morphology on SS Agar

E.coli---------- Slight growth, pink or red

Enterobacter/Klebsiella------- Slight growth, pink

Proteus---------- Slight growth, colourless, may have black center

Salmonella---------- Good growth, colourless with black centers

Shigella---------- Slight or good growth, colourless

Pseudomonas---------- Irregular, slight growth

Gram-positive bacteria-------- No growth

Precautions / Limitations

1. This medium is highly selective; some strains of *Shigella* may not grow on it and therefore must not be used for primary isolation of shigellae.
2. Inoculate plates of less inhibitory media like Deoxycholate Citrate Agar or XLD Agar for better isolation of *Shigella* species.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

SS Agar, Modified

AM50931

Use

SS Agar is a differential and selective medium used for the isolation of Salmonella and some *Shigella* species from clinical and non-clinical specimens.

Summary

SS Agar is a modification of Deoxycholate Citrate Agar described by Leifson (68) and is an example of media used in the plating of samples for the detection of enteric pathogens that contain bile salt mixtures. SS Agar, Modified is especially useful in isolating *Shigella,* because it is many times inhibited in SS Agar. By reducing the bile salt quantity and increasing the pH SS Agar has been modified such a way that easily it helps to isolate *Shigella* without interfering the growth of commensal organisms.

Principle

Peptone and beef extract provide sources of carbon, nitrogen and other growth factors. Brilliant green, bile salt mixture, thiosulphate and citrates selectively inhibit gram-positive organisms and coliforms. Lactose is the fermentable carbohydrate and differentiation of enteric organisms is achieved based on lactose fermentation in the presence of neutral red. On fermentation of lactose by a few lactose fermenting normal intestinal flora, acid is produced, which is indicated by change in colour from yellow to red by the pH indicator neutral red and these organisms grow as red-pigmented colonies. Non-lactose fermenters grow as translucent colourless colonies. Sodium thiosulphate and ferric citrate enable the detection of H_2S production. Sodium thiosulphate is reduced by certain species to sulphide and H_2S gas in the presence of the enzyme thiosulphate reductase. Production of H_2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon the reaction of H_2S with ferric citrate, indicated as a black dot in the center of the colony. The high selectivity of SS Agar allows the use of large inocula directly from faeces, rectal swabs or other material suspected of containing the enteric pathogens.

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	5.0
Beef extract	5.0
Lactose	10.0
Bile salts mixture	5.50
Sodium citrate	10.0
Sodium thiosulfate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	12.0

Final pH (at 25ºC) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 57.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. AVOID OVERHEATING. DO NOT AUTOCLAVE.
5. Cool the medium to approximately 45-50^{0}C, pour into sterile petri plates.
6. Allow the plates to dry for about 2 hours with the covers partially removed under aseptic conditions.

Quality Control

Dehydrated Appearance

Pinkish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish orange coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Poor to good	Cream pink	More than 70%
Enterococcus faecalis (29212)	none to poor	Colourless	0% or More than 70%
Escherichia coli (25922)	Poor to good	Pink with bile precipitate	More than 70%
Proteus mirabilis (25933)	Poor to good	Colourless may have black centre	More than 70%
Salmonella serotype Enteritidis (13076)	Good	Colourless with black centre	More than 70%
Salmonella serotype Typhimurium (14028)	Good	Colourless with black centre	More than 70%
Shigella flexneri (12022)	Good	Colourless without black centre	More than 70%
Salmonella typhi (6538)	Good	Colourless without black centre	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small area of the agar surface and streak for isolation with a sterile loop.
3. Incubate plates aerobically, protected from light, at 35-37ºC for 18-24 hours.
4. If negative incubate for an additional 24 hours.
5. Examine colony morphology.

6. **Note:** A non-selective medium should also be streaked to increase the chances of recovery when the population of Gram negative organisms is low and to provide an indication of other organisms present in the specimen.
7. In parallel with the SS Agar plate, inoculate a tube of Selenite Broth enrichment medium, incubate for 12 hours at 35°C, and subculture onto another SS Agar plate.

Interpretation of Results

Typical colonial morphology on SS Agar

Escherichia coli ---	Slight growth,	Pink or red
Enterobacter/ Klebsiella ---	Slight growth,	Pink
Proteus---	Slight growth,	Colourless, may have black center
Salmonella---	Good growth,	Colourless with black centers
Shigella---	Slight or good growth,	Colourless
Pseudomonas---	Irregular, slight growth	
Gram-positive bacteria---	No growth	

Precautions / Limitations

1. This medium is highly selective; some strains of Shigella may not grow on it and therefore must not be used for primary isolation of shigellae.
2. Inoculate plates of less inhibitory media like Deoxycholate Citrate Agar or XLD Agar for better isolation of Shigella species.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Standard Nutrient Agar AM509311

Use

Standard Nutrient Agar is used as a general utility medium for cultivation and enumeration of not particularly fastidious microorganisms.

Summary

Standard Nutrient Agar is formulated as per the recommendation APHA as a general purpose medium for the cultivation of nonfastidious organisms from water and wastewater, dairy and food products.

Principle

Peptic digest of lean meat provides the amino acids and large chain peptides. Beef extract (meat infusion) provides water soluble substances like carbohydrates, vitamins, organic nitrogen compounds and salts. Sodium chloride maintains osmotic equilibrium.

Formula*

Ingredients in grams per liter

Peptic digest of lean meat from	500.0
Beef extract	10.0
Sodium Chloride	5.0
Agar	20.0

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Directions

1. Suspend 45 gms of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.**Quality Control**

Dehydrated Appearance

Yellowish brown coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth	RGI
Escherichia coli (25922)	Good to luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Standard Nutrient Broth (H.S. Vaccine Medium) AM509313

Use

A highly nutritive medium recommended for the large scale cultivation of bacteria for production of vaccine preparations

Principle

Peptic digest of lean meat is the principal source of organic nitrogen while meat infusion solids provides carbohydrates, vitamins, organic nitrogen compounds and salts. Sodium chloride maintains osmolality of the medium.

Formula*

Ingredients in grams per liter

Peptic digest of lean meat infusion, solids	10.00
Meat infusion, solids	10.00
Sodium chloride	5.00

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Directions

1. Suspend 25 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics observed after an incubation at 35 – 37°C for 18-48 hours.

Organisms(ATCC)	Growth
Enterobacter aerogenes (13048)	Good-luxuriant
Escherichia coli (25922)	Good-luxuriant
Salmonella typhi (6539)	Good-luxuriant
Staphylococcus aureus (25923)	Good-luxuriant
Staphylococcus Epiderimidis (12228)	Good-luxuriant
Staphylococcus pyogenes (19615)	Good-luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Staphylococcus Agar No. 110 (Gelatin Mannitol Salt Agar) AM10932/AM50932

Use

Staphylococcus Agar No. 110 is used as a selective medium for the isolation and testing of pathogenic *Staphylococci.*

Summary

Staphylococcus Agar No. 110 is formulated as described by chapman(16, 14.1, 16.5) for selective isolation and enumeration of *Staphylococci* from clinical as well as nonclinical specimens. This media is recommended by APHA (103.4). Addition of blood in the medium enables to study haemolytic reaction and with egg yolk enables to study lecithinase production by *Staphylococcus* aureus (12.2). Media is selective due to high concentration of salts and differential on the basis of ability of organisms to ferment the mannitol, pigment production and gelatin liquefaction.

Principle

Staphylococcus Agar No. 110 is very nutritive media as they contain casein enzymic hydrolysate, yeast extract which provide essential growth factors like vitamins, nitrogen, carbon compounds, sulphur and trace nutrient etc. to the organisms. High concentration of the sodium chloride inhibits many bacterial species expect *staphylococci.*

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	10.00
Yeast extarct	2.50
Gelatin	30.00
Lactose	2.00
D-Mannitol	10.00
Sodium Chloride	75.00
Dipotassium phosphate	5.00
Agar	15.00

Final pH (at 25°C) 7.0± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 149.5 gms of the powder in 1000 ml distilled water. Mix Thoroughly.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. Resuspend the precipitately gentle agitation to avoid bubbles and pour the plates while the medium to 45-50°C and add blood or egg yolk if

desired.

5. Medium also can be used without sterilization; it should be boiled for 5 minutes and used at once

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, opalescent gel with precipitate forms in petri plates..

Cultural Response

Cultural characteristics after 48 hours at 35-37ºC.

Organisms (ATCC)	Growth	Pigment production	Gelatinase production	Mannitol fermentation	RGI
Staphylococcus aureus (25923)	Luxuriant	+	+	+	More than 70%
Staphylococcus epidermidis (12228)	Luxuriant	–	+	v	More than 70%
Enterococcus faecalis (29212)	None-poor	–	v	+	0% or More than 70%
Escherichia coli (25922)	Inhibited	–	–	–	0%

Key: + = Positive reaction

– = Negative reaction

v = Variable reaction

+ = Slight reaction

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Streptococcus Selection Agar AM50933

Use

Streptococcus Selection Agar is recommended for selective isolation and enumeration of all types of *Streptococci,* including group A beta haemolytic strains.

Summary

Streptococcus Selection Agar is based on the suggestion of Pike (88.1), for the selective isolation of *Streptococci* from various materials, specially those which are heavily contaminated with accompanying microbial flora (28.2). Welch et al., (118.1) also reported the ability of these media to recover group A β-haemolytic *Streptococci.*

Principle

Casein enzymic hydrolysate, papaic digest of soyabean meal, dextrose and salts provide nutrients essential for the growth of streptococci. Sodium azide, sodium sulphite inhibits gram-negative rods and the crystal violet suppresses Staphylococci. However, *Streptococci* are not affected by these inhibitors at these concentrations. Due to this reason, this media is useful in studies of streptococcal flora from nutritional, dental and epidemiological research.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Dextrose	5.00
Sodium Chloride	4.00
Sodium Citrate	1.00
Sodium sulphite	0.20
L-cystine	0.20
Sodium azide	0.20
Crystal violet	0.0002
Agar	15.00

Final pH (at 25°C) 7.4± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.6 gms of the powder in 1000 ml distilled water. Mix Thoroughly.
2. Heat to boiling to dissolve the medium completely.
3. Autoclaving is not required if medium is used on the same day.
4. If storage is desired, Sterilize by autoclaving at 118°C (12 lbs pressure) for 15 minutes.
5. Avoid overheating.

Caution: Sodium azide has a tendency to form explosive metal-azide with plumbing material. It is advisable to use enough water to flush off the disposable.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms(ATCC)	Growth	RGI
Streptococcus pyrogenes (19615)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	None-poor	0% or more than 70%
Escherichia coli (25922)	None-poor	0% or more than 70%
Bacillus subtilis (6633)	Inhibited	0%
Pseudomonas aeruginosa (27853)	Inhibited	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Streptococcus Selection Broth AM50934

Use

Streptococcus Selection Broth is recommended for selective isolation and enumeration of all types of *Streptococci*, including group A beta haemolytic strains

Summary

Streptococcus Selection Broth is based on the suggestion of Pike (88.1), for the selective isolation of *Streptococci* from various materials, specially those which are heavily contaminated with accompanying microbial flora (28.2). Welch et al., (118.1) also reported the ability of these media to recover group A β-haemolytic *Streptococci.*

Principle

Casein enzymic hydrolysate, papaic digest of soyabean meal, dextrose and salts provide nutrients essential for the growth of streptococci. Sodium azide, sodium sulphite inhibits gram-negative rods and the crystal violet suppresses *Staphylococci.* However, *Streptococci* are not affected by these inhibitors at these concentrations. Due to this reason, this media is useful in studies of streptococcal flora from nutritional, dental and epidemiological research.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Dextrose	5.00
Sodium chloride	4.00
Sodium citrate	1.00
Sodium sulphite	0.20
L-cystine	0.20
Sodium azide	0.20
Crystal violet	0.0002

Final pH (at 25°C) 7.4± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30.6 gms of the powder in 1000 ml distilled water. Mix Thoroughly.
2. Heat to boiling to dissolve the medium completely.
3. Autoclaving is not required if medium is used on the same day.
4. If storage is desired, Sterilize by autoclaving at 118°C (12 lbs pressure) for 15 minutes.
5. Avoid overheating.

Caution: Sodium azide has a tendency to form explosive metal-azide with plumbing material. It is advisable to use enough water to flush off the disposable.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to medium amber coloured, clear to slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms(ATCC)	Growth
Streptococcus pyrogenes (19615)	Luxuriant
Enterococcus faecalis (29212)	Luxuriant
Staphylococcus aureus (25923)	None-poor
Escherichia coli (25922)	None-poor
Bacillus subtilis (6633)	Inhibited
Pseudomonas aeruginosa (27853)	Inhibited

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Stuart Transport Medium AM1094/AM5094

Use

Stuart Transport Medium is used for collecting, transporting and preserving microbiological specimens, particularly *Neisseria* species and other fastidious organisms.

Summary

Stuart (104) originally designed Stuart Transport Medium when studying

gonococci. Stuart et al modified this medium for the transportation of gonococcal specimens for culturing. Transport media are basically chemically defined, semisolid, non-nutritive, phosphate buffered media that provide a reduced environment and are designed to maintain the viability of organisms without much increase in growth. Ringertz (92) included thioglycollate in the Stuart Medium and omitted charcoal. This medium is currently recommended for throat, vaginal and wound samples.

Principle

This medium by virtue of its composition prevents microbial proliferation but ensures that the organisms present survive for sufficiently long period of time. Calcium chloride provides essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Sodium thioglycollate is a reducing agent providing anaerobiosis, which can be monitored by means of the redox indicator; methylene blue. Sodium glycerophosphate is a buffer for use with calcium chloride.

Formula*

Ingredients in grams per liter

Sodium Glycerophosphate	10.0
Sodium Thioglycollate	0.9
Calcium Chloride	0.1
Methylene Blue	0.002
Agar	3.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 14 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in small screw cap bottles or vials, filling them almost to the top.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. Tighten caps immediately and cool the tubes in an upright position.

Note: The water used should be free from chlorine.

Quality Control

Dehydrated Appearance

White, homogeneous, free flowing powder.

Prepared Appearance

Colourless to whitish coloured, slightly opalescent butt with upper 10% or less becoming blue on standing.

Cultural Response

Cultural characteristics after 72 hours at 35°C when subcultured onto a suitable medium.

Organisms (ATCC)	Growth	Subculture Medium
Haemophilus influenzae (35056)	Good	*Chocolate Agar
Neisseria gonorrhoeae (19424)	Good	*Chocolate Agar
Streptococcus pneumoniae (6303)	Good	Soyabean Casein Digest Agar with 5% sheep blood

Key:

* = Incubation in CO_2 atmosphere.

Procedure

1. Obtain the specimen with a sterile swab and insert into the upper third of the medium in the transport container.
2. Break off the protruding portion of the swab stick and tightly screw the lid of the container.
3. Submit to laboratory within 24 hours for culture and analysis.

Interpretation of Results

1. The survival of organisms depends on many factors including the type and concentration of bacteria in the specimen, the temperature and duration of transport and inoculation to appropriate culture media within 24 hours.
2. Optimal growth and typical morphology can only be expected following direct inoculation and cultivation under appropriate conditions.

Precautions / Limitations

1. Prepared sterile media may undergo a slight degree of oxidation at the upper periphery of the medium, however, if the vials or tubes show a distinct blue colour throughout the medium, it must be discarded.
2. Fill the vials or tubes with the medium almost to capacity. Leave only enough space to allow insertion of the small swab.
3. For transportation of specimens that may contain *N.gonnorhoeae*, a selective medium may be used.
4. Viability of cells will diminish over time and contaminants may overgrow during prolonged periods of transit. This is particularly true of faecal specimens that contain high numbers of coliform organisms.
5. Specimens taken from transport media will not exhibit the optimal or comparative growth as expected from direct inoculation and cultivation. However, these media provide an adequate degree of preservation for those specimens, which cannot be immediately forwarded to the laboratory.
6. The condition of the specimen received by the laboratory for culture is an important variable in recovery and final identification of the suspect pathogen.
7. A specimen overgrown by contaminants or having the number of suspect pathogens greatly diminished may lead to wrong or inconclusive results.

Storage

Store below 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Super Broth AM50935

Use

Super Broth is used for the mass cultivation of *Escherichia coli*.

Summary

Escherichia coli is a bacterium that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. Some strains however, such as *Enterohaemorrhagic E. coli* (EHEC) can cause severe foodborne disease. Super Broth has a formulation slightly different from that described by Atlas (1.1.1) and it is used for the mass cultivation of E. coli .

Principle

Casein enzymic hydrolysate and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Sodium chloride maintains osmotic equilibrium. Super Broth is nutritionally rich hence other organisms can also grow in it easily.

Formula*

Ingredients in grams per liter	Ingredients Gms/Liter
Casein enzymic hydrolysate	35.00
Yeast extract	20.00
Sodium chloride	5.00

Final pH (at 25°C) 7.0±0

* Formula adjusted to suit performance parameters

Directions

1. Suspend 60 gms of the powder in 1000 ml distilled water. Mix Thoroughly.
2. Heat if necessary to dissolve the medium completely.
3. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream to yellow homogeneous free flowing powder

Prepared Appearance

Light yellow coloured clear solution without any precipitate

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Growth
Escherichia coli (23724)	Good - luxuriant
Escherichia coli (25922)	Good - luxuriant
Staphylococcus aureus (25923)	Good - luxuriant

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

TB Broth Base AM50941

Use

TB Broth Base is used as a liquid medium for cultivation of *Mycobacterium tuberculosis.*

Summary

TB Broth media are based on the medium formulated by Dubos and Davis (21.1) and are used as liquid media for the cultivation of *Mycobacterium tuberculosis.* Media provides dispersed growth of tubercle bacilli which is free of excessive clumps and so it can be used to prepare a uniform suspension of Mycobacteria. The media can be used without the additives and supplements however sterile dextrose and sterile serum can be added for the enrichment. Glycerol addition helps in the cultivation of *Mycobacterium tuberculosis* though some bovine strains are inhibited by it.

Principle

Proteose peptone and yeast extract provide nitrogenous nutrients like amino acids and peptides, vitamin B complex and other essential nutrients. The media are well buffered by phosphates. The salts present in the media supply ions required for the mycobacterial metabolism. Polysorbate 80, an oleic acid ester provides essential fatty acids for the replication of *Mycobacteria.*

Formula*

Ingredients in Gms/Liter	
Proteose peptone	4.00
Yeast extract	2.00
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	1.50
Magnesium sulphate	0.60
Polysorbate 80	0.50

Final pH (at 25^{0}C) 7.0± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 12.1 gms of the powder in 1000 ml distilled water. Which if desired contains 5 ml glycerol (tested to be non-inhibitory to typical cultures).
2. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.

3. Cool to 45°C and enrich with dextrose to a final concentration of 0.5% and eiter bovine albumin fraction V or serum as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 2-4 weeks at 35-37ºC.

Organisms (ATCC)	Growth
Mycobacterium tuberculosis H37 RV (25618	Luxuriant
Mycobaterium kansasi (12478)	Luxuriant
Mycobacterium smegmatis (14468)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

TAT Broth Base AM509411

Use

T.A.T. Broth Base with added polysorbate 20 is used for cultivating microorganisms from highly viscous or gelatinous materials such as salves, ointments.

Summary

T.A.T. Broth is prepared according to the formula recommended by United States Food and Drug Administration (31.1.1) for enrichment and further isolation and cultivation of gram-negative bacteria in cosmetics, tropical drugs and in the sterility testing of viscous or gelatinous substances. It is especially adapted for the testing of cosmetics. Cosmetics and pharmaceutical products are subject to contamination during manufacturing and subsequent use by consumers (85.1.1). Preservatives are used in aqueous products to make them self-sterilizing for vegetative bacteria, yeasts and moulds, and bacteriostatic or bactericidal for spores (85.1.1).

Principle

Pancreatic digest of casein provides the nitrogen, vitamins, amino acids and carbon in T.A.T. Broth Base. Soy lecithin and polysorbate 20 neutralize preservatives in the cosmetics or pharmaceutical products, allowing bacteria to grow. Prepare decimal dilutions of the sample to be tested from 10-1 to 10-6. Inoculate 1 gram (1 ml) sample and 1 ml of each dilution into 40 ml of T.A.T. Broth. After incubation, subculture the growth on MacConkey Agar and TSI Agar.

Formula*

Ingredients in grams per liter	Ingredients Gms/Liter
Pancreatic digest of casein	20.00
Soy lecithin	5.00
Final pH (at 25°C) 7.2±0.2	

* Formula adjusted to suit performance parameters

Directions

1. Suspend 25 grams in 960 ml distilled water and add 40 ml of polysorbate 20.
2. Heat if necessary to dissolve the medium completely.
3. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Off-white to yellow homogeneous free flowing powder

Prepared Appearance

Light amber coloured clear to slightly opalescent solution

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Growth
Bacillus subtilis ATCC 6633	Good- luxuriant
Candida albicans ATCC 10231	Fair- luxuriant
Pseudomonas aeruginosa *ATCC* 27853	Good-luxuriant
Salmonella Typhi ATCC 6539	Good- luxuriant
Staphylococcus aureus ATCC 25923	Good- luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tartoff-Hobbs (Terrific) Broth AM509412

Use

Tartoff-Hobbs (Terrific) Broth is recommended for the cultivation of recombinant strains of *Escherichia coli.*

Summary

Tartoff Hobbs developed the Terrific Broth Medium for cultivation of recombinant *Escherichia coli* strains. These strains have extended growth phase when cultivated in this medium (107.1). Tartoff-Hobbs Medium supports high cellular

density and mass and maintains the growth in the logarithmic phase for a long time. Due to this fact, it provides greater yields of recombinant proteins and plasmid DNA. Often, Tartoff-Hobbs (Terrific) Broth substitutes Luria Bertani Broth (AM50574), to get enhanced yields of plasmid DNA and recombinant proteins. The procedures for inoculation, incubation and generation of recombinant strains are detailed by *Sambrook et al* (102.1).

Principle

Casein enzymic hydrolysate and yeast extract supply the necessary nutrients and cofactors for the excellent growth of recombinant strains of E. coli. The addition of extra peptone and yeast extract in the medium allows higher plasmid yield per volume. Two phosphates provide good buffering action to the medium. Glycerol is used as the carbohydrate source. Unlike glucose, glycerol is not fermented to acetic acid.

Formula*

Ingredients in Gms/Liter

Casein enzymic hydrolysate	12.00
Yeast extract	24.00
Monopotassium phosphate	9.4
Dipotassium phosphate	2.2

Final pH (at 25°C) 7.2±0.2

* Formula adjusted to suit performance parameters

Directions

1. Dissolve 47.6 g of the powder in 1 L of purified water.
2. Add 4 ml of glycerol to the medium.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Quality Control

Dehydrated Appearance

Light yellow to beige homogeneous free flowing powder

Prepared Appearance

Light to medium amber coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Growth
Escherichia coli (23724)	Good
Escherichia coli (39403)	Good
Escherichia coli (47014)	Good
Escherichia coli (53868)	Good

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

TCBS Agar AM1095/AM5095

Use

TCBS Agar is a selective medium used for isolating and cultivating *vibrios* causing cholera and food poisoning from clinical and non-clinical specimens.

Summary

Thiosulphate Citrate Bile Salts Sucrose (TCBS) Agar is a primary plating medium for the selective isolation of vibrios that cause cholera, diarrhoea and food poisoning. It was developed by Nakanishi et al (83) and was modified by Kobayashi (59). The combination of alkaline peptone water and TCBS Agar is used in many procedures for the isolation of *V.cholerae* and other *Vibrio* species from foods (20), water (36) and faeces. This medium is included in the Bacteriological Analytical Manual for food testing (113).

Principle

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Oxgall and sodium citrate inhibits gram-positive bacteria. Sodium thiosulphate serves as the sulphur source and in combination with ferric citrate detects hydrogen sulphide production. Sucrose is the fermentable carbohydrate for the metabolism of vibrios. The alkaline pH of the medium enhances the recovery of *V.cholerae*. Thymol blue and bromothymol blue are included as indicators of pH change. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sucrose	20.0
Proteose Peptone	10.0
Sodium Citrate	10.0
Sodium Thiosulphate	10.0
Sodium Chloride	10.0
Oxgall	8.0
Yeast Extract	5.0
Ferric Citrate	1.0
Thymol Blue	0.04
Bromothymol Blue	0.04
Agar	15.0

Final pH (at 25°C) 8.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 89 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. DO NOT AUTOCLAVE.
5. Cool to 45-50^{0}C and pour into sterile petri plates, use immediately.

Quality Control

Dehydrated Appearance

Yellow coloured with tan cast, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35^{0}C.

Organisms (ATCC)	Growth	Colour of Colonies	RGI
Escherichia coli (25922)	Inhibited	-	0%
Proteus vulgaris (13315)	Inhibited	-	0%
Shigella flexneri (12022)	Inhibited	-	0%
Streptococcus faecalis (29212)	Inhibited	-	0%
Vibrio cholerae (15748)	Good to luxuriant	Yellow	More than 70%
Vibrio parahaemolyticus (17802)	Good to luxuriant	Blue	More than 70%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate the plates, protected from light in an inverted position at 35^{0}C for 24-48 hours.

Interpretation of Results

Colony morphology on TCBS Agar is as follows:

V.cholerae ---------- Large yellow colonies

V.parahaemolyticus ---------- Colonies with blue to green centers

V.alginolyticus ---------- Large yellow colonies.

Proteus/Enterococcus ---------- Partial inhibition. If growth occurs, colonies are small, yellow and translucent.

Pseudomonas/ Aeromonas ---------- Partial inhibition. If growth occurs, colonies are blue.

Precautions / Limitations

1. Cultures grown on TCBS Agar should be examined immediately after removal from the incubator as yellow colonies of vibrios, e.g. *V.cholerae* may revert to a green colour when left at room temperature.
2. The identification of the various Vibrio species on TCBS Agar is presumptive and further tests are required for confirmation.
3. Yellow colonies on TCBS Agar will give unsatisfactory oxidase reactions.
4. Initial isolation of *V.parahaemolyticus* may be confused with *Aeromonas hydrophila, Plesiomonas shigelloids* and *Pseudomonas* species.
5. Sucrose fermenting *Proteus* species produce yellow colonies, which may resemble those of *Vibrio.*
6. A few strains of *V.cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation.
7. Colonies taken from TCBS Agar are 'sticky' and react poorly in slide agglutination tests. Subculture to Nutrient Agar before slide agglutination tests are carried out.

Storage

Store below 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Teepol Broth (Twin Pack) AM509511

Use

Teepol Broth (Twin Pack) is used for selective isolation and identification of enteric lactose fermenting bacteria.

Summary

Teepol Broth is formulated as described by Burman, where he substituted teepol in place of bile salts in the formulation of Membrane Enrichment Teepol Broth. The use of Teepol in place of bile salts was previously recommended by Jameeson and Emberley. Burman showed that if a preliminary incubation is carried out at a lower temperature resuscitation is not required. Non-chlorinated organisms benefit from 4 hour incubation at 30^{0}C but chlorinated organisms require 6 hours incubation at 25^{0}C.

Principle

The coliform and *Escherichia coli* count are made on separate volumes of water. The water samples are filtered through membrane filter and this filter is placed face upwards on an absorbent pad saturated with Teepol Borth. The yellow

colonies formed are further identified. Presumptive coliform organisms: Yellow colonies from membranes incubated at 35°C, when subcultured in Lactose Peptone Water produce gas at 35°C after 43 hours. Presumptive *Escherichia coli* Yellow colonies from membrane at 44°C produce gas and indole after 24 hours.

Formula*

Ingredients in grams per liter

Part A :	Peptic digest of animal tissue	20.00
	Lactose	10.00
	Sodium chloride	5.00
	Phenol red	0.02
Part B:	Teepol	1.00

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 35 grams of Part A in 1000 ml distilled water containing 1 grams of Part B.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in tubes containing inverted Durham's tubes.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Dehydrated Appearance

Part A: Pink coloured, homogeneous, free flowing powder.

Part B: Colourless viscous solution.

Prepared Appearance

Red coloured, clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 24-48 hours at .....

Organisms (ATCC)	Growth at 35°C	Growth at 44°C
Escherichia coli (25922)	+	+
Enterobacter aerogenes (13048)	+	–

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tergitol-7 Agar Base AM10951/AM50951

Tergitol-7 Agar Base BIS AM10952/AM50952

Use

Tergitol-7 Agar is used for enumeration, differentiation and selective isolation of coliform bacteria.

Summary

Tergitol-7(Sodium heptadecyl sulphate) Agar or T-7 Agar is formulated as described by Chapman (16.2). Tergitol-7 (sodium heptadecyl sulfate) inhibits the growth of gram positive bacteria, gram negative sporeformers and swarming of Proteus while allowing the growth of coliform bacteria. Chapman modified his original T-7 agar by adding 40mg of triphenyl tetrazolium chloride (TTC). On modified media he found that surface colonies of *Escherichia coli* produce greenish yellow colonies surrounded by yellow halo while other coliforms produce dark red colonies (16.3). Tergitol-7 Agar with TTC is used for routine analysis of water and food (63.1, 32.1).

Principle

Proteose Peptone and Yeast extract serve as a source of nitrogen and vitamins. Tergitol-7 (sodium heptadecyl sulfate) as a selective agent inhibits the growth of gram-positive bacteria, gram-negative sporeformers and swarming of Proteus spp. Lactose is the fermentable sugar. Lactose fermentation is indicated by a colour change of the pH indicator, Bromothymol blue. Lactose fermenting organisms form yellow colored colonies surrounded with yellow zones while *Klebsiella* and *Enterobacter* form greenish yellow colonies. Non-Lactose fermenting organisms form blue colonies. Agar is the solidifying agent. When TTC is added to the medium, it is rapidly reduced to insoluble red formazan by most organisms. Non-lactose fermenters appear red due to uptake and reduction of TTC while lactose fermenting organisms continue to form yellow to greenish-yellow colonies.

Formula*

Ingredients in grams per liter

Proteose peptone	5.00
Yeast extract	3.00
Lactose	10.00
Tergitol-7 (sodium heptadecyl sulfate)	0.10
Bromthymol blue	0.025
Agar	15.00

Final pH: 6.9 ± 0.2 at 25°C

* Formula adjusted to suit performance parameters**Directions**

1. Suspend 33.13 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. If desired, cool Tergitol-7 Agar to 50°C. Add 4.0ml of a filter sterilized 1% solution of TTC (AS0271).

6. Cool the medium to approximately 45-50°C, pour in to sterile petriplates.

Quality Control

Dehydrated Appearance

Yellow coloured w/ green tinge, homogeneous, free flowing powder.

Prepared Appearance

Green coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Escherichia coli (25922)	Luxuriant	Yyellow	More than 70%
Enterobacter aerogenes (13048)	Luxuriant	Yellow	More than 70%
S. serotype Typhimurium (14028)	Luxuriant	Blue	More than 70%
Shigella flexneri (12022)	Luxuriant	Blue	More than 70%
Staphylococcus aureus (25923)	Lnhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. Incubate plates aerobically, protected from light, at 35-37°C for 18-48 hours.
3. Examine colony morphology.

Interpretation of Results

Tergitol-7 Agar without TTC

E.coli produces yellow colonies with yellow halos; other coliforms produce yellow to yellow-green colonies. Non-lactose fermenters produce blue colonies.

Tergitol-7 Agar with TTC

E.coli produces yellow colonies; other coliforms produce yellow-green colonies while Non-lactose fermenters produce red colonies.

Limitations

1. It is preferable that biochemical and / or serological tests be performed on colonies from pure culture for complete identification.
2. Pour plates do not give satisfactory results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tergitol-7 Broth AM50953

Use

Tergitol-7 Broth is used for enumeration, differentiation and selective isolation of coliform bacteria.

Summary

Tergitol-7(Sodium heptadecyl sulphate) Broth or T-7 Broth is formulated as described by Chapman (16.2). Tergitol-7 (sodium heptadecyl sulfate) inhibits the growth of gram positive bacteria, gram negative sporeformers and swarming of Proteus while allowing the growth of coliform bacteria. Chapman modified his original T-7 agar by adding 40mg of triphenyl tetrazolium chloride (TTC). On modified media he found that surface colonies of *Escherichia coli* produce greenish yellow colonies surrounded by yellow halo while other coliforms produce dark red colonies. Tergitol-7 Agar with TTC is used for routine analysis of water and food.

Principle

Proteose Peptone and Yeast extract serve as a source of nitrogen and vitamins. Tergitol-7 (sodium heptadecyl sulfate) as a selective agent inhibits the growth of gram-positive bacteria, gram-negative sporeformers and swarming of Proteus spp. Lactose is the fermentable sugar. Lactose fermentation is indicated by a colour change of the pH indicator, Bromothymol blue. Lactose fermenting organisms form yellow colored colonies surrounded with yellow zones while *Klebsiella* and *Enterobacter* form greenish yellow colonies. Non-Lactose fermenting organisms form blue colonies.

Formula*

Ingredients in grams per liter

Proteose peptone	5.00
Yeast extract	3.00
Lactose	10.00
Tergitol-7 (sodium heptadecyl sulfate)	0.10
Bromthymol blue	0.025

Final pH: 6.9 ± 0.2 at 25°C

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.13 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C aseptically add 3 ml of 1% 2,3,5, Triphenyl Tetrazolium chloride (TTC) solution (AS0271) if desired.

Quality Control

Dehydrated Appearance

Yellow coloured w/ green tinge, homogeneous, free flowing powder.

Prepared Appearance

Green coloured, slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of medium
Escherichia coli (25922)	Luxuriant	Yellow
Enterobacter aerogenes (13048)	Luxuriant	Yellow
S. serotype Typhimurium (14028)	Luxuriant	Blue
Shigella flexneri (12022)	Luxuriant	Blue
Staphylococcus aureus (25923)	Inhibited	-

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tetrathionate Brilliant Green Bile Broth AM50954

Tetrathionate Brilliant Green Bile Broth IP AM50955

Tetrathionate Brilliant Green Bile Broth (Broth Medium I) EP AM50956

Tetrathionate Brilliant Green Bile Broth (Broth Medium I) BP AM50957

Use

Tetrathionate Brilliant Green Bile Broth is used for the selective enrichment and isolation of *Salmonella* for the examination of pharmaceutical products in raw materials, foodstuffs, meat etc.

Summary

Tetrathionate Brilliant Green Bile Broth is a selective enrichment broth used for the isolation of *Salmonella*. Tetrathionate Brilliant Green Bile Broth is recommended in Indian Pharmacopoeia for isolation of Salmonella (46).

Principle

Peptic Digest of Animal Tissue supply nutrients, nitrogen compounds and amino acids. Ox bile supports the growth of enteric bacteria and inhibits other bacteria, which do not normally live in the intestine. Brilliant-green specifically inhibits the Gram-positive accompanying flora. Potassium tetrathionate inhibits normal flora of faecal specimens. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. The Calcium carbonate is a neutralizer that will absorb any toxic metabolites.

Formula*

Ingredients in grams/ liter	Microxpress	IP	EP	BP
Peptone	-	8.6	8.6	8.6
Peptic digest of animal tissue	8.6	-	-	-
Potassium tetrathionate	20.0	20.0	20.0	20.0
Brilliant green	0.07	0.07	0.07	0.07
Ox bile, dried	8.0	-	8.0	8.0
Dehydrated ox bile	-	8.0	-	-
Sodium chloride	6.4	6.4	6.4	6.4
Calcium carbonate	20.0	20.0	20.0	20.0

Final pH (at 25°) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 63 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. Do not boil. DO NOT AUTOCLAVE OR REHEAT.
4. Pour into adequate containers homogenizing the medium well enough to distribute the calcium carbonate.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, opalescent solution with white precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Recovery on MacConkey Agar AM1059 / AM5059	Colour of Colony
S. serotype Typhimurium (14028)	Luxuriant	Colourless
S. serotype Enteritidis (13076)	Luxuriant	Colourless
Escherichia coli (25922)	Fair	Pink with bile precipitate
Staphylococcus aureus (25923)	Inhibited	-

Procedure

1. Add 1-2 ml enriched sample to Tetrathionate Brilliant Green Bile Broth and incubate at 37°C for 18-24 hours.
2. Sub-culture to at least 2 of the following media for confirmation of Salmonellae spp:

a. Deoxycholate Citrate Agar (Cat. AM1031/5031);

b. XLD Agar (Cat. AM1112/5112); or Brilliant Green Agar (Cat. AM1018/5018).

Interpretation of Results

Deoxycholate Citrate Agar well-developed, colorless colonies

XLD Agar well-developed, red colonies, with black centers

Brilliant Green Agar small, transparent, colorless or pink or opaque-white colonies, often surrounded by a pink or red zone.

Storage

Storeat 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tetrathionate Broth Base, Hajna AM1096/AM5096

Use

Tetrathionate Broth Base, Hajna is a medium used for selective enrichment of *Salmonella*, particularly in food and dairy products prior to isolation.

Summary

Tetrathionate Broth Base, with added iodine-iodide solution is used as a selective enrichment medium for the isolation of *Salmonella* from faeces, urine, foods and other materials of sanitary importance. Salmonellae can be injured in food-processing procedures like exposure to low temperatures, sub-marginal heat, drying, radiation, preservation and sanitizers. Although injured cells may not form colonies on selective media, they can cause disease if ingested; causing many types of infections from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common type of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Tetrathionate Broth Base, Hajna conforms to the formulation of Hajna and Damon (41). The medium is a modification of the enrichment described by Kauffmann (51) and Knox (58). It is specified in the Standard Methods (20, 36, 39), in the USP (114) and IP (46) as well as in the Bacteriological Analytical Manual for food testing (113).

Principle

Peptone Special, provides nitrogen and amino acids, while yeast extract supplies growth factors and vitamins. Dextrose and mannitol are fermentable carbohydrates. Sodium thiosulphate and tetrathionate suppress coliforms. Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium. Sodium deoxycholate and brilliant green are selective agents that inhibit gram-positive organisms. Sodium chloride maintains the osmotic balance while calcium carbonate is a neutralizer that absorbs toxic metabolites.

Formula*

Ingredients in grams per liter

Sodium Thiosulphate	38.0
Calcium Carbonate	25.0
Peptone Special	18.0
Sodium Chloride	5.0
D-Mannitol	2.5
Yeast Extract	2.0
Dextrose	0.5
Sodium Deoxycholate	0.5
Brilliant Green	0.01

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 91.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely or place in flowing steam for 30 minutes.
4. DO NOT AUTOCLAVE.
5. Cool to 45°C.
6. Mix and add 40 ml of iodine solution (8 gms potassium iodide and 5 gms of iodine per 40 ml).
7. Mix and dispense 10 ml aliquots in tubes.
8. Do not heat after the addition of iodine.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light green coloured opalescent solution with heavy white precipitate.

Cultural Response

Cultural characteristics after18-24 hours at 35-37°C.

Organisms (ATCC)	Recovery on MacConkey Agar AM1059 / AM5059	Colour of Colony
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Colourless
Shigella dysenteriae (13313)	Good to luxuriant	Colourless
Escherichia coli (25922)	Fair to good	Pink with bile precipitate

Procedure

1. After preparation, add 1-3 gms of fecal specimen to each tube (heavy inoculum).

2. Incubate tubes for 18-24 hours at 35-37°C in an aerobic atmosphere.

Interpretation of Results

1. Growth is indicated by turbidity in the medium.
2. Subculture to selective and differential enteric plating media for further investigations.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tetrathionate Broth Base IP — AM10961/AM50961

Use Uulture Media, Bases, Supplements

Tetrathionate Broth Base is a medium used for selective enrichment of Salmonella, in Accordance with I P.

Summary

Tetrathionate Broth Base, with added iodine-iodide solution is used as a selective enrichment medium for the isolation of Salmonella from foods, faeces, urine, and other materials. Salmonellae can be injured in food-processing procedures like exposure to low temperatures, sub-marginal heat, drying, radiation, preservation and sanitizers. Although injured cells may not form colonies on selective media, they can cause disease if ingested; causing many types of infections from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common type of Salmonella disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days. The medium is a modification of the enrichment described by Kauffmann and Knox. It is specified in the Standard Methods in the IP.

Principle

Peptone, provides nitrogen and amino acids, while yeast extract and beef extract supplies growth factors and vitamins. Sodium thiosulphate and tetrathionate suppress coliforms. Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium. Sodium chloride maintains the osmotic balance while calcium carbonate is a neutralizer that absorbs toxic metabolites.

Formula*

Ingredients in grams per liter

Beef extract	0.9
Peptone	4.5
Yeast extract	1.8
Sodium chloride	4.5
Calcium carbonate	25.0
Sodium thiosulphate	40.7

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 77.40 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to dissolve the powder completely or place in flowing steam for 30 minutes.
4. DO NOT AUTOCLAVE.
5. Cool to 45°C.
6. Mix and add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of water.
7. Mix and dispense in tubes.
8. Do not heat after the addition of iodine.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

Light green coloured opalescent solution with heavy white precipitate.

Cultural Response

Cultural characteristics after18-24 hours at 35-37°C.

Organisms (ATCC)	Recovery on MacConkey Agar AM1059 / AM5059	Colour of Colony
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Colourless
Shigella dysenteriae(13313)	Poor to good	Colourless
Escherichia coli (25922)	none to poor	Pink with bile precipitate

Procedure

1. After preparation, add specimen to each tube.
2. Incubate tubes for 18-24 hours at 35-37°C in an aerobic atmosphere. Interpretation of Results

1. Growth is indicated by turbidity in the medium.
2. Subculture to selective and differential enteric plating media for further investigations.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tetrathionate Broth Base Medium USP AM10962/AM50962

Use Uulture Media, Bases, Supplements

Tetrathionate Broth Base is a medium used for selective enrichment of *Salmonella*, in compliance with USP.

Summary

Tetrathionate Broth Base, with added iodine-iodide solution is used as a selective enrichment medium for the isolation of *Salmonella* from faeces, urine, foods and other materials of sanitary importance. Salmonellae can be injured in food-processing procedures like exposure to low temperatures, sub-marginal heat, drying, radiation, preservation and sanitizers. Although injured cells may not form colonies on selective media, they can cause disease if ingested; causing many types of infections from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common type of Salmonella disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Tetrathionate Broth Base, Hajna conforms to the formulation of Hajna and Damon. The medium is a modification of the enrichment described by Kauffmann and Knox. It is specified in the Standard Methods, in the USP and IP as well as in the Bacteriological Analytical Manual for food testing .

Principle

Pancreatic digest of casein & peptic digest of animal tissue provides nitrogen, amino acids and vitamins. Sodium thiosulphate and tetrathionate suppress coliforms. Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium. Sodium deoxycholate and brilliant green are selective agents that inhibit gram-positive organisms. Sodium chloride maintains the osmotic balance while calcium carbonate is a neutralizer that absorbs toxic metabolites.

Formula*

Ingredients in grams per liter

Bile salts	1.0
Calcium carbonate	10.0
Pancreatic digest of casein	2.5
Peptic digest of animal tissue	2.5
Sodium thiosulphate	30.0

Final pH (at 25°C) 8.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 46.0 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to boiling. Cool to below 60°C.
4. Add 20 ml of iodine solution (6.0 g of iodine crystals and 5.0 g of potassium iodide in 20.0 ml of water).
5. Then add 10 ml of a solution of Brilliant Green (1 in 1000) and mix.
6. DO NOT REHEAT MEDIUM AFTER ADDING BRILLIANT GREEN.
7. DO NOT AUTOCLAVE.
8. Mix and dispense 10 ml aliquots in tubes.
9. Use immediately.

Quality Control

Dehydrated Appearance

Cream coloured, homogeneous, free flowing powder.

Prepared Appearance

White to off white coloured opalescent solution with heavy white precipitate.

Cultural Response

Cultural characteristics after18-24 hours at 35-37°C.

Organisms (ATCC)	Recovery on MacConkey Agar AM1059 / AM5059	Colour of Colony
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Colourless
Shigella dysenteriae(13313)	Poor to good	Colourless
Escherichia coli (25922)	None to poor	Pink with bile precipitate

Procedure

1. After preparation, add specimen to each tube.
2. Incubate tubes for 18-24 hours at 35-37°C in an aerobic atmosphere. Interpretation of Results

1. Growth is indicated by turbidity in the medium.
2. Subculture to selective and differential enteric plating media for further investigations.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tinsdale Agar Base AM50963

Use

Tinsdale Agar Base with supplement is used for selective isolation and differentiation of *Corynebacterium diphtheriae*.

Summary

Tinsdale Agar was originally formulated by Tinsdale (110.2) and further modified by Billings (77.1) for selective isolation and differentiation of *Corynebacterium*

diptheriae.

Principle

Peptic digest of animal tissue provides nitrogenous compounds. L-cystein and sodium thiosulphate form H_2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

Corynebacterium diptheriae forms greyish black colonies by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H_2S production from cystein combining with the tellurite salt. Moore and Parsons (80.3) found Tinsdale medium as an ideal medium for the routien cultivation and isolation of *Corynebacterium diptheriae.* They also confirmed the stability of halo formation on clear medium and its specificity for *Corynebacterium diptheriae* and *Corynebacterium ulcerans.* Corynebacterium ulcerans found in nasopharynx form colonies same as *Corynebacterium diptheriae* and require further biochemical testing.

Do not incubate the plate in 5-10 % CO_2 as it retards the development of characteristic halos (69).

Formula*

Ingredients in grams per liter

Peptic digest of animal tissue	20.0
Sodium chloride	5.00
L-Cystine	0.24
Sodium thiosulphate	0.34
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.7 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat to boiling to dissolve the medium completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 50°C and aseptically and Diphtheria Virulence Supplement (Part A & B, AS0091)
6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured opalescent gel.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Corynebacterium diptheriae			
type gravis	Good-luxuriant	Brown to black with halo	More than 70%
type intermedius	Good-luxuriant	Brown to black with halo	More than 70%
type mitis	Good-luxuriant	Brown to black with halo	More than 70%
Streptococcus pyogenes (19615)	Good	Black pin point, without halo	More than 70%
Klebsiella pneumoniae (13883)	Inhibited	–	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Todd Hewitt Broth AM10964/AM50964

Use

Todd Hewitt Broth is recommended for the Cultivation of group A haemolytic *Streptococci* used for serological studies.

Summary

Todd hewitt Broth was originally formulated by Todd and Hewitt (112.1) for the haemolysin production by *Streptococci.* Updyke and Nickle (112.2) modified it for the cultivation of beta-haemolytic Streptococci used for fluorescent antibody procedures (80.4, 49.4) and serotyping based on M protein production (77.1).

Principle

The medium is very nutritious due to the presence of peptic digest of animal tissue and beef heart infusion. Dextrose stimulates haemolysin production. This medium is well buffered by sodium phosphate and sodium carbonate to neutralize the acid produced during dextrose fermentation. This restricts destruction of antigenic streptococcal haemolysin. It is also found that sodium phosphate have a stimulating effect on the pneumococcal growth. Todd and Hewitt broth can be employed as an alternative to serum broth or horse flesh digest broth for the cultivation of streptococci prior to serological typing.

Formula*

Ingredients Gms/Liter

Beef heart, infusion form	500.00

Peptic digest of animal tissue	20.00
Dextrose	2.00
Sodium chloride	2.00
Disodium phosphate	0.40
Sodium carbonate	2.50

Final pH (at 250C) 7.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 37.0 gms of the powder in 1000 ml distilled water.
2. Mix well and dispense as desired.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance:

Medium amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth
Neisseria meningitidis (13090)	Good to luxuriant
Streptococcus pneumoniae (6303)	Good to luxuriant
Streptococcus pyogenes (19615)	Good to luxuriant
Streptococcus mitis (9895)	Good to luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tomato Juice Agar AM1097/AM5097

Tomato Juice Agar, Special AM1098/AM5098

Use

Tomato Juice Agar and Tomato Juice Agar, Special are used for the cultivation and enumeration of *Lactobacillus* species. Tomato Juice Agar Special is also used for the cultivation and enumeration of acidophilic microorganisms from clinical and non-clinical specimens.

Summary

Mickle and Breed (80) first reported the use of tomato juice in culture media for lactobacilli. Kulp (64) investigated the use of tomato juice on bacterial development and found that the growth of *L.acidophilus* was enhanced. Kulp and White (63) formulated Tomato Juice Agar, a modification of the original formula, which yields high counts of lactobacilli from foodstuffs and clinical specimens.

Tomato Juice Agar Special is a formulation of Jay (47) and is recommended for the direct plate counts of lactobacilli from saliva and acidophilic organisms in foodstuffs. The acidic pH of Tomato Juice Agar Special enhances growth of lactobacilli while inhibiting growth of accompanying bacteria. The number of lactobacilli in saliva is an index of a predisposition to dental caries as described by Jay. Many dentists use the direct count of lactobacilli for the diagnosis of caries. This medium is more selective for lactobacilli than Tomato Juice Agar.

Principle

Peptone provides nitrogen, amino acids and carbon. Tomato juice provides carbon, proteins and other nutrients. Peptonized milk contains lactose, which is the energy source.

Formula*

Ingredients in grams per liter	Tomato Juice Agar	Tomato Juice Agar Special
Tomato Juice (from 400 ml)	20.0	20.0
Peptonized Milk	10.0	10.0
Peptone	10.0	10.0
Agar	11.0	20.0
Final pH (at 25°C)	6.1 ± 0.2	5.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.
   Tomato Juice Agar - 51 gms
   Tomato Juice Agar, Special - 60 gms
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Avoid overheating Tomato Juice Agar, Special as it could cause a softer medium.

Quality Control

Dehydrated Appearance

Yellow coloured, free-flowing, homogeneous powder.

Prepared Appearance

Medium to dark amber coloured, very slightly opalescent gel.

Cultural Response

Cultural characteristics after 40-48 hours at 35 ± 2 °C

Organisms (ATCC)	Growth on Tomato Juice Agar	Growth on Tomato Juice Agar, Special
Lactobacillus casei (9595)	Luxuriant	Luxuriant
Lactobacillus acidophilus (4356)	Luxuriant	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Triple Sugar Iron Agar AM1099/AM5099

Use

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Summary

Sulkin and Willett originally developed Triple Sugar Iron Agar, which was later modified by Hajna by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose fermenting bacilli as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube.

The medium complies with the recommendations of APHA for examination of food (20), dairy (39), water and wastewater (36) and for microbial limit test (46, 114) in confirming the presence of *Salmonella* and in the identification of gram-negative bacilli. Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing (113).

Principle

Tryptone, peptone, yeast extract and beef extract provides nitrogenous compounds, sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous sulphate make the H_2S indicator system. Phenol red is the pH indicator.

Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose). A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose. A red colour in the slant and butt indicates that the organism being tested is a non-fermenter.

Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment. Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI Agar suppresses the enzyme pathway that results in H_2S production. Splitting and cracking of the medium indicates gas production.

Formula*

Ingredients in grams per liter

Lactose	10.0
Sucrose	10.0
Peptone	10.0
Tryptone	10.0
Sodium Chloride	5.0
Yeast Extract	3.0
Beef Extract	3.0
Dextrose	1.0
Sodium Thiosulphate	0.3
Ferrous Sulphate	0.2
Phenol Red	0.024
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 64.52 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.
6. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella serotype Typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-

Key:

A = acidic, yellow

K = alkaline, no change

+ = blackening (H_2S), positive reaction

- = no reaction

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulphide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

1. Compare reactions produced by the unknown isolate with those produced by the known control organisms.
2. Carbohydrate fermentation is indicated by a yellow colouration of the medium. If the medium in the butt of the tube becomes yellow (acidic), while the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose.
3. A yellow (acidic) colour in the slant and butt indicates that the organisms being tested ferment dextrose, lactose and/or sucrose.
4. A red (alkaline) colour in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulphide production results in a black precipitate in the butt of the tube.
6. Splitting and cracking of the medium indicates gas production.

Precautions / Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive Salmonellae are positive on Triple Sugar Iron Agar.
4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as *Proteus* species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Triple Sugar Iron Agar IP

AM10991/AM50991

Use

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Summary

Sulkin and Willett (105.2) originally developed Triple Sugar Iron Agar, which was later modified by Hajna (40) by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose fermenting bacilli as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube. The medium complies with the recommendations of APHA for examination of food (115.1), dairy, water and wastewater and for microbial limit test in confirming the presence of Salmonella (31.1, 36.1) and in the identification of gram-negative bacilli (77.1). Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing.

Principle

Peptone, yeast extract and beef extract provides nitrogenous compounds,

sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous sulphate make the H_2S indicator system. Phenol red is the pH indicator. Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose). A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose. A red colour in the slant and butt indicates that the organism being tested is a non-fermenter. Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment.

Some members of the Enterobacteriaceae and H_2S producing Salmonella may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI

Agar suppresses the enzyme pathway that results in H_2S production. Splitting and cracking of the medium indicates gas production.

Formula*

Ingredients in grams per liter

Lactose	10.0
Sucrose	10.0
Peptone	20.0
Sodium chloride	5.0
Yeast extract	3.0
Beef extract	3.0
Dextrose monohydrate	1.0
Ferrous sulphate	0.2
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 64.52 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.
6. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella serotype Typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-

Key:

A = acidic, yellow

K = alkaline, no change

+ = blackening (H_2S) , positive reaction

- = no reaction

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulphide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

1. Compare reactions produced by the unknown isolate with those produced by the known control organisms.
2. Carbohydrate fermentation is indicated by a yellow colouration of the medium. If the medium in the butt of the tube becomes yellow (acidic), while the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose.
3. A yellow (acidic) colour in the slant and butt indicates that the organisms being tested ferment dextrose, lactose and/or sucrose.
4. A red (alkaline) colour in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulphide production results in a black precipitate in the butt of the tube.

6. Splitting and cracking of the medium indicates gas production.

Precautions / Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive Salmonellae are positive on Triple Sugar Iron Agar.
4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as Proteus species.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Triple Sugar Iron Agar USP

AM10992/AM50992

Use

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production in complaince with USP.

Summary

Sulkin and Willett (105.2) originally developed Triple Sugar Iron Agar, which was later modified by Hajna (40) by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose fermenting *bacilli* as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube. The medium complies with the recommendations of APHA for examination of food (115.1), dairy, water and wastewater and for microbial limit test in confirming the presence of Salmonella (31.1, 36.1) and in the identification of gram-negative bacilli (77.1). Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing.

Principle

Pancreatic Digest of Casein and Pancreatic Digest of animal tissue provides nitrogenous compounds, sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous sulphate make the H_2S indicator system. Phenol red is the pH indicator. Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose). A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose. A red colour in the slant and butt indicates that the organism being tested is a non-fermenter.

Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment. Some members of the Enterobacteriaceae and H_2S producing *Salmonella* may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI Agar suppresses the enzyme pathway that results in H_2S production. Splitting and cracking of the medium indicates gas production.

Formula*

Ingredients in grams per liter

Lactose	10.0
Sucrose	10.0
Pancreatic digest of casein	10.0
Pancreatic digest of animal tissue	10.0
Sodium chloride	5.0
Dextrose	1.0
Sodium thiosulphate	0.2
Ferrous ammonium sulphate	0.2
Phenol red	0.025
Agar	13.0

Final pH (at 25^{0}C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 59.42 gms of the powder in 1000 ml distilled water.

2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.
6. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Enterobacter aerogenes(13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae(13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella serotype Typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-

Key:

A = acidic, yellow

K = alkaline, no change

+ = blackening (H_2S) , positive reaction

- = no reaction

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulphide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

1. Compare reactions produced by the unknown isolate with those produced by the known control organisms.
2. Carbohydrate fermentation is indicated by a yellow colouration of the medium. If the medium in the butt of the tube becomes yellow (acidic), while the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose.
3. A yellow (acidic) colour in the slant and butt indicates that the organisms being tested ferment dextrose, lactose and/or sucrose.
4. A red (alkaline) colour in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulphide production results in a black precipitate in the butt of the tube.
6. Splitting and cracking of the medium indicates gas production.

Precautions / Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive Salmonellae are positive on Triple Sugar Iron Agar.
4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as Proteus species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Triple Sugar Iron Agar EP — AM10993/AM50993

Use

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production in compliance with EP.

Summary

Sulkin and Willett (105.2) originally developed Triple Sugar Iron Agar, which was later modified by Hajna (40) by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased

the sensitivity of the medium by facilitating the detection of sucrose fermenting *bacilli* as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube. The medium complies with the recommendations of APHA for examination of food (115.1), dairy, water and wastewater and for microbial limit test in confirming the presence of *Salmonella* (31.1, 36.1) and in the identification of gram-negative bacilli (77.1). Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing .

Principle

Tryptone, peptone, yeast extract and beef extract provides nitrogenous compounds, sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose,

sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous sulphate make the H_2S indicator system. Phenol red is the pH indicator. Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose). A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose. A red colour in the slant and butt indicates that the organism being tested is a non-fermenter. Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment.

Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI

Agar suppresses the enzyme pathway that results in H_2S production. Splitting and cracking of the medium indicates gas production.

Formula*

Ingredients in grams per liter

Lactose monohydrate	10.0
Sucrose	10.0
Peptone (casein & beef)	20.0
Sodium chloride	5.0
Yeast extract	3.0
Beef extract	3.0
Glucose monohydrate	1.0
Sodium thiosulphate	0.3
Ferric ammonium citrate	0.3
Phenol red	0.025
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 64.63 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.
6. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S	RGI
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-	More than 70%
Escherichia coli (25922)	Luxuriant	A	A	+	-	More than 70%
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	-	More than 70%
Proteus vulgaris (13315)	Luxuriant	K	A	-	+	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	K	A	+	+	More than 70%
Shigella flexneri (12022)	Luxuriant	K	A	-	-	More than 70%

Key:
A = acidic, yellow
K = alkaline, no change
+ = blackening (H2S) , positive reaction
- = no reaction

For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for

carbohydrate fermentation, gas production and hydrogen sulphide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

1. Compare reactions produced by the unknown isolate with those produced by the known control

   organisms.
2. Carbohydrate fermentation is indicated by a yellow colouration of the medium. If the medium in the butt of the tube becomes yellow (acidic), while the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose.
3. A yellow (acidic) colour in the slant and butt indicates that the organisms being tested ferment dextrose, lactose and/or sucrose.
4. A red (alkaline) colour in the slant and butt indicates that the organism being tested is a non- fermenter.
5. Hydrogen sulphide production results in a black precipitate in the butt of the tube.
6. Splitting and cracking of the medium indicates gas production.

Precautions / Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive Salmonellae are positive on Triple Sugar Iron Agar.
4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as Proteus species.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Triple Sugar Iron Agar BIS — AM10994/AM50994
Triple Sugar Iron Agar ISO — AM50995

Use

Triple Sugar Iron Agar is used for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Summary

Sulkin and Willett (105.2) originally developed Triple Sugar Iron Agar, which was later modified by Hajna (40) by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar. The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose fermenting *bacilli* as well as lactose and/or dextrose fermenters. Acid and gas production is an indication of carbohydrate fermentation, which gives a visible colour change from red to yellow due to change in the phenol red indicator. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube. The medium complies with the recommendations of APHA for examination of food (115.1), dairy, water and wastewater and for microbial limit test in confirming the presence of *Salmonella* (31.1, 36.1) and in the identification of gram-negative bacilli (77.1). Triple Sugar Iron Agar is also included in the Bacteriological Analytical Manual for food and cosmetics testing.

Principle

Peptone, yeast extract and beef extract provides nitrogenous compounds, sulphur, trace elements, vitamin B complex, etc. while sodium chloride maintains the osmotic equilibrium. Lactose, sucrose and glucose are the fermentable carbohydrates. Sodium thiosulphate and Iron (III) citrate make the H_2S indicator system. Phenol red is the pH indicator. Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. More amounts of acids are liberated in the butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amount of acid present in the butt, therefore, if the medium in the butt of the tube becomes yellow (acidic) while the medium in the slant becomes red (alkaline) the organism being tested only ferments dextrose (glucose). A yellow colour in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose. A red colour in the slant and butt indicates that

the organism being tested is a non-fermenter. Hydrogen sulphide results in a black precipitate in the butt of the tube because reduction of thiosulphate proceeds in an acid environment.

Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on Triple Sugar Iron Agar (may be H_2S positive on Kligler Iron Agar) because utilization of sucrose in TSI

Agar suppresses the enzyme pathway that results in H_2S production. Splitting and cracking of the medium indicates gas production.

Formula*

Ingredients in grams per liter

Lactose	10.0
Sucrose	10.0
Peptone	20.0
Sodium chloride	5.0
Yeast extract	3.0
Meat extract	3.0
Glucose	1.0
Sodium thiosulphate	0.3
Iron (III) citrate	0.3
Phenol red	0.024
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 64.52 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.
6. Allow the medium to set in sloped form with a butt about 1 inch long.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Pinkish red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Slant	Butt	Gas	H_2S
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae(13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella serotype Typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-

Key:
A = acidic, yellow
K = alkaline, no change
+ = blackening (H_2S) , positive reaction
- = no reaction
For growth RGI should be more than 70%
RGI- Relative Growth Index

Procedure

1. Touch only the center of an isolated colony on an enteric plated medium with a cool and sterile needle, stab into the medium and then streak back and forth along the surface of the slant.
2. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
3. Incubate at 35°C with caps loosened and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulphide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

1. Compare reactions produced by the unknown isolate with those produced by the known control organisms.
2. Carbohydrate fermentation is indicated by a yellow colouration of the medium. If the medium in the butt of the tube becomes yellow (acidic), while the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose.
3. A yellow (acidic) colour in the slant and butt indicates that the organisms being tested ferment dextrose, lactose and/or sucrose.
4. A red (alkaline) colour in the slant and butt indicates that the organism being tested is a non-fermenter.
5. Hydrogen sulphide production results in a black precipitate in the butt of the tube.
6. Splitting and cracking of the medium indicates gas production.

Precautions / Limitations

1. It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar because while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Caps must be loosened during this test or erroneous results will occur.
2. Triple Sugar Iron Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.
3. Hydrogen sulphide production may be evident on Kligler Iron Agar but

negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanism responsible for H_2S production. Not all H_2S positive Salmonellae are positive on Triple Sugar Iron Agar.

4. Sucrose is added to Triple Sugar Iron Agar to eliminate some sucrose fermenting, lactose non-fermenters such as Proteus species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Salt Broth — AM50996/AM50996-5K

Use

Tryptone Salt Broth is recommended for preparation of specimens, stock suspensions and decimal dilutions for the purpose of microbiological tests of food specimens.

Summary

Tryptone Salt Broth is recommended by ISO Committee (46.6) for preparation of specimens, stock suspensions and decimal dilutions used in various microbiological tests of food specimens.

Principle

Casein enzymic hydrolysate provides nitrogenous compounds and other essential growth nutrients. Sodium chloride maintains the osmotic equilibrium. For ten-fold serial dilutions, dispense the diluents in volume necessary for the preparation of the decimal dilutions into test tubes or flasks in quantities such that after sterilization each tube or flask contains 9.0 ml. Transfer 1 ml of the initial suspension by means of a pipette into a tube containing 9 ml of sterile diluent at the appropriate temperature. For optimal precision, avoid any contact between the pipette containing the inoculum and the sterile diluent. Mix thoroughly to obtain dilutions until the appropriate number of microorganisms has been obtained.

Formula*

Ingredients in grams per liter	Ingredients Gms/Liter
Casein enzymic hydrolysate	1.00
Sodium chloride	8.50

Final pH (at 25°C) 7.0±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 9.5 grams in 1000 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Mix well and dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth
Escherichia coli (25922)	Luxuriant
Salmonella Typhimurium (14028)	Luxuriant
Staphylococcus aureus (25923)	Luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptic Digest Broth — AM1100/AM5100

Use

Tryptic Digest Broth is used for the cultivation of fastidious microorganisms.

Summary

Tryptic Digest Broth was formulated by Field (31). It may be supplemented with serum, blood or ascitic fluid to support the growth of fastidious organisms such as *Neisseria meningitidis, Haemophilus influenzae,* and *Streptococcus pneumoniae,* etc. Shepard et al described its use for culturing *Streptococcus pneumoniae* and *Actinomycetes.*

Principle

Tryptic digest of beef heart provides carbon, nitrogen and other growth nutrients. Blood, serum or ascitic fluid provides additional growth factors. Sodium chloride maintains the osmotic equilibrium.

Formula*

Ingredients in grams per liter	
Tryptic Digest of Beef Heart	10.0
Sodium Chloride	5.0

Dextrose	1.0

Final pH (at 25°C) 7.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 16 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool below 50°C and add sterile enrichment such as blood, serum or ascitic fluid as required.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured clear solution.

With addition of blood - Cherry red coloured clear to slightly opalescent solution.

With addition of serum or ascitic fluid - Dark yellow coloured clear to slightly opalescent solution.

Cultural Response

Cultural characteristics after 24-48 hours at 35°C.

Organisms (ATCC)	Growth	RGI
Haemophilus influenzae (35056)	Luxuriant	More than 70%
Neisseria meningitidis (13090)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Luxuriant	More than 70%
Streptococcus pyogenes (19615)	Luxuriant	More than 70%

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Agar — AM11001/AM51001

Use

Tryptone agar is a general-purpose medium for the growth of non-fastidious microorganisms.

Summary

Tryptone Agar is a general-purpose agar medium, containing Tryptone, which will support the growth of a wide variety of microorganisms. It is suitable for the cultivation of both aerobes and anaerobes. With the addition of 7% sterile blood to the medium in molten state cooled to approximately 45° C, the medium serves as a good blood agar base. The medium can also be used for the preparation of chocolate agar.

Principle

Tryptone in the medium is a source of nitrogen and carbon. Sodium Chloride maintains the osmotic equilibrium. Agar is the solidification agent.

Formula*

Ingredients in grams per liter

Tryptone	10.0
Sodium Chloride	8.0
Agar	15.0

Final pH (at 25° C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 33.0 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121° C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37° C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Good to luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Good to luxuriant	More than 70%
Staphylococcus aureus (25923)	Good to luxuriant	More than 70%

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Glucose Beef Extract Agar — AM11002/AM51002

Use

Tryptone Glucose Beef Extract Agar (TGB) is used for the enumeration of bacteria in water, air, milk and dairy products.

Summary

Accurate methods are essential in the determination of the number of bacteria found in milk. The composition of medium is the most important factor that

affects this accuracy. Bowers and Huckers (8) originally developed Tryptone Glucose Extract Agar, which was initially called Tryptone Glucose Skim Milk Agar. It was later modified to the present composition for the cultivation and enumeration of bacteria in air, water, milk and dairy products. Tryptone Glucose Beef Extract Agar has been used for the study of various aspects like study of thermophilic bacteria in milk, influence of incubation temperature etc (122.1).

Tryptone Glucose Beef Extract Agar used for the standard plate count of milk and ice cream has been adopted by the committee on standard Methods for the examination of dairy products (84.5). It is also recommended in Compendium of methods for the Microbiological Examination of Foods for performing the heterotrophic plate count procedure in testing bottled water (20).

Principle

Tryptone, Beef extract and glucose supply nutrients, amino acids, carbon compounds, carbohydrates, minerals and trace elements. Glucose is the energy source.

Formula*

Ingredients in grams per liter

Tryptone	5.0
Beef extract	3.0
Glucose	1.0
Agar	15.0

Final pH (at 25°C) 7.0 ±0.2

Formula adjusted to suit performance parameters

Directions

1. Suspend the 24 gms of powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Bacillus subtilis (6633)	Good – luxuriant	More than 70%
Enterobacter aerogenes (13048)	Good – luxuriant	More than 70%
Escherichia coli (25922)	Good – luxuriant	More than 70%
Lactobacillus casei (9595)	Good – luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Good – luxuriant	More than 70%
Staphylococcus aureus (25923)	Good – luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Glucose Extract Agar AM1101/AM5101

Tryptone Glucose Extract Broth AM1102/AM5102

Use

Tryptone Glucose Extract Agar is used for the enumeration of bacteria in water, air, milk and dairy products while Tryptone Glucose Extract Broth is used as a general purpose enrichment medium for a wide variety of microorganisms.

Summary

Bowers and Huckers (8) originally developed Tryptone Glucose Extract Agar, which was initially called Tryptone Glucose Skim Milk Agar. It was later modified to the present composition for the cultivation and enumeration of bacteria in air, water, milk and dairy products. Tryptone Glucose Extract Agar has been used for the study of various aspects like study of thermophilic bacteria in milk, influence of incubation temperature etc. It is used as a standard medium for the bacteriological plate count of milk and dairy products.

Tryptone Glucose Extract Agar is recommended by APHA for the enumeration of microorganisms in milk (39) during microbiological examination of food material by MPN technique (20).

Principle

Tryptone, yeast extract and glucose supply nutrients, amino acids, carbon compounds, carbohydrates, minerals and trace elements. Glucose is the energy source. Dipotassium phosphate is the buffer.

Formula*

Ingredients in grams per liter	Tryptone Glucose Extract Agar	Tryptone Glucose Extract Broth
Tryptone	5.0	10.0
Yeast Extract	3.0	1.0
Glucose	1.0	5.0
Dipotassium Phosphate	-	1.25
Agar	15.0	-
Final pH (at 25°C)	7.0 ± 0.2	6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water and mix thoroughly.

Tryptone Glucose Extract Agar - 24 gms

Tryptone Glucose Extract Broth - 17.25 gms

2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Tryptone Glucose Extract Agar - Light yellow coloured, clear to slightly opalescent gel.

Tryptone Glucose Extract Broth - Light yellow coloured clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth on Tryptone Glucose Extract Agar and in Tryptone Glucose Extract Broth	RGI
Bacillus subtilis (6633)	Luxuriant	More than 70%
Enterobacter aerogenes (13048)	Luxuriant	More than 70%
Enterococcus faecalis (29212)	Luxuriant	More than 70%
Escherichia coli (25922)	Luxuriant	More than 70%
Lactobacillus casei (9595)	Luxuriant	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Phosphate Broth AM1103/AM5103

Use

Tryptone Phosphate Broth is used for the enrichment of enteropathogenic *E.coli.*

Summary

Tryptone Phosphate Broth is recommended by APHA (20) and is also included in the Bacteriological Analytical Manual for enriching pathogenic *E.coli* (113).

Principle

Tryptone is the source of nitrogen. Polysorbate 80 provides the fatty acids required for bacterial metabolism. The inorganic phosphates serve as the buffer while sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams/liter

Tryptone	20.0
Sodium Chloride	5.0
Monopotassium Phosphate	2.0
Dipotassium Phosphate	2.0
Polysorbate 80	1.50

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 30.5 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Dispense in 100 ml aliquots.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light amber coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Soya Agar with Lecithin and Tween 80 AM11031/AM51031

Use

Tryptone soya agar with lecithin and tween 80 is used for detection of microorganisms on surfaces sanitized with quaternary ammonium compounds.

Summary

Tryptone soya agar with lecithin and tween 80 is recommended for validation of cleanliness on surface of containers, equipment surfaces and water miscible cosmetics.

For the microbiological examination of surfaces RODAC (Replicate Organism Detection and Counting) and surface plates are used (115.4). Microbiological

examination of surfaces before and after treatment with disinfectant provides data about cleanliness, which is used for validation of cleaning procedures in environmental sanitation (9.2).

Principle

Casein enzymic hydrolysate and papaic digest of soyabean meal serves as a source of nitrogen. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Lecithin and tween 80 inactivates disinfectant. Lecithin neutralizes quaternary ammonium compounds and tween 80 neutralizes substituted phenolic disinfectant. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

TCasein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Sodium chloride	5.00
Lecithin	0.70
Polysorbate 80 (Tween 80)	5.00
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 45.7 gms of the powder in 1000 ml distilled water
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool the medium to approximately 45-50°C, pour in to sterile petriplates.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Light to slightly amber, clear to slightly opalescent gel, may have a precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	RGI
Staphylococcus aureus (6538)	Luxuriant	Yellow to gold	More than 70%
Pseudomonas aeruginosa (27853)	Luxuriant	Yellow to green	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

To Check Neutralizing activity of the medium:

- Prepare the medium as per label directions.
- Test the medium in parallel with Plate Count Agar (AM1081/AM5018), by using the pour plate method.
- Apply disks impregnated with varying dilutions of quaternary ammonium compound to the medium surface.
- Incubate plates at 35-370C for 40-48 hours and inspect for zone of inhibition.

Organism (ATCC)	Growth*
Escherichia coli (11229)	Smaller zone of inhibition of growth compare to Plate Count Agar
Staphylococcus aureus (6538)	Smaller zone of inhibition of growth compare to Plate Count Agar

* Interpretation- The smaller zone of inhibition indicate neutralization of the quaternary ammonium compound by the medium.

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies.
2. If the specimen to be cultured is on a swab, roll the swab on a small area of the agar surface and streak for isolation with a sterile loop.
3. Incubate plates aerobically, protected from light, at 35-37°C for 18-24 hours or as required.
4. After incubation count the colonies.
5. Examine colony morphology and carry out biochemical testing for identification.

Interpretation of Results

1. Count all developing colonies.
2. Interpretation of results are relative, each laboratory should establish its own values for cleanness and compare the counts for results.

Limitations

1. Neutralization of disinfectant depends on its concentration and type.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Soya Yeast Extract Agar ISO — AM51032

Use

Tryptone Soya Yeast Extract Agar is recommended for confirmation of *Listeria* in Henry's light, in compliance with ISO specifications ISO 10560: 1993.

Summary

Listeriosis has been recognized as an important public health problem. *Listeria monocytogenes* is the causative agent of listeriosis. Tryptone Soya Yeast Extract Agar is recommended by APHA (115.1) for the isolation and cultivation of *Listeria monocytogenes* from foods. ISO Committee (46.2)has recommended these media for confirmation of Listeria species.

Principle

Casein enzymic hydrolysate, papaic digest of soyabean meal and yeast extract provide the carbon, nitrogen and other growth factors. Dextrose is the source of energy. Dipotassium hydrogen phosphate acts as a buffering agent.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Sodium chloride	5.00
Lecithin	0.70
Polysorbate 80 (Tween 80)	5.00
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 51 grams of the powder in 1000 ml of Distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs) for 15 minutes

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear gel forms in petri plates.

Cultural Response

Cultural characteristics after after 24-48 hours at 30°C.

Organisms (ATCC)	Growth	RGI
Listeria monocytogenes (19111)	Good to luxuriant	More than 70%
Listeria monocytogenes (19118)	Good to luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Soya Yeast Extract Broth ISO — AM51033

Use

Tryptone Soya Yeast Extract Broth is recommended for confirmation of *Listeria* in Henry's light, in compliance with ISO specifications ISO 10560: 1993.

Summary

Listeriosis has been recognized as an important public health problem. *Listeria monocytogenes* is the causative agent of listeriosis. Tryptone Soya Yeast Extract Broth is recommended by APHA (115.1) for the isolation and cultivation of *Listeria monocytogenes* from foods. ISO (46.2) Committee has recommended these media for confirmation of Listeria species.

Principle

Casein enzymic hydrolysate, papaic digest of soyabean meal and yeast extract provide the carbon, nitrogen and other growth factors. Dextrose is the source of energy. Dipotassium hydrogen phosphate acts as a buffering agent.

Formula*

Ingredients in grams per liter

Casein enzymic hydrolysate	17.00
Papaic digest of soyabean meal	3.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	2.50
Dextrose	2.50
Yeast extract	6.00

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 36 grams of the powder in 1000 ml of Distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear solutions forms in tubes.

Cultural Response

Cultural characteristics after after 24-48 hours at 30°C.

Organisms (ATCC)	Growth
Listeria monocytogenes (19111)	Good to luxuriant
Listeria monocytogenes (19118)	Good to luxuriant

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Water AM1104/AM5104

Use

Tryptone Water is used for the detection of indole production especially by coliforms.

Summary

Tryptone Water is based on the formula described in an ISO standard, where it is used with Brilliant Green Bile Broth 2% to determine the most probable number (MPN) of *E.coli* present in meat and meat products. Growth and gas production in Brilliant Green Bile Broth 2% and indole production in Tryptone Water followed by incubation of both media at 44 ± 1°C is used as the basis for the presumptive *E.coli* test. Tryptone Water is also used for the differentiation of other bacteria based on indole production and is recommended by APHA (20, 36). It is also included in the Bacteriological Analytical Manual for food testing (113). Pure cultures are used for indole production in tryptophan-containing media to differentiate bacteria and to identify *E.coli* isolated from food and water samples.

Principle

Tryptone is suitable for detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia. Indole can be detected by the addition of either Kovac's or Ehrlich's Reagent, which contains an aldehyde group. The aldehyde group combines with indole to produce a red colour in the alcohol layer. Sodium chloride is added to the medium to provide a suitable osmotic environment.

Formula*

Ingredients in grams per liter

Tryptone	10.0
Sodium Chloride	5.0

Final pH (at 25°C) 7.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 15 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat if necessary to dissolve the powder completely.
4. Dispense in tubes as per requirements.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogenous, free flowing powder.

Prepared Appearance

Yellow coloured clear solution, without any precipitate.

Cultural Response

Cultural characteristics after 24 hours at 37°C.

Organisms	Growth	Indole reaction
Escherichia coli (25922)	Good to luxuriant	+
Enterobacter aerogenes (13048)	Good to luxuriant	-

Procedure

Indole determination using pure cultures.

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 37°C with loosened caps for 24 hours.
3. Add 0.5 ml of Indole Reagent (Kovac's) directly to the tube and agitate.
4. Allow tubes to stand for 5-10 minutes.

Interpretation of Results

1. Examine the tubes for the formation of a red ring at the top of the tube indicating indole production.

Precautions / Limitations

1. Detection of *E.coli* in meats using Tryptone Water is a presumptive test.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. Other biochemical tests need to be performed for complete identification.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptone Water without Sodium Chloride AM110411/AM510411

Use

Tryptone Water without Sodium Chloride is used for the detection of *Vibrio cholerae* and Vibrio parahaemolyticus in compliance with BIS specification IS: 5887 (Part 5) 1976 reaffirmed 1986.

Summary

Tryptone Water without Sodium Chloride, is used for differentiation of *Vibrio cholerae* and *Vibrio parahaemolyticus,* Vibrio cholerae and Vibrio parahaemolyticus isolates food and water samples.

Principle

Tryptone provides all essential growth nutrient.

Formula*

Ingredients in grams per liter

Tryptone	10.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 10 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Sodium chloride in requisite amount is to be added if desired.
4. Dispense in 5 ml amount into sterilized tubes as per requirements.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured clear solution, without any precipitate.

Cultural Response

Cultural characteristics after 24 hours at 35-37°C.

Organisms (ATCC)	Growth
Vibrio cholerae	Good to luxuriant
Vibrio parahaemolyticus	Good to luxuriant

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Tryptose Agar AM11041/AM51041

Use

Tryptose Agar is recommended for the cultivation of *Brucella* species and other fastidious microorganisms.

Summary

Tryptose Agar is prepared with Tryptose, and recommended for the cultivation and isolation of pathogenic and saprophytic bacteria. Historically, it was considered necessary to include meat extract or infusion as a nutritional supplement in culture media. Tryptose was developed while studying growth requirements of *Brucella* species. Huddleson found Tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious microorganisms. Tryptose Agar is particularly well suited for the isolation of *Brucella* from blood.

Principle

Tryptose in the medium serves as a nitrogen source. Dextrose is a source of energy. Sodium Chloride maintains the osmotic equilibrium. Agar is the solidification agent.

Formula*

Ingredients in grams per liter

Tryptose	20.0
Dextrose	1.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.0 grams of the powder in 1000 ml distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated sheep, horse or rabbit blood.
5. Dispense as desired.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48-72 hours at 35-37°C under 10% CO_2.

Organisms (ATCC)	Growth	RGI
Brucella abortus (4315)	Good-luxuriant	More than 70%
Brucella melitensis (4309)	Good-luxuriant	More than 70%
Brucella suis (4314)	Good-luxuriant	More than 70%
Streptococcus pneumoniae (6303)	Good-luxuriant	More than 70%
Streptococcus pyogenes (19615)	Good-luxuriant	More than70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Refer to U.S.P. and other appropriate references for procedure and interpretation of results.

Precautions / Limitations

1. Tryptose Agar is a general-purpose, non-selective medium. Therefore, a number of non-pathogenic, bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.
2. When preparing blood agar, hemolytic reactions of some strains of Group D Streptococci may be effected due to differences in animal blood.
3. The incubation atmosphere may affect the hemolytic reactions of some Beta hemolytic Streptococci. For optimal performance, incubate the medium supplemented with blood under increased CO_2 or anaerobic conditions.
4. Dextrose has been shown to inhibit hemolysin production by some microorganisms.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Universal Beer Agar AM11042

Use

Universal Beer agar is recommended for cultivation of microorganisms related to brewing industry.

Summary

Universal Beer Agar is designed according to the formula developed by Kozulis and Page (61.1) for culturing microorganisms which are significant in the brewing industry. They had suggested that the beer should be added to the medium to stimulate the growth of beer spoilage organisms, thereby increasing the selectivity of the medium. Beer contains hop constituents and ethyl alcohol which eliminates many airborne contaminants and thus help in minimizing false positive results.

Principle

Peptonized milk, yest extract, dextrose and salts provide all essential growth nutrient. Tomato juice gives acidic environment. The organisms which survive or grow in wort and beer during the beer manufacturing can be recovered due to this particular composition of the medium (77.1).

Formula*

Ingredients in grams per liter

Peptonized milk	15.00
Yeast extract	6.10
Dextrose	16.10
Tomato juice	12.20
Dipotassium phosphate	0.31
Monopotassium phosphate	0.31
Magnesium sulphate	0.12
Sodium chloride	0.006
Ferrous sulphate	0.006
Manganese sulphate	0.006
Agar	12.00

Final pH (at 25°C) 6.3 + 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 62.16 grams in 750 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Add 250 ml Beer, without degassing, to the hot medium and mix gently.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 10 minutes.

* If required, add 5 mcg/ml of Amphotercin B to sterile medium to dispensing.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance:

Medium amber coloured, clear to slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after after 40-48 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Acinetobacter calcoaceticus (19606)	Good-luxuriant	More than 70%
Lactobacillus fermentum (9338)	Good-luxuriant	More than 70%
Lactobacillus acidophilus (4356)	Good-luxuriant	More than 70%
Proteus vulgaris (13315)	Fair-good	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Urea Agar Base, Christensen AM1105/AM5105

Use

Urea Agar Base with added urea is used for the detection of urease production, particularly by the genus *Proteus*.

Summary

Christensen (17) devised Urea Agar Base for use as a solid medium for the differentiation of enteric bacilli. It differentiates between rapid urease-positive *Proteeae* organisms (*Proteus* species, *Morganella morganii* subspecies *morganii*, *Providencia rettgeri*, and some *Providencia stuartii*) and other urease-positive organisms: *Citrobacter*, *Enterobacter* and *Klebsiella* and bacteria other than *Enterobacteriaceae*, i.e., some *Bordetella* and *Brucella* species. Urea Agar Base is

included in the Bacteriological Analytical Manual for food and cosmetics testing (113), in IP (46) and is recommended by APHA for the examination of foods (20).

Principle

Rustigian and Stuart (98) had originally formulated Urea Broth to differentiate *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea; the latter were unable to do so because of limited nutrients and the high buffering capacity of the Urea Broth. To provide a medium with greater use Christensen devised Urea Agar Base with the addition of peptone, dextrose and reduced content of buffer to promote rapid growth of many of the *Enterobacteriaceae* and permit a reduction in incubation time.

Formula*

Ingredients in grams per liter

Sodium Chloride	5.0
Disodium Phosphate	1.2
Dextrose	1.0
Peptone	1.0
Monopotassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24 gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 115°C (10 lbs pressure) for 20 minutes.
5. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea (AS028) solution and mix well.
6. Dispense into sterile test tubes and allow to set in a slanting position.
7. Do not reheat the medium after the addition of Urea 40%, as urea decomposes very easily.

Quality Control

Dehydrated Appearance

Yellowish orange coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow orange coloured, clear gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Urease	RGI
Enterobacter aerogenes (13048)	Luxuriant	-	More than 70%
Escherichia coli (25922)	Luxuriant	-	More than 70%
Proteus vulgaris (13315)	Luxuriant	+	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	-	More than 70%

Key :

+ = positive, pink-red colour

- = negative, no change

Procedure

1. Using a heavy inoculum of growth from an 18-24 hour pure culture, inoculate the agar by streaking back and forth over the entire surface of the slant.
2. Do not stab the butt since it serves as a colour control.
3. Incubate tubes with loosened caps at 35°C in an incubator or water bath.
4. Observe reactions after 2, 4, 6, 18, 24 and 48 hours.
5. Continue to check everyday for a total of 6 days; even longer incubation periods may be necessary.

Interpretation of Results

1. When organisms utilize urea, ammonia is formed which makes the medium alkaline, producing an intense pink-red colour on the slant.
2. The colour may penetrate into the agar butt; the extent of colour indicates the rate of urea hydrolysis.
3. A negative reaction is no colour change. The agar medium remains pale yellow to buff.

Precautions / Limitations

1. Do not reheat the medium as urea decomposes very easily.
2. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (*P.aeruginosa* for e.g.) or other proteins, which raises the pH due to protein hydrolysis, and the release of excess amino acid residue. To eliminate possible protein hydrolysis, perform a control test without urea.
3. Urea Agar rapidly detects urease activity of only the urease positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within first 2-6 hours after incubation.
4. Urease positive *Enterobacter, Citrobacter* or *Klebsiella* in contrast, hydrolyze urea much more slowly, showing only slight reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Storage

Store at 22-30°C and prepared medium at 2-8°C

Shelf Life

Use before expiry date as mentioned on the label.

Urea Agar Base, Christensen BIS

AM11051/AM51051

Use

Urea Agar Base with added urea is used for the detection of urease production, particularly by the genus Proteus in compliance with BIS specification IS: 5887 (Part 1), 1976 and IS: 5887 (Part 3): 1999.

Summary

Christensen (17, 77.1) devised Urea Agar Base for use as a solid medium for the differentiation of enteric bacilli. It differentiates between rapid urease-positive Proteeae organisms (*Proteus* species, *Morganella morganii subspecies morganii, Providencia rettgeri*, and *some Providencia stuartii*) and other urease-positive organisms: *Citrobacter, Enterobacter* and *Klebsiella* and bacteria other than Enterobacteriaceae, i.e., some Bordetella and Brucella species. Urea Agar Base is included in the Bacteriological Analytical Manual for food and cosmetics testing, in IP and is recommended by APHA and BIS for the examination of foods.

Principle

Rustigian and Stuart (98) had originally formulated Urea Broth to differentiate Proteus species from other gram-negative enteric bacilli capable of utilizing urea; the latter were unable to do so because of limited nutrients and the high buffering capacity of the Urea Broth. To provide a medium with greater use Christensen devised Urea Agar Base with the addition of peptone, dextrose and reduced content of buffer to promote rapid growth of many of the *Enterobacteriaceae* and permit a reduction in incubation time.

Formula*

Ingredients	Gms/Liter
Sodium chloride	5.0
Glucose	1.0
Peptone	1.0
Potassium dihydrogen phosphate	2.0
Phenol red	0.012
Agar	15.0

Final pH (at 25ºC) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 24.012gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 115ºC (10 lbs pressure) for 20 minutes.
5. Cool to 50ºC and aseptically add 50 ml of sterile 40% Urea (AS028) solution and mix well.
6. Dispense into sterile test tubes and allow to set in a slanting position.
7. Do not reheat the medium after the addition of Urea 40%, as urea decomposes very easily.

Quality Control

Dehydrated Appearance

Yellowish orange coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow orange coloured, clear gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC.

Organisms (ATCC)	Growth	Urease
Enterobacter aerogenes (13048)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	-
Proteus vulgaris (13315)	Luxuriant	+
Salmonella serotype Typhimurium (14028)	Luxuriant	-

Key:

+ = positive, pink-red colour

- = negative, no change

Procedure

1. Using a heavy inoculum of growth from an 18-24 hour pure culture, inoculate the agar by

   streaking back and forth over the entire surface of the slant.
2. Do not stab the butt since it serves as a colour control.
3. Incubate tubes with loosened caps at 35ºC in an incubator or water bath.
4. Observe reactions after 2, 4, 6, 18, 24 and 48 hours.
5. Continue to check everyday for a total of 6 days; even longer incubation periods may be necessary.

Interpretation of Results

1. When organisms utilize urea, ammonia is formed which makes the medium alkaline, producing an

   intense pink-red colour on the slant.
2. The colour may penetrate into the agar butt; the extent of colour indicates the rate of urea hydrolysis.
3. A negative reaction is no colour change. The agar medium remains pale yellow to buff.

Precautions / Limitations

1. Do not reheat the medium as urea decomposes very easily.
2. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (P. aeruginosa for e.g.) or other proteins, which raises the pH due to protein hydrolysis, and the release of excess amino acid

residue. To eliminate possible protein hydrolysis, perform a control test without urea.

3. Urea Agar rapidly detects urease activity of only the urease positive Proteus species. For results to be valid for the detection of Proteus, the results must be read within first 2-6 hours after incubation.
4. Urease positive Enterobacter, Citrobacter or Klebsiella in contrast, hydrolyze urea much more slowly, showing only slight reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Urea Agar Base, Christensen ISO — AM51052

Use

Urea Agar Base with added urea is used for the detection of urease production, particularly by the genus *Proteus* in compliance with ISO.

Summary

Christensen (17, 77.1) devised Urea Agar Base for use as a solid medium for the differentiation of enteric bacilli. It differentiates between rapid urease-positive *Proteeae organisms (Proteus species, Morganella morganii* sub species morganii, Providencia rettgeri, and some Providencia stuartii) and other urease-positive organisms: *Citrobacter, Enterobacter* and *Klebsiella* and *bacteria* other than *Enterobacteriaceae*, i.e., some *Bordetella* and *Brucella* species. Urea Agar Base is included in the Bacteriological Analytical Manual for food and cosmetics testing, in IP and is recommended by APHA for the examination of foods.

Principle

Rustigian and Stuart (98) had originally formulated Urea Broth to differentiate *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea; the latter were unable to do so because of limited nutrients and the high buffering capacity of the Urea Broth. To provide a medium with greater use Christensen devised Urea Agar Base with the addition of peptone, dextrose and reduced content of

buffer to promote rapid growth of many of the Enterobacteriaceae and permit a reduction in incubation time.

Formula*

Ingredients Gms/Liter

Sodium chloride	5.0
Glucose	1.0
Peptone	1.0
Potassium dihydrogen phosphate	2.0
Phenol red	0.012
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. S. Suspend 24.012 gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 115°C (10 lbs pressure) for 20 minutes.
5. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea (AS028) solution and mix well.
6. Dispense into sterile test tubes and allow to set in a slanting position.
7. Do not reheat the medium after the addition of Urea 40%, as urea decomposes very easily.

Quality Control

Dehydrated Appearance

Yellowish orange coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellowish orange coloured, clear gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Urease
Enterobacter aerogenes (13048)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	-
Proteus vulgaris (13315)	Luxuriant	+
Salmonella serotype Typhimurium (14028)	Luxuriant	-

Key:

+ = positive, pink-red colour

- = negative, no change

Procedure

1. Using a heavy inoculum of growth from an 18-24 hour pure culture, inoculate the agar by streaking back and forth over the entire surface of the slant.
2. Do not stab the butt since it serves as a colour control.
3. Incubate tubes with loosened caps at 35°C in an incubator or water bath.
4. Observe reactions after 2, 4, 6, 18, 24 and 48 hours.
5. Continue to check everyday for a total of 6 days; even longer incubation periods may be necessary.

Interpretation of Results

1. When organisms utilize urea, ammonia is formed which makes the medium alkaline, producing an intense pink-red colour on the slant.
2. The colour may penetrate into the agar butt; the extent of colour indicates the rate of urea hydrolysis.
3. A negative reaction is no colour change. The agar medium remains pale yellow to buff.

Precautions / Limitations

1. Do not reheat the medium as urea decomposes very easily.
2. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (*P.aeruginosa* for e.g.) or other proteins, which raises the pH due to protein hydrolysis, and the release of excess amino acid residue. To eliminate possible protein hydrolysis, perform a control test without urea.
3. Urea Agar rapidly detects urease activity of only the urease positive Proteus species. For results to be valid for the detection of Proteus, the results must be read within first 2-6 hours after incubation.
4. Urease positive *Enterobacter, Citrobacter* or *Klebsiella* in contrast, hydrolyze urea much more slowly, showing only slight reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Urea Broth Base AM1106/AM5106

Use

Urea Broth Base with added urea is used for the detection of urease production, to differentiate *Proteus* species from *Salmonella* and *Shigella* species.

Summary

Rustigian and Stuart (98) developed Urea Broth Base for the identification of bacteria on the basis of urea utilization and is particularly recommended for the differentiation of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections. The broth is positive for *Proteus, Morganella morganii* subspecies *morganii, Providencia rettgeri,* and a few *Providencia stuartii* strains with the reclassification of the members of the genus *Proteeae.* Urea Broth Base is included in the Bacteriological Analytical Manual for food and cosmetics testing (113), in IP (46) and is recommended by APHA in the examination of milk (39) and foods (20).

Principle

Yeast extract provides trace elements, vitamins and amino acids. Gram-negative enteric bacilli are unable to utilize urea because of less nutrients and high buffering capacity of the medium. Urea Broth becomes alkaline as utilization of urea by the organisms liberates ammonia during incubation, which is indicated by pink red colour. Since this test relies on the alkalinity formation, it is not specific for urease testing. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids results in false positive reaction.

Formula*

Ingredients in grams per liter

Yeast Extract	0.1
Dipotassium Phosphate	9.5
Monopotassium Phosphate	9.1
Phenol Red	0.01

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 18.7 gms of the powder in 950 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 55°C.
6. Aseptically add 50 ml of sterile 40% Urea (AS028) Solution and distribute 10 ml aliquots into sterile tubes.

Quality Control

Dehydrated Appearance

Light orange coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow orange coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Urease	RGI
Enterobacter aerogenes (13048)	Luxuriant	-	More than 70%
Escherichia coli (25922)	Luxuriant	-	More than 70%
Proteus vulgaris (13315)	Luxuriant	+	More than 70%
Salmonella serotype Typhimurium (14028)	Luxuriant	-	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Key:

+ = Positive, pink-red colour
- = Negative, no change

Procedure

1. Using a heavy inoculum of growth from an 18-24 hour pure culture, inoculate the broth.
2. Shake tubes gently to suspend the bacteria.
3. Incubate tubes with loosened caps at 35 -37^{0}C in an incubator or water bath.
4. Observe reactions after 2, 4, 6, 18, 24 and 48 hours.

Interpretation of Results

1. The production of urease is indicated by an intense pink-red colour throughout the broth.
2. A negative reaction is no colour change. The broth medium remains pale yellow to buff.

Precautions / Limitations

1. It is preferable that the medium be used on the day of preparation. If not, examine the tubes carefully to ensure sterility.
2. Do not reheat the medium after the addition of Urea 40% as urea decomposes very easily.
3. To rule out false-positives due to protein hydrolysis (as opposed to urea hydrolysis) that may occur in the medium after prolonged incubation, perform a control test without urea.
4. The high buffering system in this medium masks urease activity in organisms that are delayed positive. This medium is therefore recommended for the detection of urease activity in all *Proteus* species. *Providencia rettgeri* and urease-positive *Providencia stuartii, M.morganii* slowly hydrolyze urea and may require approximately a 36-hour incubation for strong urease positive reaction to occur. When in doubt as to a result, compare with an un-inoculated tube or incubate for an additional 24 hours.
5. Variations in the size of the inoculum can affect the time required to reach positive results.

Storage

Store at 22-30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Urea Broth IP — AM51061

Use

Urea Broth is recommended for the identification of bacteria on the basis of urea utilization, specifically for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in compliances with IP.

Summary

Urea Broth was developed by Rustigian and Stuart (98). This medium is especially recommended for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in the enteric infection diagnosis, based on urea utilization.

Principle

Gram-negative enteric bacilli are unable to utilize urea because of less nutrients and high buffering capacity of the medium. Urea Broth becomes alkaline as the utilization of urea by the organisms liberate ammonia during the incubation, indicated by pink colour. All urea test media rely on the alkalinity formation and so they are not specific for urease testing.

The utilization of protein may raise the pH to the alkalinity due to protein hydrolysis and excess of amino acids results in false-positive reaction.

Formula*

Ingredients Gms/Liter

Potassium dihydrogen ortho phosphate	9.10
Anhydrous disodium hydrogen phosphate	9.50
Yeast extract	0.10
Phenol red	0.01
Urea	20.00

Final pH (at 25ºC) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38.7 gms of the powder in1000 ml distilled water.
2. Mix well and sterilize by filtration..
3. DO NOT AUTOCLAVE OR HEAT the medium.
4. Dispense into sterile test tubes.

Quality Control

Dehydrated Appearance

Light orange coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow orange coloured, clear solution without any precipitate.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organisms (ATCC)	Growth	Urease
Enterobacter aerogenes (13048)	Luxuriant	-
Escherichia coli (25922)	Luxuriant	-
Klebsiella pneumoniae (13883)	Luxuriant	+
Proteus vulgaris (13315)	Luxuriant	+
Salmonella serotype Typhimurium (14028)	Luxuriant	-

Key:
+ = Positive, cerise colour
- = Negative, no change

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Agar AM1107/AM5107

Use

Violet Red Bile Agar is a selective medium used for the detection and enumeration of coliforms.

Summary

Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliforms in water (36), dairy (39) and other food products (20). Druce *et al* (21) found that this medium was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey Broth.

Principle

Peptone provides nitrogen and carbon required for growth while yeast extract supplies B complex vitamins. Bile salts mixture and crystal violet inhibit gram-positive organisms especially *staphylococci* and makes the medium selective. Sodium chloride maintains the osmotic equilibrium. Lactose is the carbohydrate source and neutral red is the pH indicator. Organisms, which rapidly ferment lactose, produce red colonies surrounded by red-purple haloes. Lactose non-fermenters and late lactose fermenters produce pale colonies. Other related gram-negative bacteria can be suppressed by incubation greater than 42°C or by anaerobic incubation.

Formula*

Ingredients in grams per liter

Lactose	10.0
Peptone	7.0
Sodium Chloride	5.0
Yeast Extract	3.0
Bile Salts Mixture	1.5
Neutral Red	0.03
Crystal Violet	0.002
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C, and pour into sterile petri plates containing the inoculum.

Quality Control

Dehydrated Appearance

Beige coloured homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink	More than 70%
Escherichia coli (25922)	Luxuriant	Pinkish red with bile precipitate.	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

Presumptive test for coliforms

1. Transfer 1 ml aliquot of test sample to a petri plate.
2. Add 10 ml of Violet Red Bile Agar cooled to 45°C and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18-24 hours. For dairy products, incubate at 32°C.
4. Do confirmatory testing of typical coliform colonies.
5. An overlay method is helpful to improve the specificity of the medium. A thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation can be carried out at greater than 42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days, depending on the temperature characteristics of the organism to be recovered. For *E.coli*, a temperature of 44°C is specifically recommended.

Interpretation of Results

1. Lactose fermenters including coliforms form purple red colonies, with or without a zone of precipitate around the colonies. (Generally surrounded by a reddish zone of precipitated bile)

2. Non-lactose fermenters form colourless to transparent colonies.
3. Gram-positive cocci, if present, form colourless, pinpoint colonies.

Precautions / Limitations

1. Boiling the medium for more than 2 minutes may decrease the ability to support growth.
2. Do not incubate inoculated plates for more than 24 hours because microorganisms that are only slightly inhibited may grow after extended incubation.
3. Prepare and use the medium within 24 hours for optimum performance.
4. This medium may not be completely inhibitory to gram-positive organisms. *Enterococci* may grow as pinpoint colonies. Perform gram stain and biochemical tests to identify isolates.
5. Gram-negative bacilli other than *Enterobacteriaceae* may also grow. Perform biochemical tests to identify isolates to genus and species.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Agar BIS — AM11071/AM51071

Use

Violet Red Bile Agar is a selective medium used for the detection and enumeration of coliforms in compliance with BIS.

Summary

Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliforms in water (36), dairy (39) and other food products (20). Druce *et al.*, (21)found that this medium was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey Broth. Recently the agar formulation is recommended by ISO committee for the enumeration of coliforms (5).

Principle

Peptone provides nitrogen and carbon required for growth while yeast extract supplies B complex vitamins. Bile salts mixture and crystal violet inhibit gram-positive organisms especially staphylococci and makes the medium selective. Sodium chloride maintains the osmotic equilibrium. Lactose is the carbohydrate source and neutral red is the pH indicator. Organisms, which rapidly ferment lactose, produce red colonies surrounded by red-purple haloes. Lactose non-fermenters and late lactose fermenters produce pale colonies. Other related gram-negative bacteria can be suppressed by incubation greater than 42°C or by anaerobic incubation.

Formula*

Ingredients in grams per liter

Lactose	10.0
Peptone	7.0
Sodium chloride	5.0
Yeast extract	3.0
Bile salts mixture	1.5
Neutral red	0.03
Crystal violet	0.002
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C, and pour into sterile petri plates containing the inoculum.

Quality Control

Dehydrated Appearance

Beige coloured homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink	More than 70%
Escherichia coli (25922)	Luxuriant	Pinkish red with bile precipitate.	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

Presumptive test for coliforms

1. Transfer 1 ml aliquot of test sample to a petri plate.
2. Add 10 ml of Violet Red Bile Agar cooled to 45°C and swirl to mix.

3. Allow medium to solidify before incubating at 35-37°C for 18-24 hours. For dairy products, incubate at 32°C.
4. Do confirmatory testing of typical coliform colonies.
5. An overlay method is helpful to improve the specificity of the medium. A thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation can be carried out at greater than 42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days, depending on the temperature characteristics of the organism to be recovered. For E.coli, a temperature of 44°C is specifically recommended.

Interpretation of Results

1. Lactose fermenters including coliforms form purple red colonies, with or without a zone of precipitate around the colonies. (Generally surrounded by a reddish zone of precipitated bile)
2. Non-lactose fermenters form colourless to transparent colonies.
3. Gram-positive cocci, if present, form colourless, pinpoint colonies.

Precautions / Limitations

1. Boiling the medium for more than 2 minutes may decrease the ability to support growth.
2. Do not incubate inoculated plates for more than 24 hours because microorganisms that are only slightly inhibited may grow after extended incubation.
3. Prepare and use the medium within 24 hours for optimum performance.
4. This medium may not be completely inhibitory to gram-positive organisms. Enterococci may grow as pinpoint colonies. Perform gram stain and biochemical tests to identify isolates.
5. Gram-negative bacilli other than Enterobacteriaceae may also grow. Perform biochemical tests to identify isolates to genus and species.

Storage

Store 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Agar (1.2%) ISO — AM510711

Use

Violet Red Bile Agar (1.2%) ISO is a selective medium used for the detection and enumeration of coliforms.

Summary

Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliforms in water (36), dairy (39) and other food products (20). Druce *et al.*, (21)found that this medium was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey Broth. Recently the agar formulation is recommended by ISO committee for the enumeration of coliforms (5).

Principle

Peptone provides nitrogen and carbon required for growth while yeast extract supplies B complex vitamins. Bile salts mixture and crystal violet inhibit gram-positive organisms especially staphylococci and makes the medium selective. Sodium chloride maintains the osmotic equilibrium. Lactose is the carbohydrate source and neutral red is the pH indicator. Organisms, which rapidly ferment lactose, produce red colonies surrounded by red-purple haloes. Lactose non-fermenters and late lactose fermenters produce pale colonies. Other related gram-negative bacteria can be suppressed by incubation greater than 42°C or by anaerobic incubation.

Formula*

Ingredients in grams per liter

Lactose	10.0
Peptone	7.0
Sodium chloride	5.0
Yeast extract	3.0
Bile salts mixture	1.5
Neutral red	0.03
Crystal violet	0.002
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C, and pour into sterile petri plates containing the inoculum.

Quality Control

Dehydrated Appearance

Beige coloured homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink	More than 70%

Escherichia coli (25922)	Luxuriant	Pinkish red with bile precipitate.	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

Presumptive test for coliforms

1. Transfer 1 ml aliquot of test sample to a petri plate.
2. Add 10 ml of Violet Red Bile Agar cooled to 45°C and swirl to mix.
3. Allow medium to solidify before incubating at 35-37°C for 18-24 hours. For dairy products, incubate at 32°C.
4. Do confirmatory testing of typical coliform colonies.
5. An overlay method is helpful to improve the specificity of the medium. A thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation can be carried out at greater than 42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days, depending on the temperature characteristics of the organism to be recovered. For *E.coli*, a temperature of 44°C is specifically recommended.

Interpretation of Results

1. Lactose fermenters including coliforms form purple red colonies, with or without a zone of precipitate around the colonies. (Generally surrounded by a reddish zone of precipitated bile)
2. Non-lactose fermenters form colourless to transparent colonies.
3. Gram-positive cocci, if present, form colourless, pinpoint colonies.

Precautions / Limitations

1. Boiling the medium for more than 2 minutes may decrease the ability to support growth.
2. Do not incubate inoculated plates for more than 24 hours because microorganisms that are only slightly inhibited may grow after extended incubation.
3. Prepare and use the medium within 24 hours for optimum performance.
4. This medium may not be completely inhibitory to gram-positive organisms. *Enterococci* may grow as pinpoint colonies. Perform gram stain and biochemical tests to identify isolates.
5. Gram-negative *bacilli* other than *Enterobacteriaceae* may also grow. Perform biochemical tests to identify isolates to genus and species.

Storage

Store 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Agar with MUG — AM510712

Use

Violet Red Bile Agar with MUG is used for enumerating *Escherichia coli* and coliform bacteria in food and dairy products.

Summary

Violet Red Bile Agar is specified in standard methods procedures to enumerate coliforms in food and dairy products (17.3, 61.2, 43.1). In 1982, Feng and Hartman (30.1) developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl-ß – D- glucuronide (MUG) into Lauryl Tryptose Broth. Incorporating MUG into Violet Red Bile Agar permits the detection of E. coli among the coliform colonies.

Principle

Peptone as a source of carbon, nitrogen, vitamin and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Bile salt and crystal violet inhibit gram-positive bacteria. Lactose is a carbohydrate source. Neutral red is a pH indicator. MUG is a substrate used for detecting glucuronidase activity. Agar is the solidifying agent.

E. coli produces the enzyme glucuronidase which hydrolyzes MUG to yield a fluorogenic compound detectable with long wave UV light (366 nm). Typical strains of E. coli (red colonies surrounded by a bile precipitate) exhibit blue fluorescence. Non- *E. coli* coliform may produce red colonies with zone of precipitated bile but they are MUG negative.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Peptone	7.0
Bile salts No. 3	1.5
Lactose	10.0
Sodium chloride	5.0
Agar	15.0
Neutral red	0.03
Crystal violet	0.002
MUG (4-methylumbelliferyl-β– D- glucuronide)	0.1

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.6 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45-50^{0}C, and use immediately.

Quality Control

Dehydrated Appearance

Reddish beige, homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organisms (ATCC)	Growth	Colour of Colony	Fluorescence	RGI
Enterobacter aerogenes (13048)	Luxuriant	Pink may have bile precipitate.	-	More than 70%
(25922) *Escherichia coli*	Luxuriant	Deep red with bile	+	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22- 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Broth AM51072

Use

Violet Red Bile Broth is selective media used for the detection and enumeration of coliform organisms from water and food.

Summary

Violet Red Bile Broth is recommended by APHA for the detection and enumeration of coliform organisms in water, milk, dairy and other food products (103.2, 91.2). Druce *et al.*,(21) found this media equally good as the indicator of coli-aerogenes in milk as MaConkey Broth. Recently, the agar formulation is recommended by ISO committee for the enumeration of coliforms (46.5).

Principle

The media is selective due to the presence of the inhibitors- bile salts and crystal violet. Crystal violet inhibit gram-positive microorganisms especially *staphylococci*. Organisms which rapidly ferment lactose produce red colonies surrounded by red-purple halo (18.4). Lactose non-fermenters and late lactose fermenters produce pale colonies.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Peptic digest of animal tissue	7.0
Bile salt mixture	1.5
Lactose	10.0
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.002

Final pH (at 25^{0}C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 26.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.
4. Cool to 45^{0}C,and pour into tubes containing the inoculum..

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37^{0}C.

Organisms (ATCC)	Growth
Enterobacter aerogenes (13048)	Luxuriant
Escherichia coli (25922)	Luxuriant
Salmonella serotype Enteritidis (13076)	Luxuriant
Staphylococcus aureus (25923)	Inhibited

For growth RGI should be more than 70%

RGI- Relative Growth Index

Storage

Store at 22- 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Glucose Agar (Harmonized) AMH51073

Violet Red Bile Glucose Agar BP AM510731

Violet Red Bile Glucose Agar USP AM510732

Violet Red Bile Glucose Agar AM51073

Use

Violet Red Bile Glucose Agar is used for detection and enumeration of *Enterobacteriaceae* in food products.

Summary

Mossel *et al.,* (81.4)added glucose and excluded lactose from the media observing improved detection of coliforms. Incubation can be carried out at different temperature and incubation time depending upon the group of *Enterobacteriaceae* to be recovered.

Principle

Pancreatic digest of gelatin and yeast extract provide nitrogenous compounds, vitamin B complex and other nutrients essential for the bacterial metabolism while glucose and neutral red helps to detect glucose fermentation. Bile salts mixture and crystal violet select the growth of gram-negative intestinal bacteria inhibiting gram-positive bacteria.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Pancreatic digest of gelatin	7.0
Bile salts	1.5
Sodium chloride	5.0
Glucose monohydrate	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	15.0

Final pH (at 25ºC) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 41.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C, and pour into sterile petri plates containing the inoculum.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colony of colour	RG
Enterobacter aerogenes (13048)	Luxuriant	Pink	More than 70%
Escherichia coli (25922)	Luxuriant	Pinkish red with bile precipitate.	More than 70%
Salmonella serotype Enteritidis (13076)	Luxuriant	Light pink with bile precipitate	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Glucose Agar without Lactose ISO AM51074

Use

Violet Red Bile Glucose Agar without Lactose ISO is selective media used for the detection and enumeration of *Enterobacteriaceae* in food product. It is also recommended by ISO committee under the specifications ISO 7402:1993.

Summary

Violet Red Bile Broth is recommended by APHA for the detection and enumeration of coliform organisms in water (36), milk, dairy (39) and other food products (20). Druce et al., (21) found this media equally good as the indicator of *coli-aerogenes* in milk as MaConkey Broth. Recently, the agar formulation is recommended by ISO committee for the enumeration of coliforms (5).

Principle

The media is selective due to the presence of the inhibitors- bile salts and crystal violet. Crystal violet inhibit gram-positive microorganisms especially staphylococci. Organisms which rapidly ferment glucose produce red colonies surrounded by red-purple halo. Glucose non-fermenters and late glucose fermenters produce pale colonies.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Peptic digest of animal tissue	7.0
Bile salt mixture	1.5
Glucose	10.0
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.00

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 38.53 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.
4. Cool to 45°C,and pour into petri plates containing the inoculum..

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colony colour	RGI
Enterobacter aerogenes (13048)	Good-luxuriant	Pink-red	More than 70%
Escherichia coli (25922)	Good-luxuriant	Pink-red with bile precipitate	More than 70%
Salmonella serotype Enteritidis(13076)	Good-luxuriant	Light pink	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Violet Red Bile Glucose Agar with Lactose (Agar Medium F) EP AM51075

Violet Red Bile Glucose Agar with Lactose (Agar Medium F) BP AM51076

Use

Violet Red Bile Glucose Agar (VRBGA) is used for the detection and enumeration of *Enterobacteriaceae* in foods and dairy products.

Summary

Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliforms in water(36), milk, dairy (39) and other food products (20). Druce *et al.*, (21) found that this medium was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey Broth. European Pharmacopoeia also recommends this medium for microbiological examination of non-sterile products.

Principle

Pancreatic digest of gelatin and Yeast extract are the source of nutrients, amino acids, carbon compounds, vitamin B -complex, minerals & trace elements. Glucose is the energy source. Bile Salts and Crystal Violet inhibit gram-positive bacteria. Neutral red is pH indicator. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Pancreatic digest of gelatin	7.0
Yeast extract	3.0
Bile salts	1.5
Sodium chloride	5.0
Glucose monohydrate	10.0
Lactose monohydrate	10.0
Crystal violet	0.002
Neutral red	0.03
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 51.53 gms of powder in 1000 ml distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. DO NOT AUTOCLAVE.

Quality Control

Dehydrated Appearance

Pink- beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Reddish purple coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37ºC

Organisms (ATCC)	Growth	Colony colour	RGI
Enterobacter aerogenes (13048)	Good-luxuriant	Pinkish purple	More than 70%
Escherichia coli (25922)	Good-luxuriant	Pink-red with bile	More than 70% precipitate
Salmonella serotype Enteritidis *(13076)*	Good-luxuriant	Colourless	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the spread or pour plate method.
2. Incubate plates aerobically, protected from light, at 35-37ºC for 18-24 hours or as required.
3. After incubation count the colonies.
4. Examine colony morphology and carry out biochemical testing for identification.

Interpretation of Results

1. *Enterobacteriaceae* ferment glucose and produce red-pink colonies.
2. Count all developing red-pink colonies.

Storage

Store at 22- 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

Vogel Johnson Agar Base w/o Tellurite IP AM11081/AM51081

Vogel Johnson Agar Base w/o Tellurite USP AM11082/AM51082

Use

Vogel Johnson Agar Base with the addition of potassium tellurite is used for the isolation of *Staphylococcus aureus* from clinical and nonclinical specimens.

Summary

Vogel Johnson (116) Agar is used for the early detection of coagulase positive and mannitol fermenting *Staphylococcus aureus* from heavily contaminated food and clinical specimens. Vogel Johnson modified the original Tellurite Glycine Agar formula of Zebovitz *et al.*, (124) by increasing the mannitol concentration and adding phenol red as the pH indicator. This medium is specified as a standard method medium for cosmetics, pharmaceutical articles and nutritional supplements and the formulation complies with recommendations by the USP and IP for microbial limit testing. It selects and differentiates the coagulase positive *staphylococci*, which ferment mannitol and reduce tellurite.

Principle

Pancreatic digest of casein is a source of carbon, nitrogen, minerals and other growth factors. Yeast extract provides B complex vitamins. Mannitol is the carbohydrate source while dipotassium phosphate is the buffer. Potassium tellurite, lithium chloride and the high glycine content inhibit non-staphylococcal organisms. *Staphylococcu s* may also be slightly inhibited by the above inhibitors; but this is compensated by the addition of mannitol and glycine. Coagulase positive strains of *S.aureus* reduce tellurite to metallic free tellurium, producing characteristic black colonies. The fermentation of mannitol is detected by a change in colour of the phenol red indicator from red (alkaline) to yellow (acid); and the colonies are surrounded by a yellow zone. Other microorganisms if present are easily distinguished by their inability to produce black colonies.

Formula*

Ingredients in grams per liter

Mannitol	10.0
Pancreatic digest of casein	10.0
Glycine	10.0
Yeast extract	5.0
Lithium chloride	5.0
Dipotassium phosphate	5.0
Phenol red	0.025
Agar	16.0

Final pH (at 25^{0}C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 61 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50^{0}C and add 20 ml of sterile 1% Potassium Tellurite Solution (AS022).

6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colony colour	RGI
Escherichia coli (25922)	Inhibited	-	0%
Proteus mirabilis (25933)	Poor	Black	More than 70%
Staphylococcus aureus (25923)	Luxuriant	Black with yellow halo.	More than 70%
Staphylococcus epidermidis (12228)	Fair	Translucent to blackish.	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies from specimens.
2. If material being cultured is directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. The medium is highly selective and the inoculum may be applied heavily.
4. Incubate the plates aerobically at 35-37°C for 18-48 hours.

Interpretation of Results

1. During the first 24 hours of incubation, most organisms other than coagulase positive *staphylococci*, are markedly or totally inhibited.
2. After 48 hours, many coagulase negative, mannitol fermenting or coagulase negative, mannitol non-fermenting *staphylococci* will grow on the medium.
3. The coagulase positive cocci form small, black colonies against the red background.
4. If mannitol is fermented, the colonies are surrounded by yellow zones due to the colour change of the phenol red indicator in response to the acid formation.

Precautions / Limitations

1. Do not heat the medium after the addition of potassium tellurite.
2. Prolonged incubation may result in the growth of black coagulase negative colonies and if these organisms also ferment mannitol they may be falsely identified from their appearence as *S.aureus.*
3. If tellurite is reduced but mannitol is not fermented, the medium surrounding colonies may be a deeper red colour due to utilization of proteins in the medium resulting in alkalinity.

Warning: Lithium chloride is harmful, bodily contact or inhalation of vapours must be avoided. On contact with skin, wash with plenty of water immediately.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Vogel Johnson Agar Base w/o Tellurite AM1108/AM5108

Use

Vogel Johnson Agar Base with the addition of potassium tellurite is used for the isolation of *Staphylococcus aureus* from clinical and non-clinical specimens.

Summary

Vogel Johnson Agar is used for the early detection of coagulase positive and mannitol fermenting *Staphylococcus aureus* from heavily contaminated food and clinical specimens. Vogel Johnson (116) modified the original Tellurite Glycine Agar formula of Zebovitz et al (124) by increasing the mannitol concentration and adding phenol red as the pH indicator. This medium is specified as a standard method medium for cosmetics (113), pharmaceutical articles and nutritional supplements and the formulation complies with recommendations by the USP (114) and IP (46) for microbial limit testing. It selects and differentiates the coagulase positive *staphylococci*, which ferment mannitol and reduce tellurite.

Principle

Tryptone is a source of carbon, nitrogen, minerals and other growth factors. Yeast extract provides B complex vitamins. Mannitol is the carbohydrate source while dipotassium phosphate is the buffer. Potassium tellurite, lithium chloride and the high glycine content inhibit non-staphylococcal organisms. *Staphylococcus* may also be slightly inhibited by the above inhibitors; but this is compensated by the addition of mannitol and glycine. Coagulase positive strains of *S.aureus* reduce tellurite to metallic free tellurium, producing characteristic black colonies. The fermentation of mannitol is detected by a change in colour of the phenol red indicator from red (alkaline) to yellow (acid); and the colonies are surrounded by a yellow zone. Other microorganisms if present are easily distinguished by their inability to produce black colonies.

Formula*

Ingredients in grams per liter

Mannitol	10.0
Tryptone	10.0
Glycine	10.0
Yeast Extract	5.0
Lithium Chloride	5.0
Dipotassium Phosphate	5.0
Phenol Red	0.025
Agar	16.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 61 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and add 20 ml of sterile 1% Potassium Tellurite Solution (AS022).
6. Mix well and pour into sterile petri plates.

Warning: Lithium chloride is harmful, bodily contact or inhalation of vapours must be avoided. On contact with skin, wash with plenty of water immediately.

Quality Control

Dehydrated Appearance

Light pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Escherichia coli (25922)	Inhibited	-	0%
Proteus mirabilis (25933)	Poor	Black	0%
Staphylococcus aureus (25923)	Luxuriant	Black with yellow halo.	More than 70%
Staphylococcus epidermidis (12228)	Fair	Translucent to blackish.	0%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies from specimens.
2. If material being cultured is directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. The medium is highly selective and the inoculum may be applied heavily.
4. Incubate the plates aerobically at 35-37°C for 18-48 hours.

Interpretation of Results

1. During the first 24 hours of incubation, most organisms other than coagulase positive *staphylococci*, are markedly or totally inhibited.
2. After 48 hours, many coagulase negative, mannitol fermenting or coagulase negative, mannitol non-fermenting *staphylococci* will grow on the medium.
3. The coagulase positive cocci form small, black colonies against the red background.
4. If mannitol is fermented, the colonies are surrounded by yellow zones due to the colour change of the phenol red indicator in response to the acid formation.

Precautions / Limitations

1. Do not heat the medium after the addition of potassium tellurite.
2. Prolonged incubation may result in the growth of black coagulase negative colonies and if these organisms also ferment mannitol they may be falsely identified from their appearence as *S.aureus*.
3. If tellurite is reduced but mannitol is not fermented, the medium surrounding colonies may be a deeper red colour due to utilization of proteins in the medium resulting in alkalinity.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Wilson Blair Agar Base AM51083

Use

Wilson Blair Agar Base is used for isolation and differentiation of *Salmonella* serotype Typhi.

Summary

Wilson Blair Agar Base is a valuable medium for the isolation of Typhi. *Salmonellae* produce hydrogen sulfide that causes the colony to be surrounded by a metallic sheen. Wilson Blair Agar Base is highly selective for *Salmonellae*, being inhibitory to *coliforms*, *Proteus* and *Shigellae* (77.2).

Principle

Peptone and beef extract provide carbon, nitrogen and other growth factors. Dextrose is the source of energy whereas sodium chloride maintains the osmotic balance. Agar is used as a solidifying agent.

Formula*

Ingredients in grams per liter

Peptone, special	10
Dextrose	10
Beef extract	5
Sodium chloride	5
Agar	30

Final pH (at 25°C) 7.3 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 60gms of powder in 1000ml-distilled water.
2. Boil to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
4. To sterile melted base, add 4 ml of 1% Brilliant Green solution and 70 ml of selected reagent.

   Selective Reagent:

   Solution 1: 40 gm Sodium sulphite in 100mld/w

   Solution 2: 21 gm Dibasic Sodium phosphate in 100 ml d/w

   Solution 3: 12.5 gm Bismuth ammonium citrate in 100 ml d/w

   Solution 4: 0.96 gm Ferrous sulphate in 20 ml d/w with 2 drops of hydrochloric acid.

   Prepare each solution separately and then combine.

   Boil the combined solution until a slate grey colour develops.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous free flowing powder.

Prepared Appearance

Light yellow coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 24-48 hours at 35-37ºC.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Escherichia coli (25922)	Inhibited	-	0%
Proteus mirabilis (25933)	Luxuriant	Green	More than 70%
Salmonella serotype Typhi (6539)	Luxuriant	Black with Sheen	More than 70%
Salmonella serotype Typhimurium	Luxuriant	Black with sheen	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

WL Differential Agar — AM1109/AM5109

Use

WL Differential Agar is used for the selective isolation and enumeration of bacteria encountered in breweries and industrial fermentation.

Summary

Green and Gray (35) developed WL (Wallerstein Laboratory) Differential Agar to study various fermentation processes. Development of this medium came about due to an exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products. By adjusting the pH to 6.5, the medium is made suitable for obtaining counts of baker and distiller's yeast.

Principle

Yeast extract provides trace elements, vitamins and amino acids. Tryptone provides carbon, nitrogen and amino acids while dextrose is the source of carbohydrate. Monopotassium phosphate buffers the medium. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is the pH indicator. Cycloheximide suppresses the growth of yeasts and moulds in brewing samples, permitting the detection and enumeration of bacteria that may be present in small numbers.

Formula*

Ingredients in grams per liter

Dextrose	50.0
Tryptone	5.0
Yeast Extract	4.0
Monopotassium Phosphate	0.55
Potassium Chloride	0.425
Magnesium Sulphate	0.125
Calcium Chloride	0.125
Bromocresol Green	0.022
Cycloheximide	0.004
Ferric Chloride	0.0025
Manganese Sulphate	0.0025
Agar	20.0

Final pH (at 25°C) 5.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 80.26 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

5. If desired, use 1% solution of sodium bicarbonate to adjust the pH to 6.5.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, very slightly opalescent gel.

Cultural Response

Cultural characteristics after 40-48 hours at 35°C.

Organisms (ATCC)	Growth	RGI
Saccharomyces cerevisiae (9763)	Inhibited	0%
Escherichia coli (25922)	Luxuriant	More than 70%
Proteus mirabilis (25933)	Luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

1. For brewing materials, incubate at 25°C.
2. For baker's yeast and alcohol fermentation mash analysis, incubate at 30°C.
3. Incubate aerobically for the growth of acetic acid bacteria, *Flavobacterium* species, *Proteus* and thermophilic bacteria.
4. Incubate anaerobically for the growth of lactic acid bacteria and *Pediococcus* species.

Precautions / Limitations

1. While reconstituting vials containing cycloheximide ensure that the vial solution does not touch the skin, also avoid the formation of aerosol and the inhalation of the compound.
2. Wear personal protective equipment.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

WL Differential Broth AM51091

Use

WL Differential Broth is used for the selective isolation and enumeration of bacteria encountered in breweries and industrial fermentation.

Summary

Green and Gray (35) developed WL (Wallerstein Laboratory) Differential Broth to study various fermentation processes. Development of this medium came about due to an exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products. By adjusting the pH to 6.5, the medium is made suitable for obtaining counts of baker and distiller's yeast.

Principle

Yeast extract provides trace elements, vitamins and amino acids. Tryptone provides carbon, nitrogen and amino acids while dextrose is the source of carbohydrate. Monopotassium phosphate buffers the medium. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is the pH indicator. Cycloheximide suppresses the growth of yeasts and moulds in brewing samples, permitting the detection and enumeration of bacteria that may be present in small numbers.

Formula*

Ingredients in grams per liter

Dextrose	50.0
Casein enzyme hydrolysate	5.0
Yeast extract	4.0
Monopotassium phosphate	0.55
Potassium chloride	0.425
Magnesium sulphate	0.125
Calcium chloride	0.125
Bromocresol green	0.022
Captan	0.004
Ferric chloride	0.0025
Manganese sulphate	0.0025

Final pH (at 25°C) 5.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 60.26 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. If desired, use 1% solution of sodium bicarbonate to adjust the pH to 6.5.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, very slightly opalescent solution form in tube.

Cultural Response

Cultural characteristics after 40-48 hours at 35-37°C.

Organisms(ATCC)	Growth
Saccharomyces cerevisiae (9763)	Inhibited

Escherichia coli (25922)	Luxuriant
Saccharomyces uvarum (9080)	Inhibited
Lactobacillus fermentum (9338)	Luxuriant
Proteus mirabilis (25933)	Luxuriant

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. For brewing materials, incubate at 25°C.
2. For baker's yeast and alcohol fermentation mash analysis, incubate at 30°C.
3. Incubate aerobically for the growth of acetic acid bacteria, Flavobacterium species, Proteus and thermophilic bacteria.
4. Incubate anaerobically for the growth of lactic acid bacteria and Pediococcus species.

Precautions / Limitations

1. While reconstituting vials containing cycloheximide ensure that the vial solution does not touch the skin, also avoid the formation of aerosol and the inhalation of the compound.
2. Wear personal protective equipment.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

WL Nutrient Agar AM51092

Use

WL Nutrient Agar is used for cultivating yeasts, moulds and bacteria encountered in brewing and fermentation processes.

Summary

WL (Wallerstein Laboratory) Nutrient Agar is based on the formulation of Green and Gray and is used for the determination of the microbiological flora in brewing and fermentation processes (35).

Principle

Yeast extract provides trace elements, vitamins and amino acids. Tryptone provides carbon, nitrogen and amino acids while dextrose is the source of carbohydrates. Monopotassium phosphate buffers the medium. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is the pH indicator.

Adding 0.004 grams/litre of cycloheximide suppresses the growth of yeast and renders the medium selective for bacteria. Adjusting the pH to 6.5 facilitates the growth of bakers' and distillers' yeasts.

Formula*

Ingredients in grams per liter

Dextrose	50.0
Tryptone	5.0
Yeast extract	4.0
Monopotassium phosphate	0.55
Potassium chloride	0.425
Magnesium sulphate	0.125
Calcium chloride	0.125
Bromocresol green	0.022
Manganese sulphate	0.0025
Ferric chloride	0.0025
Agar	20.0

Final pH (at 25°C) 5.5 ± 0.2
* Formula adjusted to suit performance parameters

Directions

1. Suspend 80.25 gms of the powder in 1000ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 121°C (15lbs pressure) for 15 minutes.
6. If desired, use 1% solution of sodium bicarbonate to adjust the pH to 6.5.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, very slightly opalescent gel forms in petri plate and tube.

Cultural Response

Cultural characteristics after 40-48 hours at 30°C.

Organisms (ATCC)	Growth	RGI
Saccharomyces cerevisiae (9763)	Good to luxuriant	More than 70%
Saccharomyces uvarum (9080)	Good-luxuriant	More than 70%
Escherichia coli (25922)	Fair to good	More than 70%
Lactobacillus fermentum (9338)	Fair to good	More than 70%
Proteus mirabilis (25933)	Fair to good	More than 70%

For growth RGI should be more than 70%
RGI- Relative Growth Index

Test Procedure

1. Prepare the medium and inoculate the medium.
2. For brewing materials, incubate at 25°C.

3. For Baker's yeast and alcohol fermentation mash analysis, incubate at 300C.
4. For bacteria incubate at 35 ± 2°C.

Interpretation of Results

Refer to appropriate references and procedures for result.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

WL Nutrient Broth — AM1110/AM5110

Use

WL Nutrient Broth is used for cultivating yeasts, moulds and bacteria encountered in brewing and fermentation processes.

Summary

WL Nutrient Broth is based on the formulation of Green and Gray (35) and is used where there are advantages for broth media e.g. using larger samples of liquid products or for enrichment cultures with cycloheximide.

Principle

Yeast extract provides trace elements, vitamins and amino acids. Tryptone provides carbon, nitrogen and amino acids while dextrose is the source of carbohydrates. Monopotassium phosphate buffers the medium. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is the pH indicator.

Adding 0.004 grams/litre of cycloheximide suppresses the growth of yeast and renders the medium selective for bacteria. Adjusting the pH to 6.5 facilitates the growth of bakers' and distillers' yeasts; the medium at pH 5.5 is used for the growth of bakers' yeasts.

Formula*

Ingredients in grams per liter

Dextrose	50.0
Tryptone	5.0
Yeast Extract	4.0
Monopotassium Phosphate	0.55
Potassium Chloride	0.425
Magnesium Sulphate	0.125
Calcium Chloride	0.125
Bromocresol Green	0.022
Manganese Sulphate	0.0025
Ferric Chloride	0.0025

Final pH (at 25°C) 5.5 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 60.25 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Dispense in desired containers as per requirements.
5. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
6. If desired, use 1% solution of sodium bicarbonate to adjust the pH to 6.5.
7. Adding 0.004 gms of cycloheximide per liter of broth will make it WL Differential Broth.

Quality Control

Dehydrated Appearance

Greenish yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Bluish green coloured, very slightly opalescent solution.

Cultural Response

Cultural characteristics after 40-48 hours at 30°C.

Organisms (ATCC)	Growth
Saccharomyces cerevisiae (9763)	Good to luxuriant
Escherichia coli (25922)	Fair to good
Proteus mirabilis (25933)	Fair to good

For growth RGI should be more than 70%

RGI- Relative Growth Index

Precautions / Limitations

1. While reconstituting vials containing cycloheximide ensure that the vial solution does not touch the skin, also avoid the formation of aerosol and the inhalation of the compound.
2. Wear personal protective equipment.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Wort Agar — AM1111/AM5111

Use

Wort Agar is used for the cultivation and enumeration of yeasts.

Summary

Parfitt (86) formulated Wort Agar for the cultivation of fungi especially yeasts in syrups and butter. Culture media containing dextrose and maltose with an acidic

pH enhance the growth of yeasts.

Principle

Malt extract and peptone provide nitrogenous compounds and other nutrients for the growth of yeasts. Maltose and dextrin are fermentable carbohydrates. The high acidic pH inhibits the growth of many bacteria.

Formula*

Ingredients in grams per liter

Malt Extract	15.0
Maltose	12.75
Peptone	0.78
Dextrin	2.75
Ammonium Chloride	1.0
Dipotassium Phosphate	1.0
Agar	15.0

Final pH (at 25°C) 4.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 48.3 gms of the powder in 1000 ml distilled water containing 2.35 gms glycerol.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, opalescent gel.

Cultural Response

Cultural characteristics after 40-48 hours at 30°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Candida albicans (10231)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Precautions / Limitations

1. Do not reliquify agar medium as it may cause alteration of the medium with hydrolysis of agar at low pH and results in failure to gel when cooled.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Xylose Lysine Deoxycholate Agar (XLD Agar) AM1112/AM5112

Use

XLD Agar is a moderately selective medium used for the isolation and differentiation of *Salmonella* and *Shigella.*

Summary

XLD Agar is a differential medium used for the isolation of salmonellae and shigellae from clinical and non-clinical specimens like faeces and foods. It was developed by Taylor (108) in order to increase the efficiency of isolation of the enteric pathogens, particularly *Shigella* from faecal specimens. The pathogens are differentiated not only from the non-pathogenic lactose fermenters but also from many non-pathogens, which do not ferment lactose or sucrose. Also, the medium was formulated to increase the frequency of growth of the more fastidious pathogens, which in other formulations have often failed to grow due to the inclusion of excessively toxic inhibitors. This medium is used in the microbial limit test for screening specimens for the detection of *Salmonella* and is recommended by APHA for the examination of foods (20), dairy products (39) and water (36). XLD Agar conforms to the specifications of the USP (114), IP (46) and is included in the Bacteriological Analytical Manual for food testing (113).

Principle

XLD Agar is both, a selective and differential medium. Yeast extract provides nutrients while sodium deoxycholate inhibits gram-positive organisms. Xylose is fermented practically by all enterics except shigellae, which enables the differentiation of *Shigella* species. Incorporation of lysine enables the *Salmonella* group to be differentiated from the non-pathogens since, without lysine, *Salmonella* would rapidly ferment xylose and be indistinguishable from non-pathogenic species. After *Salmonella* exhausts the supply of xylose, lysine is attacked, with reversion to an alkaline pH, which mimic the *Shigella* reaction. However, to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence non-pathogenic H_2S producers do not decarboxylate lysine. The acid reaction produced by them prevents the blackening of the colonies. Sodium thiosulphate and ferric ammonium citrate are included for the visualization of hydrogen sulphide production, resulting in the formation of colonies with black centers. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sucrose	7.5
Lactose	7.5
Sodium Thiosulphate	6.8
L-Lysine	5.0

Sodium Chloride	5.0
Xylose	3.5
Yeast Extract	3.0
Sodium Deoxycholate	2.5
Ferric Ammonium Citrate	0.8
Phenol Red	0.08
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 56.68 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation until the medium just boils to dissolve the powder completely.
4. DO NOT OVERHEAT OR AUTOCLAVE. Overheating causes precipitation.
5. Cool immediately in a water bath at 45-50°C and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony
Enterobacter aerogenes (13048)	Fair	Yellow
Escherichia coli (25922)	Fair	Yellow
Proteus vulgaris (13315)	Good to luxuriant	Yellow
Salmonella serotype Enteritidis (13076)	Good to luxuriant	Red with black centers
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Red with black centers
Shigella dysenteriae (13313)	Good to luxuriant	Red
Staphylococcus aureus (25923)	Inhibited	-

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies from specimens.
2. If material being cultured is directly from a swab, roll the swab over a small area on the agar surface and streak for isolation.
3. Incubate the plates, protected from light at 35-37°C for 18-24 hours.
4. Colonies on XLD Agar may require 48 hours incubation for full colour development.
5. Examine colonial morphology, characteristics and haemolytic reactions.
6. It is recommended that selective enrichment broth, such as Selenite Cystine Broth be used in conjunction with other selective plating media to maximize the recovery of enteric pathogens.

Interpretation of Results

1. Degradation of xylose, lactose and sucrose leads to formation of acid products, causing a colour change in the medium from red to yellow.
2. H_2S production under alkaline conditions causes colonies to form black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.
3. Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to alkaline condition and the colour of the medium changes back to red.

Typical colonial morphology and reactions on XLD Agar:

E.coli----------	Large, flat, yellow; some strains may be inhibited.
Enterobacter/ Klebsiella----------	Mucoid, yellow.
Proteus----------	Red to yellow, most strains have black centers.
Salmonella----------	Red- yellow with black centers.
H_2S negative *Shigella*, *Salmonella* ----------	Red.
Pseudomonas----------	Red.
Gram-positive bacteria----------	No growth or slight growth.

Precautions / Limitations

1. It is advisable not to prepare large volumes, which will require prolonged heating.
2. Longer incubation may result in false positive results.
3. Some species of *Salmonella* like *S.paratyphi A, S.choleraesuis, S.gallinarum* and *S.pullorum* form red colonies without black centers, which resemble *Shigella* colonies.
4. Also, a few species of *Shigella* that ferment lactose, and *Salmonella* that fail to decarboxylate lysine would not be detected on this medium.
5. Red, false positive colonies may occur with some *Proteus* and *Pseudomonas* species. Some *Proteus* strains will give black centered colonies on XLD Agar.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Xylose Lysine Deoxycholate Agar (XLD Agar) Harmonized AMH5112

Xylose Lysine Deoxycholate Agar (XLD Agar) IP AM51121

Xylose Lysine Deoxycholate Agar (XLD Agar)USP AM51122

Xylose Lysine Deoxycholate Agar (XLD Agar) EP AM51123

Xylose Lysine Deoxycholate Agar (XLD Agar) BP AM51124

Use

XLD Agar is a moderately selective medium used for the isolation and differentiation of *Salmonella* and *Shigella.*

Summary

XLD Agar is a differential medium used for the isolation of *salmonellae* and *shigellae* from clinical and non-clinical specimens like faeces and foods. It was developed by Taylor (108) in order to increase the efficiency of isolation of the enteric pathogens, particularly *Shigella* from faecal specimens. The pathogens are differentiated not only from the non-pathogenic lactose fermenters but also from many nonpathogens, which do not ferment lactose or sucrose. Also, the medium was formulated to increase the frequency of growth of the more fastidious pathogens, which in other formulations have often failed to grow due to the inclusion of excessively toxic inhibitors. This medium is used in the microbial limit test for screening specimens for the detection of *Salmonella* (111.2) and is recommended by APHA for the examination of foods, dairy products and water. XLD Agar conforms to the specifications of the USP, IP and is included in the Bacteriological Analytical Manual for food testing.

Principle

XLD Agar is both, a selective and differential medium. Yeast extract provides nutrients while sodium deoxycholate inhibits gram-positive organisms. Xylose is fermented practically by all enterics except

shigellae, which enables the differentiation of *Shigella* species. Incorporation of lysine enables the *Salmonella* group to be differentiated from the non-pathogens since, without lysine, *Salmonella* would rapidly ferment xylose and be indistinguishable from non-pathogenic species. After *Salmonella* exhausts the supply of xylose, lysine is attacked, with reversion to an alkaline pH, which mimic the *Shigella* reaction. However, to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence non-pathogenic H_{25} producers do not decarboxylate lysine. The acid reaction produced by them prevents the blackening of the colonies. Sodium thiosulphate and ferric ammonium citrate are included for the visualization of hydrogen sulphide production, resulting in the formation of colonies with black centers. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sucrose	7.5
Lactose	7.5
Sodium thiosulphate	6.8
L-Lysine	5.0
Sodium chloride	5.0
Xylose	3.5
Yeast extract	3.0
Sodium deoxycholate	2.5
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	13.5

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 55.18 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation until the medium just boils to dissolve the powder completely.
4. DO NOT OVERHEAT OR AUTOCLAVE. Overheating causes precipitation.
5. Cool immediately in a water bath at 45-50°C and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Fair	Yellow	More than 70%
Escherichia coli (25922)	Fair	Yellow	More than 70%
Proteus vulgaris (13315)	Good to Luxuriant	Yellow	More than 70%

Salmonella serotype Enteritidis (13076)	Good to Luxuriant	Red with black centers	More than 70%
Salmonella serotype Typhimurium (14028)	Good to Luxuriant	Red with black centers	More than 70%
Shigella dysenteriae (13313)	Good to Luxuriant	Red	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%

For growth RGI should be more than 70%
For Inhibition RGI should be 0%
RGI- Relative Growth Index

Procedure

1. Use standard procedures like the streak plate method to obtain isolated colonies from specimens.
2. If material being cultured is directly from a swab, roll the swab over a small area on the agar surface and streak for isolation.
3. Incubate the plates, protected from light at 35-37^{0}C for 18-24 hours.
4. Colonies on XLD Agar may require 48 hours incubation for full colour development.
5. Examine colonial morphology, characteristics and haemolytic reactions.
6. It is recommended that selective enrichment broth, such as Selenite Cystine Broth be used in conjunction with other selective plating media to maximize the recovery of enteric pathogens.

Interpretation of Results

1. Degradation of xylose, lactose and sucrose leads to formation of acid products, causing a colour change in the medium from red to yellow.
2. H_2S production under alkaline conditions causes colonies to form black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.
3. Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to alkaline condition and the colour of the medium changes back to red.

Typical colonial morphology and reactions on XLD Agar:

E.coli----------Large, flat, yellow; some strains may be inhibited.

Enterobacter/Klebsiella-----Mucoid, yellow.

Proteus----------Red to yellow, most strains have black centers.

Salmonella----------Red- yellow with black centers. H_2S negative *Shigella,*

Salmonella ---------- Red.

Pseudomonas----------Red.

Gram-positive bacteria------ No growth or slight growth.

Precautions / Limitations

1. It is advisable not to prepare large volumes, which will require prolonged heating.
2. Longer incubation may result in false positive results.
3. Some species of *Salmonella* like *S.paratyphi A, S.choleraesuis, S.gallinarum* and *S.pullorum* form red colonies without black centers, which resemble *Shigella* colonies.
4. Also, a few species of *Shigella* that ferment lactose, and *Salmonella* that fail to decarboxylate lysine would not be detected on this medium.
5. Red, false positive colonies may occur with some *Proteus* and *Pseudomonas* species. Some Proteus strains will give black centered colonies on XLD Agar.

Storage

Store at 22- 30^{0}C and prepared medium at 2-8^{0}C.

Shelf Life

Use before expiry date as mentioned on the label.

XLD Agar, Modified ISO

AM51125

Use

XLD Agar, Modified is a moderately selective medium used for the isolation and differentiation of *Salmonella* and *Shigella* in compliances with ISO specification ISO 6579:2002..

Summary

XLD Agar is a differential medium used for the isolation of *salmonellae* and *shigellae* from clinical and non-clinical specimens like faeces and foods. It was developed by Taylor (108) in order to increase the efficiency of isolation of the enteric pathogens, particularly *Shigella* from faecal specimens. The pathogens are differentiated not only from the non-pathogenic lactose fermenters but also from many nonpathogens, which do not ferment lactose or sucrose. Also, the medium was formulated to increase the frequency of growth of the more fastidious pathogens, which in other formulations have often failed to grow due to the inclusion of excessively toxic inhibitors. This medium is used in the microbial limit test for screening specimens for the detection of *Salmonella* (111.2) and is recommended by APHA for the examination of foods, dairy products and water. XLD Agar conforms to the specifications of the ISO 6579:2002 and is included in the Bacteriological Analytical Manual for food testing.

Principle

XLD Agar is both, a selective and differential medium. Yeast extract provides nutrients while sodium deoxycholate inhibits gram-positive organisms. Xylose is fermented practically by all enterics except

shigellae, which enables the differentiation of *Shigella* species. Incorporation of lysine enables the *Salmonella* group to be differentiated from the non-pathogens since, without lysine, *Salmonella* would rapidly ferment xylose and be indistinguishable from non-pathogenic species. After Salmonella exhausts the supply of xylose, lysine is attacked, with reversion to an alkaline pH, which mimic the *Shigella* reaction. However, to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence non-pathogenic H_2S producers do not decarboxylate lysine. The acid reaction produced by them prevents the blackening of the colonies. Sodium thiosulphate and ferric ammonium citrate are included for the visualization of hydrogen sulphide production, resulting in the formation of colonies with black centers. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients in grams per liter

Sucrose	7.5
Lactose	7.5
Sodium thiosulphate	6.8
L-Lysine	5.0
Sodium chloride	5.0
Xylose	3.75
Yeast extract	3.0
Sodium deoxycholate	1.0
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	15.0

Final pH (at 25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 55.43 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation until the medium just boils to dissolve the powder completely.
4. DO NOT OVERHEAT OR AUTOCLAVE. Overheating causes precipitation.
5. Cool immediately in a water bath at 45-50°C and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Pink coloured, homogeneous, free flowing powder.

Prepared Appearance

Red coloured, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterobacter aerogenes (13048)	Fair	Yellow	More than 70%
Escherichia coli (25922)	Fair	Yellow	More than 70%
Proteus vulgaris (13315)	Good to luxuriant	Yellow	More than 70%
Salmonella serotype Enteritidis (13076)	Good to luxuriant	Red with black centers	More than 70%
Salmonella serotype Typhimurium (14028)	Good to luxuriant	Red with black centers	More than 70%
Shigella dysenteriae (13313)	Good to luxuriant	Red	More than 70%
Staphylococcus aureus (25923)	Inhibited	-	0%
Proteus mirabilis (25933)	Good to luxuriant	Yellow	More than 70%
Salmonella typhi (6539)	Good to luxuriant	Red with black center	More than 70%
Shigella sonnei (25931)	Good to luxuriant	Red	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

XLT4 Agar AM571251

Use

XLT4 Agar Base medium is recommended for selective isolation of *Salmonella* species other than *Salmonella* Typhi.

Summary

Salmonella is a genus of gram-negative enterobacteria commonly implicated in foodborne illness and is the causative agent of typhoid and paratyphoid fever. Although most *Salmonella* cannot be distinguished by biochemical characteristics, one serotype, namely *S. Typhi* produce only a trace amount of hydrogen sulphide and is less active biochemically than the more common serotypes (60.1). XLT4 Agar Base is formulated as described by Miller and Tate (79.5) for isolating Salmonella from faecally contaminated farm samples, which contains other bacteria as well. XLT4 Agar Base enhances the recovery of

Salmonella species other than *Salmonella* Typhi (79.6, 79.7, 20.1 & 20.2).

Principle

Proteose peptone is a source of carbon, nitrogen and other essential amino acids and growth factors. Yeast extract supplies nitrogenous requirements and vitamins required for growth. The sugars namely lactose, saccharose and xylose are the fermentable carbohydrates. Salmonella rapidly utilize xylose, producing acidity. Subsequently they decarboxylate lysine and revert to alkalinity. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate is included for the visualization of the hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylate lysine; therefore, the acid reaction generated by them prevents the blackening of the colonies. XLT4 Agar is both selective and differential.

Formula*

Ingredients in grams per liter

Proteose peptone	1.60
Yeast extract	3.00
L-Lysine	5.00
Xylose	3.75
Lactose	7.50
Saccharose	7.50
Ferric ammonium citrate	0.80
Sodium thiosulphate	6.80
Sodium chloride	5.00
Phenol red	0.08
Agar	18.00

Final pH (at 25°C) 7.4±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 59.03 grams in 1000 ml distilled water.
2. Add 4.6 ml XLT4 Supplement (AS0281).
3. Heat to boiling to dissolve the medium completely.
4. DO NOT AUTOCLAVE OR OVERHEAT.

Quality Control

Dehydrated Appearance

Light yellow to pink homogeneous free flowing powder

Prepared Appearance

Red coloured clear to slightly opalescent gel forms in Petri plates.

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added XLT4

Organisms (ATCC)	Growth	Colour of Colony	RGI
Salmonella Enterococcus faecalis (29212)	Inhibited	0%	-
Escherichia coli (25922)	Fair-good	Yellow	More than 70%
Salmonella Enteritidis (13076)	Good-luxuriant	Red with black centers	More than 70%
Salmonella Typhimurium (14028)	Good-luxuriant	Red with black centers	More than 70%
Staphylococcus aureus (25923)	Inhibited	0%	-

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast and Mould Broth AM51126

Use

Yeast and Mould Broth is used for isolation and cultivation of yeasts and moulds.

Summary

Yeast and Mould Broth is formulated on the basis of the description given by Wickerham (119.1). The medium is recommended for the isolation and maintenance of yeasts and moulds.

Principle

Peptone, yeast extract and malt extract provide all the essential nutrients for growth. Dextrose is the source of energy. Acidic pH inhibits the bacterial growth and favours the growth of mould.

Formula*

Ingredients in grams per liter

Yeast extract	3.0
Malt extract	3.0
Peptone	5.0
Dextrose	10.0

Final pH (at 25°C) 6.2 ±0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 21.0gms of powder in 1000 ml distilled water.
2. Mix thoroughly.

3. Heat with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Cream coloured, homogenous, free flowing powder.

Prepared Appearance

Light amber couloured, slightly opalescent solution forms in tubes.

Cultural Response

Cultural characteristics after 48-72 hours at 20-25°C.

Organisms (ATCC)	
Aspergillus niger (16404)	Good
Candida albicans (10231)	Good
Saccharomyces cerevisiae (9763)	Good

Procedure

Refer to appropriate references for specific procedures for the cultivation of yeast and mould.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Dextrose Agar AM51127

Use

Yeast Dextrose Agar is used for the cultivation of a variety of heterotrophic microorganisms.

Summary

Yeast Dextrose Agar is recommended for the cultivation of a variety of heterotrophic microorganisms.

Principle

Yeast extract supplies carbon, nitrogen and other ingredients for the growth of microorganisms. Dextrose is the energy source. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter	
Yeast extract	10.0
Dextrose	10.0
Agar	15.0

Final pH (at 25°C) 7.0 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 35 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C.

Organisms (ATCC)	**Growth**	**RGI**
Candida albicans (10231)	Luxuriant	More than 70%
Saccharomyces cerevisiae (9763)	Luxuriant	More than 70%
Aspergillus niger (16404)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Extract Agar AM1113/AM5113

Use

Yeast Extract Agar is a highly nutritive medium used for cultivation of a wide variety of bacteria.

Summary

Windle Taylor (109) formulated Yeast Extract Agar for the plate count of microorganisms in water and is also used for the cultivation of a wide variety of bacteria. It is included in the Bacteriological Analytical Manual for food and cosmetics testing (113).

Principle

Yeast extract and peptone provide nitrogenous compounds, vitamin B complex and other nutrients. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Peptone	5.0
Yeast Extract	3.0
Agar	15.0

Final pH (at 25°C) 7.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 23 gms of the powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yellow coloured, clear to very slightly opalescent gel.

Cultural Response

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	RGI
Escherichia coli (25922)	Luxuriant	More than 70%
Staphylococcus aureus (25923)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

1. Incubate plates at 35-37°C for 18-24 hours.
2. Separate counts are also made of the organisms forming visible colonies after 24 hours at 35°C and organisms forming visible colonies after 3 days at 20-22°C.

Interpretation of Results

1. Select plates containing 20-300 colonies.
2. Record results as colony forming units (CFU) per volume of sample taking into account the dilution factor.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Extract Chloramphenicol Dextrose Agar AM51131

Use

Yeast Extract Chloramphenicol Dextrose Agar recommended for isolation and enumeration of fungi-yeasts and moulds in milk and milk products

Summary

The antibiotic method for enumerating yeasts and molds in dairy products has become the method of choice, replacing the traditional acidified method. The use of antibiotics for supressing bacteria results in better recovery of injured fungal cells. Which are sensitive to an acid environment, and in less interference from precipitated food particles during the counting. Yeast Extract Chloramphenicol Dextrose Agar is a nutrient medium that inhibits the growth of organisms other than yeasts and molds due to the presence of Chloramphenicol.

Principle

Yeast extract provides basic nutrient. Dextrose is a carbon energy source. Chloramphenicol inhibits bacterial growth. Agar is the solidifying agent.

Formula*

Ingredients in grams per liter

Yeast extract	5.0
Dextrose	20.0
Chloramphenicol	0.10
Agar	14.90

Final pH (at 25°C) 6.6 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 40.0 gms of the powder in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Light yellow colour, homogeneous, free flowing powder.

Prepared Appearance:

Yellow coloured, clear to slightly opalescent gel forms in petriplates.

Cultural Response

Cultural characteristics after 2-5 days at 20-25°C.

Organisms (ATCC)	Growth	RGI
Aspergillus niger (16404)	Good-luxuriant	More than 70%
Candida albicans (10231)	Good-luxuriant	More than 70%
Escherichia coli (25922)	Inhibited	0%
Saccharomyces cerevisiae (9763)	Good-luxuriant	More than 70%

For growth RGI should be more than 70%

For Inhibition RGI should be 0%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Malt Agar AM1114/AM5114

Yeast Malt Broth AM1115/AM5115

Use

Yeast Malt Agar and Yeast Malt Broth are used for the isolation and cultivation of yeasts, moulds and aciduric bacteria.

Summary

Wickerham (119) formulated Yeast Malt Agar. Media selectivity can be enhanced by adding selective agents or by acidifying the medium. Yeast Malt Agar and Yeast Malt Broth should be sterilized without prior pH adjustment and sterile acid should be added to sterile cooled molten agar. Antibiotics may be aseptically added to the medium. Fungistatic material like sodium propionate and diphenyl may be added to eliminate moulds thus permitting the enumeration of yeasts in a mixed population.

Principle

Yeast extract provides trace elements, vitamins and amino acids. Malt extract provides carbon, protein and other nutrients. Peptone is an additional source of carbon and provides amino acids and nitrogen. Dextrose provides carbon as energy source.

Formula*

Ingredients in grams per liter	Yeast Malt Agar	Yeast Malt Broth
Dextrose	10.0	10.0
Peptone	5.0	5.0
Malt Extract	3.0	3.0
Yeast Extract	3.0	3.0
Agar	20.0	-
Final pH (at 25°C)	6.2 ± 0.2	6.2 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the powder in 1000 ml distilled water.

   Yeast Malt Agar - 41 gms

   Yeast Malt Broth - 21 gms
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. To make the medium selective: acidify the medium to pH 3.0 to 4.0 by adding sterile acid (10% HCl, tartaric acid or 10% citric acid) or antibiotics.
6. DO NOT REHEAT the media after the addition of antibiotics or acid.

Quality Control

Dehydrated Appearance

Light yellow coloured, homogeneous, free flowing powder.

Prepared Appearance

Yeast Malt Agar - Light amber coloured, slightly opalescent gel.

Yeast Malt Broth - Light amber coloured, slightly opalescent solution.

Cultural Response

Cultural characteristics after 40-72 hours at 25-30°C.

Organisms (ATCC)	Growth at pH 3.0-4.0	Growth at pH 6.2
Aspergillus niger (16404)	Good to luxuriant	Good to luxuriant
Candida albicans (10231)	Good to luxuriant	Good to luxuriant
Saccharomyces cerevisiae (9763)	Good to luxuriant	Good to luxuriant
Escherichia coli (25922)	Inhibited	Good to luxuriant

Procedure

1. Inoculate the Yeast Malt Agar plates and Yeast Malt Broth tubes with samples to evaluate the presence of yeasts, moulds or aciduric organisms.
2. Incubate at 25-30°C for 40-72 hours.
3. To favour isolation of fermentative species in Yeast Malt Broth, add a layer of sterile paraffin oil 1 cm deep on the surface of the inoculated broth. Incubate the culture till growth appears then streak onto Yeast Malt Agar to obtain isolated yeast colonies.
4. To isolate oxidative or fermentative strains, place the acidified Yeast Malt Broth on a rotary shaker for 1-2 days. This favours yeast recovery while preventing the sporulation of moulds.

Interpretation of Results

For Yeast Malt Agar

1. Examine the plates for growth.
2. Record as colony forming units (CFU) per volume of the sample.

For Yeast Malt Broth

1. Record result as growth or no growth on the basis of turbidity of the medium.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Mannitol Agar with 1.5% Agar AM51151

Use

Yeast Mannitol Agar with 1.5% agar is used for cultivation, isolation and enumeration of soil microorganism like *Rhizobium* species.

Summary

Yeast Mannitol Agar with 1.5% agar is recommended for the cultivation of the symbiotic nitrogen-fixing organisms like *Rhizobium* species (106.1).

Principle

Yeast extract provides amino acids and vitamins while sodium chloride maintains the osmotic balance. Mannitol is the source of energy. Calcium and magnesium supports the growth of *rhizobia.*

Formula*

Ingredients in grams per liter

Yeast extract	1.0
Mannitol	10.0
Dipotassium phosphate	0.50
Magnesium sulphate	0.20
Sodium chloride	0.10
Calcium carbonate	1.00
Agar	15.00

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 27.8 gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Yellow coloured, homogenous, free flowing powder.

Prepared Appearance

Whitish buff coloured, opalescent solution forms in petri plates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C.

Organisms (ATCC)	Growth	RGI
RRhizobium meliloti (9930)	Luxuriant	More than 70%
Rhizobium leguminosarum (10004)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Mannitol Agar with Congo Red AM51152

Use

Yeast Mannitol Agar with Congo Red is used for the cultivation of soil microorganisms like *Rhizobium* species.

Summary

Yeast Mannitol Agar with Congo Red is recommended for the cultivation of the symbiotic nitrogen-fixing organisms like *Rhizobium* species (106.1).

Principle

Yeast extract provides amino acids and vitamins while sodium chloride maintains the osmotic balance. Mannitol is the source of energy. Magnesium ion supports the growth of *rhizobia.*

Formula*

Ingredients in grams per liter

Yeast extract	1.0
Mannitol	10.0
Dipotassium phosphate	0.5
Magnesium sulphate	0.2
Sodium chloride	0.1
Congo red	0.025
Agar	20.0

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 31.83gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Orangish red colour. homogeneous, free flowing powder.

Prepared Appearance

Orangish red coloured slightly opalescent gel forms in petri plates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C.

Organisms (ATCC)	Growth	RGI
Rhizobium meliloti (9930)	Luxuriant	More than 70%
Rhizobium leguminosarum (10004)	Luxuriant	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Procedure

Refer to appropriate references for specific procedures for the cultivation of *Rhizobium* species.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yeast Mannitol Broth AM51153

Use

Yeast Mannitol Broth is used for cultivation of *Rhizobium* species.

Summary

Yeast Mannitol Broth is recommended for the cultivation of the symbiotic nitrogen-fixing organisms like *Rhizobium* species (106.1).

Principle

Yeast extract provides amino acids and vitamins while sodium chloride maintains the osmotic balance. Mannitol is the source of energy. Calcium and magnesium supports the growth of *rhizobia.*

Formula*

Ingredients in grams per liter

Yeast extract	1.0
Mannitol	10.0
Dipotassium phosphate	0.50
Magnesium sulphate	0.20
Sodium chloride	0.10
Calcium carbonate	1.00

Final pH (at 25°C) 6.8 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend the 12.8gms of powder in 1000 ml distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

Dehydrated Appearance

Yellow coloured, homogenous, free flowing powder.

Prepared Appearance

Whitish buff coloured, opalescent solution forms in petri plates.

Cultural Response

Cultural characteristics after 5 days at 20-25°C.

Organisms (ATCC)	Growth
Rhizobium meliloti (9930)	Luxuriant
Rhizobium leguminosarum (10004)	Luxuriant

Procedure

Refer to appropriate references for specific procedures for the cultivation of phosphate solubilizing soil microorganisms.

Interpretation of Results

Refer to appropriate references and procedures for results.

Storage

Store at 22- 30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Yersinia Selective Agar Base AM1116/AM5116

Use

Yersinia Selective Agar Base with added supplements is recommended for isolation and enumeration of *Yersinia enterocolitica* from clinical and non-clinical specimens.

Summary

Yersinia Selective Agar Base was first described by Schiemann (102) as an alternative to MacConkey Agar and other commonly used media for isolation of *Y.enterocolitica,* a causative agent of gastroenteritis and was found to be far superior. The formula is based on CIN (Cefsulodin-Igrasan-Novobiocin) Agar of Schiemann and is recommended by the ISO committee and APHA (20).

Principle

The medium differentiates between mannitol fermenting and non-mannitol

fermenting bacteria. Fermentation of mannitol in the presence of neutral red results in a characteristic "bull's-eye" colony, colourless with red center. Sodium deoxycholate and crystal violet inhibit most gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for *Yersinia* by inhibiting normal enteric pathogens. Organisms that do not metabolize mannitol to acid end products form colourless, translucent colonies.

Formula*

Ingredients in grams per liter

Mannitol	20.00
Peptone, Special	20.00
Sodium Pyruvate	2.00
Yeast Extract	2.00
Sodium Chloride	1.00
Sodium Deoxycholate	0.50
Neutral Red	0.03
Magnesium Sulphate	0.01
Crystal Violet	0.001
Agar	12.50

Final pH at (25°C) 7.4 ± 0.2

* Formula adjusted to suit performance parameters

Directions

1. Suspend 29 gms of the powder in 500 ml distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 lbs pressure) for 15 minutes.
5. Cool to 45-50°C and add reconstituted contents of 1 vial of Yersinia Selective Supplement (AS029).
6. Mix well and pour into sterile petri plates.

Quality Control

Dehydrated Appearance

Beige coloured, homogeneous, free flowing powder.

Prepared Appearance

Orange red coloured, clear to slightly opalescent gel.

Cultural Response

Cultural characteristics after 48 hours at 32°C.

Organisms (ATCC)	Growth	Colour of Colony	RGI
Enterococcus faecalis (29212)	Inhibited	-	0%
Escherichia coli (25922)	Inhibited	-	0%
Pseudomonas aeruginosa (27853)	Inhibited	-	0%
Yersinia enterocolitica (27729)	Good to luxuriant	Translucent with dark pink center and bile precipitate.	More than 70%

For growth RGI should be more than 70%

RGI- Relative Growth Index

Interpretation of Results

1. Typical *Y.enterocolitica* colonies will have deep red centers surrounded by a transparent border giving the appearance of a "bull's-eye". Growth of other organisms is markedly inhibited.

Precautions / Limitations

1. *Serratia liquefaciens, Citrobacter freundii* and *Enterobacter agglomerans* may grow on this medium and resemble *Y.enterocolitica*, which needs to be differentiated by biochemical tests.

Storage

Store at 22-30°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.