Oxidase Discs

DD018

Oxidase Discs are used for detection of oxidase production by microorganisms like Neisseria, Alcaligenes, Aeromonas, Vibrio's, Campylobacter and Pseudomonas, which give positive reactions and for excluding Enterobacteriaceae, which give negative reactions.

Directions

Oxidase reaction is carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction is observed within 5-10 seconds at 25-30°C. A change later than 10 seconds or no change at all is considered negative reaction.

Precautions

1. ,,Do not use stainless steel or nichrome inoculating wires, as false positive reaction may result from surface oxidation products formed during flame sterilization.

2. ,,Growth from media containing dyes is not suitable for testing.

3. ,,Timing is critical (5-10 sec) for interpretation of results.

4. ,,Perform oxidase test on all gram-negative bacilli.

5. ,,Cytochrome oxidase production may be inhibited byacid production. False negative reactions may be exhibited by Vibrio, Aeromonas and Plesiomonas species when grown on a medium containing fermentable carbohydrate e.g. MacConkey Agar (M081). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto-oxidation which may be avoided by adding 0.1% ascorbic acid (3).

Principle And Interpretation

Certain bacteria posses either cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein), which catalyzes the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). In the oxidase test, a colourless dye such as N, N-dimethy-p-phenylenediamine serves as an artificial electron acceptor for the enzyme oxidase. The dye is oxidized to form indophenol blue, a coloured compound. The test is useful in the initial characterization of aerobic gram-negative bacteria of the genera Aeromonas, Plesiomonas, Pseudomonas, Campylobacter and Pasteurella.

Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. These discs overcome the neccessity of daily preparation of fresh reagent. Gordon and McLeod (1) introduced oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and a-naphthol. Gaby and Hadley (2) introduced a more sensitive method by using N, N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative. In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural response

Typical oxidase reaction given by 18-48 hour culture observed within 5-10 seconds at 25-30°C.

Organism	Reaction Observed
Pseudomonas aeruginosa ATCC 27853	positive : deep purplish blue colouration of disc

Neisseria gonorrhoeae ATCC 19424	positive : deep purplish blue colouration of disc
Escherichia coli ATCC 25922	negative : purplish blue colouration after 10 sec/ no colour change
Staphylococcus aureus ATCC 25923	negative : no colour change

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1.Gordon J. and Mcleod J.W., 1928, J. Path. Bact., 31:185
2.Gaby W.L and Hadley C., 1957. J. Bact., 74:356
3.Steel. K.J. 1962. J. Appl. Bact. 25:445

Revision : 1 / 2011

Spore Strips (Steam Sterilization Monitor Strips)

DD032

Steam Sterilization Monitor Strips are used for evaluating sterilization process. These indicators which are specified by the U.S. military specification MIL-S- 36586 are GMP requirements of U.S. FDA.

Directions

Place indicators in the areas of the pack or load least accessible to steam. Places such as the geometrical center, and the upper and lower regions of both front and rear of the load to be sterilized are considered suitable areas for placement of these indicators. A standard procedure should be established for the routine evaluation of each sterilizer. On completion of the sterilization cycle, remove the indicators from the test loads and deliver them to the laboratory for testing. All sterility tests should be performed in a clean dust free transfer area, preferably under positive air pressure, using rigid aseptic technique throughout the test procedure.

Using sterile scissors, cut open one end of the envelope. Thereafter remove the indicator with sterile tweezers and aseptically transfer it to a tube of sterile Soyabean Casein Digest Medium w/ Yeast Extract and Ferric pyrophosphate (M207) or Soyabean Casein Digest Medium (M011). Incubate the tubes for seven days at 55 - 60°C. Observe the tubes daily. If turbidity develops, failure of the sterilization process is indicated.

Precautions

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Principle And Interpretation

Bacillus stearothermophilus is a thermophilic bacteria which can grow at 65°C and above. The spores are highly heat resistant and are used to monitor autoclave performance (1).

Sterilisation is the freeing of an article from all living organisms including viable spores(1). Sterilization quality control can only be achieved through the use of calibrated biological indicators (endospores). These indicators consist of *Bacillus stearothermophilus* spores impregnated on chromatography paper strips, individually placed into envelopes. Number of spores present per strip : 10^6. These organisms are difficult to destroy because they are more resistant to heat than other vegetative bacteria and viruses. Therefore, if they are destroyed during sterilization,it is assumed that all other life forms are also destroyed. This test is considered the most sensitive check of the autoclaves efficiency.

Precautions :

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Quality Control

Appearance
Filter paper strip impregnated with spores of standard culture of B.stearothermophilus

Number of spores
1000000 spores/strip

Cultural response
Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of st. Soyabean Casein Digest Medium(M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml M011

Growth	Unexposed Spore Strip	Exposed Spore Strip	Positive control	Negative control
Growth in M011	Luxuriant	No growth	Luxuriant	No growth

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B, P., Simmons A (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

Technical Data

Egg Yolk Tellurite Emulsion (50 ml/100 ml per vial) FD046

Sterile stabilized tellurite emulsion of egg yolk recommended for identification of *Staphylococcus* species.

Composition

Ingredients	Concentration
Egg yolk	30ml
Sterile saline	64ml
Sterile 3.5%potassium tellurite solution	6ml
Final pH (at 25°C)	7.6±0.2

Directions:

Warm up the refrigerated Egg Yolk Tellurite Emulsion to 40-45°C. Shake well to attain uniform emulsion (since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043 / M043S / Baird Parker HiVeg™ Agar Base MV043 / Baird Parker Agar Base w/ Sulpha M1140 / HiCrome Aureus Agar Base M1468 . Mix well and pour into sterile petri plates.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Revision : 1 / 2012

Technical Data

Urea 40% (5 ml per vial) FD048

Filter sterilized urea solution recommended for detection of urease activity.

Composition

Per vial sufficient for 100 ml medium

Ingredients	Concentration
Urea	2g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea Solution to room temperature and aseptically add 5 ml in 95 ml sterile, molten, cooled (45-50ºC) Urea Broth Base M111 / Urea Agar Base (Christensen) M112 / M112S / M112I / Urea HiVeg™ Agar Base (Christensen) MV112 / MIU Medium Base M1076 / Hemmes Medium Base M775 or 25 ml in 975 ml Kohn Two Tube Medium No. 1 Base M142 / Kohn Two Tube HiVeg™ Medium No.1 Base MV142 or to Yersinia Identification Broth Base M1221 as desired. Mix well and dispense in sterile tubes.

Storage and Shelf Life

Store at 2-8°C. Use before expiry period on the label.

Revision : 1 / 2012

Technical Data

Potassium Tellurite 1%

FD052

(Final concentration after addition of 8.9 ml sterile distilled water = 1%)

Recommended for the selective isolation of Staphylococci and Corynebacteria.

Composition

To achieve 1% solution dilute the contents in 8.9 ml sterile distilled water.

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

Warm up the refrigerated contents of one vial to room temperature. Add aseptically 8.9 ml sterile distilled water, mix well and add in sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043B / MM043 / MU043 / ME043 / Vogel Johnson Agar Base w/o Tellurite M023 / MM023 / MU023 / Vogel Johnson HiVeg™ Agar Base w/o Tellurite MV023 / Vogel Johnson Agar w/1.5% Agar M023F / Vogel Johnson HiCynth™ Agar Base w/o Tellurite (V.J. HiCynth™ Agar) MCD023 / Mycoplasma Broth Base w/ CV M268 / Mycoplasma HiVeg™ Broth Base w/ CV MV268 / TPEY Agar Base M402 / TPEY HiVeg™ Agar Base MV402 / Tellurite Glycine Agar Base M448 / Cholera Medium Base M558 / Cholera HiVeg™ Medium Base MV558 / Giolitti-Cantoni Broth Base M584I /Dextrose Proteose Peptone Agar Base M734 / Dextrose Proteose Peptone HiVeg™ Agar Base MV734 / Cystine Tellurite Agar Base M881 / Diphtheria Virulence Agar Base M882 / Diphtheria Virulence HiVeg™ Agar Base MV882 / Tryptone Tellurite Agar Base M1056 / Baird Staphylococcus Enrichment Broth Base M1091 / Baird Staphylococcus Enrichment Broth Base, Granulated GM1091 / Tellurite Blood Agar Base M1260 / Mitis Salivarius Agar Base M259 / Mitis Salivarius HiVeg™ Agar Base MV259 / Monsur Medium Base M474 / HiCrome™ ECO157:H7 Agar, Modified M1574A / as desired. Mix well and dispense in sterile Petri plates or tubes.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Revision : 2 / 2017

Nalidixic Selective Supplement

FD130

An antibiotic supplement recommended for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients	Concentration
Nalidixic acid	15mg

Directions:

Rehydrate the content of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add to 1000 ml of sterile, molten, cooled (45-50ºC) Cetrimide Agar Base M024 / Cetrimide HiVeg™ Agar Base MV024 . Mix well and pour into sterile petri plates.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

*** Not For Medicinal Use**

Technical Data

Brucella Selective Supplement, Modified

FD161

An antibiotic supplement recommended for the selective isolation of *Brucella* species from milk.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Polymyxin B sulphate	2500IU
Bacitracin	12500IU
Nystatin	50000IU
Natamycin	50mg
Naildixic acid	2.500mg
Vancomycin	10mg

Directions:

Rehydrate the contents of 1 vial aseptically with 10 ml of 50% methanol. Shake to form a uniform suspension. Add the contents to 500 ml of sterile, molten, cooled (45-50ºC) media such as, Blood Agar Base No.2 M834 / M834A / Blood Agar Base No.2, HiVeg™ MV834 / MV834A / Columbia Blood Agar Base M144 / M144A /Columbia Blood Agar Base HiVeg™ MV144 / MV144A with 5-10% v/v inactivated horse serum RM1239 and 1% w/v sterile dextrose or Brucella Agar Base M074 / Brucella HiVeg™ Agar Base MV074 /Brucella Broth Base M348 /Brucella HiVeg™ Broth Base MV348 / Brucella Selective Medium Base M822 with 5-10% v/v inactivated horse serum RM1239 .Mix well and pour into sterile petri plates / tubes.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Revision : 02 / 2016

*** Not For Medicinal Use**

Technical Data

Anthracis Selective Supplement

FD185

Anthracis Selective Supplement is recommended for the selective isolation of *Bacillus anthracis* .

Composition

Per vial sufficient for 1000 ml medium

*Ingredients	Concentration
Lysozyme	300000Unit
Polymyxin B sulphate	30000Unit

Directions:

Rehydrate the contents of 1 vial aseptically with 10 ml sterile distilled water. Mix well and add aseptically to sterile molten, cooled to (45-50°C) PLET Agar Base M1446 / PLET Agar Base, Modified M1451 .Mix well and dispense as desired.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

*** Not For Medicinal Use**

Technical Data

IMRV/RV Selective Supplement

FD193

An antibiotic supplement recommended for isolation of *Salmonella* from food stuffs and other materials.

Composition

Per vial sufficient for 500ml / 1000ml medium

*Ingredients	Concentration
Novobiocin	10mg

Directions:

Rehydrate the contents of one vial aseptically with 5 ml of sterile distilled water and aseptically add it to 1000 ml sterile, molten, cooled (45-50°C) Semisolid IMRV Medium Base M1427 / Semisolid IMRV HiVeg™ Medium Base MV1427 / Modified Semisolid RV Medium Base M1482 & 500 ml of Semisolid RV Medium Base M1428 / Semisolid RV HiVeg™ Medium Base MV1428 . Mix well and pour into sterile petri plates.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

*** Not For Medicinal Use**

MKTT Novobiocin Supplement

FD203

A selective supplement for enrichment and isolation of *Salmonella* species.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients	Concentration
Novobiocin	40mg

Directions:

Rehydrate contents of 1 vial aseptically with 5 ml of sterile distilled water and aseptically add to sterile, cooled (45-50ºC) Mueller Kauffman Tetrathionate Novobiocin Broth Base M1496I . Mix well and dispense as desired.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

*** Not For Medicinal Use**

Iron Sulphate Supplement

FD237

A supplement recommended for preparation of Modifide Iron Sulphite Agar which is used for the detection and enumeration of Clostridia species.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
Iron sulphate	0.700g

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add it to 500 ml sterile Modified Iron Sulphite Agar Base M1629 . Mix well and pour in to sterile Petri plates.

Storage and Shelf Life

Store at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

Starch soluble, Hi-AR™/ACS GRM3029

Product Identifier

CAS No.	:	9005-84-9
EC No.	:	232-679-6
Molecular Formula	:	$(C_6H_{10}O_5)n$
Molecular Weight	:	(162.14)n
HS Code	:	3505 10 90
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	White powder or solid
Solubility	:	33.3 mg soluble in 1 mL of hot water
pH (2% in water at 25°C)	:	5.00 - 7.00
Clarity	:	1% in boiling water gives clear solution
Residue after ignition	:	<= 0.40%
Sensitivity	:	Passes test

Safety Information

UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	GM5090000
WGK	:	1

Durham Tubes **GW163**

Durham Tubes are very small size test tubes made from Neutral Glass. The Durham Tube is inverted tube inside the fermentation tube which actually captures the gas produced by microorganisms.

Application : Chemical laboratory, Biological laboratory , Microbiology & Diagnostic sectors , Industrial laboratory and various other laboratories.

Product Name	Product Code	Description	Size (mm)
Durham Tubes	**GW163**	These tube are made of neutral Glass. and they are autoclavable.	Length = 25-27 Diameter = 6-7

Product features :

- Neutral Glass.
- Glass is very clear and transparent.
- The edges are properly polished and hence smooth.
- The bottoms of these Durham tubes are rounded.
- Pack Size : 1 X 100 Nos.

Technical Data

Capsule Stains-Kit

K004

Capsule Stain-Kit is recommended for staining bacterial capsule against dark background.

Composition**

Methylene Blue (aqueous)(S021)

Ingredients

Methylene blue	0.500gm
Distilled water	100.000 ml

Nigrosin stain, 10% w/v (S025)

Ingredients

Nigrosin	10.000 gm
Formalin	0.500 ml
Distilled water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

For Capsule Staining: Using Nigrosin (S025)
1) To a loopful of cerebrospinal fluid, or to a light aqueous or saline suspension of growth from an agar culture, add a loopful of Nigrosin (S025).
2) Mix well and cover with a thin cover glass. If only a few organisms are present, centrifugation of the cerebrospinal fluid may be necessary.
3) Examine promptly with a high power lens. Light may have to be reduced by lowering the condenser. Oil immersion may be used, if higher magnification is required.

For Capsule Staining:Using Methylene Blue (S021)
1) Transfer aseptically a loopful of culture on a clean and dry slide.
2) Mix it with a loopful of aqueous Methylene Blue (S021).
3) Make a smear by using a glass slide.
4) Allow it to air dry slowly.
5) Observe under oil immersion objective.

Principle And Interpretation

Capsules are composed of mucoid polysaccharides of polypeptides. Extracellular capsules are detected by capsule staining. A generally accepted technique for staining capsules employs India ink, nigrosin or congo red (all negative stains) as background material against which the unstained organisms stand out. By counterstaining with dyes like crystal violet or methylene blue, bacterial cell wall takes up the dye. Capsules appear colourless with stained cells against dark background.

Quality Control

Microscopic Examination

Negative staining is carried out and observed under oil immersion lens.

Results

Capsule: Clear halos against dark background

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Revision : 1 / 2015

CE

HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email:techhelp@himedialabs.com Website: www.himedialabs.com

Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

Intended Use:

Recommended as a general purpose medium used for cultivation of a wide variety of microorganisms and recommended for sterility testing of moulds and lower bacteria.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dextrose (Glucose)	2.500
Dipotassium hydrogen phosphate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.

Principle And Interpretation

Soyabean Casein Digest Medium is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium (1,2,3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4).

The combination of Tryptone and soya peptone makes the medium nutritious by providing nitrogenous, carbonaceous substances, amino acids and long chain peptides for the growth of microorganisms. Dextrose/glucose serve as the carbohydrate source and dibasic potassium phosphate buffer the medium. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

Pharmaceutical samples, Clinical samples - urine, pus, wound samples.

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (5,6). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (2).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate.

Reaction
pH of 3.0% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

pH
7.10-7.50

Stability test
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu (at 30-35°C for 18-24 hours for bacteria and 5days for fungal) Growth promotion is carried out as per USP/ EP/BP/JP/IP.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Micrococcus luteus ATCC 9341	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	20 -25 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Sterility Testing- Growth promotion+Validation				
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	20 -25 °C	<=3 d

# *Aspergillus brasiliensis ATCC 16404 (00053*)*	50 -100	luxuriant	20 -25 °C	<=5 d
Candida albicans ATCC 2091 (00055)*	50 -100	luxuriant	30 -35 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	20 -25 °C	<=3 d
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Abony NCTC 6017 (00029)*	50 -100	luxuriant	20 -25 °C	<=3 d
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli NCTC 9002	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	20 -25 °C	<=3 d
Micrococcus luteus ATCC 9341	50 -100	luxuriant	20 -25 °C	<=3 d

Key : (#) Formerly known as *Aspergillus niger*, (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period.

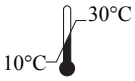
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Indian Pharmacopeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, M.d.
3.The United States Pharmacopeia, 2019, The United States Pharmacopeial Convention, Rockville, MD.
4.Forbes B. A., Sahm D. F. and Weissfeld A. S., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/2022

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, Plot No. C-40, Road No.21Y, MIDC,Wagle Industrial Area, Thane (W) -400604, MS, India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

Disclaimer :

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Brilliant Green Agar Base, Modified

M016

Intended Use:

Recommended for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.0 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (8) and further modified by Kauffmann (7). Brilliant Green Agar is also recommended by APHA (9,10) FDA (2) and described in EP, BP and IP (4,11,12).

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar.

The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphaacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species.

Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

Type of specimen

Clinical : blood, faeces; Foodstuffs & dairy samples; Water samples; Pharmaceutical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,9,13).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus, Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* isolates.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petriplates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli ATCC 8739 (00012*)	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli NCTC 9002	50 -100	none-poor	0 -10 %	yellowish green
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Salmonella Typhi *ATCC 6539*	50 -100	fair-good	30 -40 %	reddish pink
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=50 %	pinkish white
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	pinkish white
Salmonella Abony *NCTC 6017* (00029*)	50-100	good-luxuriant	>=50 %	pinkish white

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6)

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Bacteriological Analytical Manual, 5th Ed, 1978, AOAC, Washington D.C.
4. Indian Pharmacopoeia, 2010, Ministry of Health and Family Welfare, Govt., of India.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
8. Kauffman F., 1935, Seit F. Hyg. 177:26.
9. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Standard Methods for the Microbiological Examination of Dairy Products, 1995, 19th Ed, APHA, Washington, D.C.
11. The British Pharmacopoeia, 2008 vol. II, London.
12. The European Pharmacopoeia, 2008, Council or Europe, Strasbourg.
13. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

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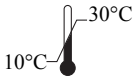

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Peptone Water

M028

Intended Use:

Peptone Water is used as a growth medium and as a base for carbohydrate fermentation media.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.0 grams in 1000 ml distilled water. Add the test carbohydrate in desired quantity and dissolve completely. Dispense in tubes with or without inverted Durhams tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Peptone Water is particularly suitable as a substrate in the study of indole production. Peptone used in Peptone Water is rich in tryptophan content. Presence of indole can be demonstrated using either Kovacs or Ehlrich reagent. Peptone Water is also utilized as a base for carbohydrate fermentation studies with the addition of sugar and indicators such as bromocresol purple, phenol red or bromothymol blue.

Peptone Water is recommended (3,6,7) for studying the ability of an organism to ferment a specific carbohydrate which aid in differentiation of genera and species. Peptone water is formulated as per Shread, Donovan and Lee (9). Peptone Water with pH adjusted to 8.4 is suitable for the cultivation and enrichment of *Vibrio* species. Peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins provides essential nutrients. Sodium chloride maintains the osmotic balance of the medium. To study the fermentation ability of carbohydrates, saccharose, rhamnose, salicin are generally added in 0.5% amount separately to the basal medium before or after sterilization. The acidity formed during fermentation can be detected by addition of phenol red indicator, which shows a colour change of the medium from red to yellow under acidic conditions. If desired, Durham's tube may be used to detect the gas production if produced.

Type of specimen

Isolated microrganism from clinical specimen , food, dairy and water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Due to nutritional variations , some strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution without any precipitate

Reaction

Reaction of 1.5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Indole test
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	negative reaction, no red ring at the interface of the medium on addition of Kovac's reagent (R008)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, red ring at the interface of the medium on addition of Kovac's reagent (R008)
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction, no red ring at the interface of the medium on addition of Kovac's reagent (R008)

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

References

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Finegold and Baron, 1986, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed, ASM, Washington, D.C.
7. MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

8. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

9. Shread P., Donovan T.J, and Lee J.V, (1981), Soc. Gen, Microbiol. Q., 8, 184.

10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 04 / 2019

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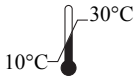

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Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031

Intended use

Recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.68 grams in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. Note : Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.

Principle And Interpretation

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (3) and for the microbiological testing. XLD Agar was formulated by Taylor (13-17) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species.of foods, water and dairy products (2,12,20,21). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (14,16,18, and 4,9,11,19). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (7). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (1).

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by Shigellae but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylate by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. 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 hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (13).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

Clinical samples - Blood, faeces; Food and dairy samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,8).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,20).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(15)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use . Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S.* Paratyphi A, *S.*Choleraesuis, *S.* Pullorum and *S.* Gallinarum may form red colonies without H_2S, thus resembling *Shigella* species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.67% w/v aqueous solution at 25°C . pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Abony *NCTC* 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs

Escherichia coli ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli NCTC 9002	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Proteus vulgaris ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	18 -72 hrs
Salmonella Paratyphi A *ATCC 9150*	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Salmonella Paratyphi B *ATCC 8759*	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis *ATCC* 13076 (00030*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Typhi *ATCC 6539*	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus subsp. *aureus ATCC* 25923 (00034*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Staphylococcus aureus subsp. *aureus ATCC* 6538 (00032*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Enterococcus faecalis ATCC 29212 (00087*)	$>=10^4$	inhibited	0	0%		>=72 hrs

Key : *Corresponding WDCM numbers.
(#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,8).

Reference

1. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, Eur. J. Clin. Microbiol., Inf. Dis. 11, 936-939.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Chadwick P., Delisle G. H and Byer M., 1974, Can. J. Microbiol., 20, 1653-1664.
4. Dunn C. and Martin W. J., 1971, Appl. Microbiol., 22, 17-22.
5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
8. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
9. MacCarthy M. D., 1966, N. Z. J. Med. Lab. Technol., 20, 127-131.
10. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
11. Rollender M. A., Beckford O., Belsky R. D and Kostroff B. 1969, Am. J. Clin. Pathol., 51, 284-286.
12. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
13. Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
14. Taylor W. L. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
15. Taylor W. L. and Harris B., 1967, Am. J. Clin. Pathol., 48:350.
16. Taylor W. L. and Schelhart B., 1967, Am. J. Clin. Pathol., 48:356.
17. Taylor W. L. and Schelhart B., 1968, Am. J. Clin. Pathol., 16:1387.
18. Taylor W. L. and Schelhart B., 1969, Appl. Microbiol., 18.393-395.
19. Taylor W. L. and Schelhart B., 1969, Appl. Micro. 18, 1387-1392.
20. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
21. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.

Revision : 04/ 2019

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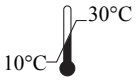

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Baird Parker Agar Base

M043

Intended Use:

Recommended for the isolation and enumeration of coagulase positive staphylococci from food and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
HM Peptone B#	5.000
Yeast extract	1.000
Glycine	12.000
Sodium puruvate	10.000
Lithium chloride	5.000
Agar	20.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 63.0 grams in 950 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (FD045) and 3 ml sterile 3.5% Potassium Tellurite solution (FD047) or 50 ml Egg Yolk Tellurite Emulsion (FD046). For additional selectivity, if desired add rehydrated contents of 1 vial of BP Sulpha Supplement (FD069). Alternatively 1 vial of Fibrinogen Plasma Trypsin Inhibitor Supplement (FD195) may be used per 90 ml medium in place of Egg yolk Tellurite Emulsion (FD046) for identification of coagulase, positive Stapylococci. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Baird Parker Agar was developed by Baird Parker (1,2) from the Tellurite-glycine formulation of Zebovitz et al (3) for isolation and enumeration of Staphylococci in food and other material since it allows a good differentiation of coagulase positive strains. A high correlation has been found between the coagulase test and the presence of clear zone of lipolysis in this medium, which is due to the lecithinase of Staphylococci that breakdown, the egg yolk. On the other hand, studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, whereas other Staphylococci cannot always do so. The medium was found to be less inhibitory to *Staphylococcus aureus* than other media at the same time being more selective (4,5,6). Subsequently the use of Baird-Parker Agar was officially adopted by AOAC International (7) and is recommended in the USP for use in the performance of Microbial Limit Tests (8). Recently, ISO committee has also recommended this medium for the isolation and enumeration of Staphylococci (9).

The identity of *Staphylococcus aureus* isolated on Baird-Parker Agar must be confirmed with a coagulase reaction. Baird-Parker Agar can also be used to detect coagulase activity by adding fibrinogen plasma (10). Fibrinogen Plasma Trypsin Inhibitor supplement (FD195) dissolved in 10 ml sterile distilled water added to 90 ml sterile molten media kept at 45-50°C. On this medium coagulase positive colonies appear white to grey-black surrounded by an opaque zone due to coagulase activity within 24-48 hours incubation at 35°C. Reduction in tellurite is necessary because of absence of egg yolk emulsion. This results in translucent agar and white to grey coloured colonies of Staphylococci. For quantitative results select 20-200 colonies. Count *Staphylococcus aureus* like colonies and test them for coagulase reaction. Report *Staphylococcus aureus* per gram of food. Smith and Baird-Parker (11) found that the addition of 50 mg/l Sulphamethazine in the medium, suppresses the growth and swarming of *Proteus* species.

Tryptone, HM peptone B and yeast extract are sources of nitrogen, carbon, sulphur and vitamins. Sodium pyruvate not only protects injured cells and helps recovery but also stimulates *Staphylococcus aureus* growth without destroying selectivity. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus*. The tellurite additive is toxic to egg yolk-clearing strains other than *S.aureus* and imparts a black colour to the colonies.

Glycine, pyruvate enhances growth of *Staphylococcus*. With the addition of egg yolk, the medium becomes yellow, opaque. The egg yolk additive, in addition to provide enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). A clear zone and grey-black colonies on this medium are diagnostic for coagulase positive Staphylococci. Upon further incubation, an opaque zone is developed around colonies, which can be due to lipolytic activity. When testing the medium, inoculate the material to be examined (0.1 ml per plate of diameter 90-100 mm), incubate at 37°C and take the first reading after 24-26 hours. The colonies of *Staphylococcus aureus* are black and shiny, with a fine white rim, surrounded by a clear zone. Incubate at 37°C for another 24 hours and perform the coagulase test on the colonies with the above characteristics, which have developed during the further incubation period. Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones. The basal medium, without the egg yolk or the tellurite, is perfectly stable. Colonies of some contaminating organisms may digest the coagulase halo reaction. Other bacteria may grow on this media but biochemical test will differentiate coagulase positive Staphylococci from the other organisms.

Type of specimen

Clinical samples : Pus, wounds, Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,13,14). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (15,16). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Though the medium is recommended for detection of coagulase positive *Staphylococcus aureus,* other bacteria may grow.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested with the standard strains, slight variation in growth may be observed depending on the source from where the organism has been isolated.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion and Tellurite Emulsion: Yellow coloured opaque gel forms in Petri plates.

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural response was observed after an incubation at 35-37°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	50 -100	luxuriant	>=50 %	grey-black shiny	Positive, opaque zone around the colony
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50 -100	luxuriant	>=50 %	grey-black shiny	Positive, opaque zone around the colony
Proteus mirabilis ATCC 25933	50 -100	good - luxuriant	>=50%	brown - black	Negative
Micrococcus luteus ATCC 10240	50 -100	poor - good	30 -40 %	shades of brown-black (very small)	Negative
Staphylococcus epidermidis ATCC 12228 (00036*)	50 -100	poor - good	30 -40 %	black	Negative
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50 -100	none - poor	0 -10 %	dark brown matt	Negative
Escherichia coli ATCC 8739 (00012*)	50 -100	none- poor	0 -10 %	large brown black	Negative
Escherichia coli ATCC 25922 (00013*)	50 -100	none- poor	0 -10 %	large brown black	Negative
Escherichia coli NCTC 9002	50 -100	none- poor	0 -10 %	large brown black	Negative

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (15,16).

Reference

1. Baird-Parker A. C., 1962, J. Appl. Bacteriol., 25:12.
2. Baird-Parker A. C. and Davenport E., 1965, J. Appl. Bacteriol., 28:390.
3. Zebovitz E., Evans J. B. and Niven C.F., 1955, J. Bacteriol., 70:686 .
4. Assoc. off. Anal. Chem., 1971, 54:401.
5. Baer, 1971, J. Assoc. Off. Anal. Chem., 54:732.
6. Tardio and Baer, 1971, J. Assoc. Off. Anal. Chem., 54:728.
7. Horwitz (Ed.), 2000, Official methods of analysis of AOAC International, 17th Ed., Vol. I., AOAC International, Gaithersburg, MD.
8. The United States Pharmacopoeia, 2018, The United States Pharmacopoeial Convention. Rockville, MD

9. International Organization for Standardization (ISO), 1983, Draft ISO/DIS 6888.

10. Beckers N. J. et al, 1984, Can. J. Microbiol., 30:470.

11. Smith B. A. and Baird-Parker A.C., 1964, J. Appl. Bacteriol., 27:78.

12. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

13. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

14. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington.

15. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

16. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03 / 2022

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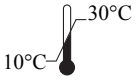

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Brucella Agar Base

M074

Intended Use:

Recommended for enrichment, isolation and cultivation of *Brucella* or *Campylobacter* species from clinical and non-clinical specimens.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Peptone	10.000
Yeast extract	2.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Sodium bisulphite	0.100
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.55 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. If required, for additional selectivity of *Brucella* species: Aseptically add sterile 5% v/v inactivated Horse Serum (RM1239, inactivated by heating at 56°C for 30 minutes) and rehydrated contents of one vial of Brucella Selective Supplement (FD005).

For *Campylobacter*: Add rehydrated contents of 1 vial of Campylobacter Supplement-I (Blaser-Wang)(FD006) or Campylobacter Supplement-II (Butzler) (FD007) or Campylobacter Supplement-III (Skirrow) (FD008) and 5-7% defibrinated sheep blood to 500 ml sterile medium. For growth enhancement add rehydrated contents of 1 vial of Campylobacter Growth Supplement (FD009). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Brucella are intracellular parasites that cause epizootic abortions in animals and septicemic febrile illness or localized infections of bone, tissue or organ systems in humans (8,12). *Brucella* species are highly fastidious and therefore require a nutrient rich medium to be able to grow. Also, *Brucella* species are highly infective and so extreme care should be taken while handling. Brucella Agar Base is used for the isolation and cultivation of *Brucella* species. The basal medium (with addition of Campylobacter Supplements) can be also used for the isolation of *Campylobacter* (9). Brucella Medium is a modified medium formulated to support luxuriant growth of fastidious bacteria like *Brucella*, streptococci, pneumococci, *Listeria, Neisseria meningitides* and *Haemophilus influenzae* (4). Brucella Agar is also recommended by APHA for isolation of *Brucella* species from foods (11).

Tryptone and peptone provide nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients Yeast extract serves as a source of vitamin B complex, and additionally it also supplies some nitrogenous nutrients. Sodium bisulphite is a reducing agent and sodium chloride helps to maintain the osmotic equilibrium of the medium. Dextrose serves as an energy source. The medium can also be enriched with 5 % v/v sterile defibrinated horse blood. For selective isolation of *Brucella* species antibiotic mixtures in the form of freeze dried supplements (FD) are incorporated into the base (3,5,10).

Type of specimen

Clinical : Blood

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,4).
Swab specimens can be directly streaked on the plate. Liquid specimens can be inoculated by means of an inoculation loop. When non-selective medium is required, Brucella Broth Base may be employed with the addition of serum only (i. e. without antibiotics).

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. All presumptive anaerobic organisms must be identified by confirmatory test.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.31% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-72 hours in presence of 10% CO2 with added sterile 5% v/v inactivated horse serum (RM1239) and Brucella Selective Supplement (FD005).

Organism	Inoculum (CFU)	Growth
Brucella melitensis ATCC 4309	50-100	luxuriant
Brucella suis ATCC 4314	50-100	luxuriant
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	>=10^4	inhibited
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,4).

Reference

1. Finegold et al, (Ed.), 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jones L. M. and Brinley M. W. J., 1958, Bull. Wld. Hlth. Org., 19:200.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5.Kuzdas C. D., and Morse E. V., 1953, J. Bacteriol., 66 (4):502

6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.

7. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

8. Moyer N. P., and Holcomb L. A., Laboratory Diagnosis and Infectious Diseases: Principles and Practice, Vol. I, Springer-Verlag, New York

9. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

10. Renoux G., 1954, Ann. Inst. Pasteur, 87 (3):325.

11. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

12. Smith L. D., and Fient T. A., 1990, Crit. Rev.Microbiol., 17 : 209-230

Revision : 04 /2019

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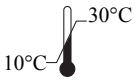

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Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for detection and enumeration of coliform bacteria in water, waste water, dairy products ,other food and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.60 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (1). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (3,4,5). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (6). Lauryl Sulphate Broth was developed by Mallmann and Darby (7). Cowls (6) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (7). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (8).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms.For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. 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. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature. Refer appropriate references for standard procedures (1,6,8).

Type of specimen

Food and dairy samples; Water samples, Clinical samples- faeces

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Due to poor nutritional variations, some strains may show poor growth.

2. Further tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
Staphylococcus aureus subsp aureus ATCC 25923 (00034)*	>=10^4	inhibited		

Key : (#) Formerly known as *Enterobacter aerogenes* (*) corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.

2.Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone

3.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

4.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

5.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

6.Cowls P. B., 1938, J. Am. Water Works Assoc., 30:979.

7.Mallmann W. C. and Darby C. W., 1941, Am. J. Public Health, 31:127

8.International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.

9.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

10.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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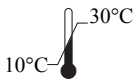

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B12 Assay Agar (Using *E. coli* Mutant Culture)

M110

Intended Use:

(Harrison et al. Medium) for microbiological assay of vitamin B12 using *Escherichia coli* mutant 113-3 Davis ATCC 11105.

Composition**

A complete dehydrated medium for microbiological assay of Vitamin B12 contains all essential nutritives except Vitamin B12 for the growth of *E.coli* mutant 113-3 Davis ATCC11105. The additon of B12 in specified increasing concentration gives a growth response, which can be measured with zone reader.

Final pH (at 25°C) 7.2 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Mix well to distribute slight precipitate evenly. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Generally satisfactory results are obtained with B12 at levels ranging from 0 to 300 ng per ml.

Caution: Over heating or over sterilization will give unsatisfactory results.

Principle And Interpretation

B12 Assay Agar is dehydrated medium devoid of Vitamin B12 but containing all the nutrients essential for the growth of *E. coli* mutant 113-3 Davis ATCC-11105. Incorporation of Vitamin B12 in specified increasing amounts gives a growth response that can be measured by the diameter of the zone of growth around the disc or cup containing Vitamin B12.

For the preparation of Standard, make sterile solutions of Vitamin B12 (Cyanocobalamine Reference Standard). For the determination of Vitamin B12 content of unknown materials the assay sample should be properly diluted and applied similarly as the dilutions of the standards.

Inoculum for the assay is prepared by sub-culturing from a stock culture previously made by stab inoculation. Freshly sub-cultured cells incubated at 35°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for this assay.

Type of specimen

Isolated microorganisms

Specimen Collection and Handling:

Inoculum for the assay is prepared by sub-culturing from a stock culture previously made by stab inoculation. Freshly sub-cultured cells incubated at 35°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for this assay.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidleines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Freshly prepared plates must be used or it may result in erroneous results.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Microbiological assay of Vitamin B12 was carried out using E.coli mutant 113-3 Davis ATCC 11105 as a test organism.Cultural characteristics observed after an incubation at 35- 37°C for 18-24 hours, good growth was obtained around cups containing Vitamin B12 showing an increase in diameter of zone of growth in proportion the increasing Vit B12 concentration in the cup.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1.Harrison,E., Lees, K.A and Wood, F. (1951) Analyst 76: 696.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :02 / 2019

Pseudomonas Agar (For Fluorescein) M120

Intended Use:
Recommended for detection of fluorescein production by *Pseudomonas* species.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Proteose peptone	10.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate	1.500
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 38 grams in 1000 ml purified / distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation
Pseudomonas Agar (For Fluorescein) is based on the formula described by King et al (3) and as modified in the U.S. Pharmacopeia (5) for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species (4). The medium enhances the elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin formation. The fluorescein pigment diffuses from the colonies of *Pseudomonas* into the agar and shows yellow fluorescent colouration. Some *Pseudomonas* strains produce small amounts of pyocyanin resulting in a yellow-green colouration.

Tryptone and proteose peptone provide the essential nitrogenous nutrients, carbon, sulphur and trace elements for the growth of *Pseudomonas*. Dipotassium hydrogen phosphate buffers the medium while magnesium sulphate provides necessary cations for the activation of fluorescein production. Salt concentration exceeding 2% affects pigment production. UV illumination may be bactericidal, so make sure that there is good growth before placing culture under UV light (4).

A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescein. It must, therefore, be differentiated from other simple fluorescent *Pseudomonads* by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C (4).

Type of specimen
Pharmaceutical samples

Specimen Collection and Handling:
For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :
1.This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.8% w/v aqueous solution (containing 1% v/v glycerol) at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added 1% glycerol after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Pseudomonas aeruginosa ATCC 17934	50-100	luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	luxuriant	>=70%	greenish yellow

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44 : 301.
4. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.

Revision : 03/ 2019

Brilliant Green Bile Broth

M121I

Intended Use:

Recommended for isolation and cultivation of coliform organismsfrom cream, yogurt and raw milk. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4831:2006.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Lactose monohydrate	10.000
Dehydrated bile	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.51 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense the medium in quantities of 10ml in test tubes of approximately 16mm x 160mm containing Durham tubes. Sterilize in an autoclave set at 121°C for 15 minutes. Cool to 45-50°C.

Note: The Durham tube shall not contain air bubbles after sterilization.

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO 4831:2006(E) for confirmation of coliform bacteria (1) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and Dehydrated bile present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (4). Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, indicates a positive evidence of faecal coliforms since nonfaecal coliforms growing in this medium do not produce gas. During examination of food samples or environmental samples, growth from presumptive positive tubes showing gas in Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth wherein gas formation within 48 ± 2 hours confirms the presumptive test (1). Gram-positive spore-formers may produce gas if the bile or brilliant green inhibition is weakened by food material.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

Reaction of 3.95% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Gas
Bacillus cereus ATCC 10876	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	positive reaction
Enterobacter aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction
Citrobacter freundii ATCC 43864 (00006*)	50-100	good-luxuriant	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Enterococcus faecalis ATCC 19433 (00009*)	50-100	none-poor	negative reaction
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Standard, ISO 4831:2006 (E). Microbiology of food and animal feeding stuff- Horizontal method for the detection and enumeration of coliforms- Most Probable number technique.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:321.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision :03 / 2019

EC Broth

M127

Intended Use:

Recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique from water samples and from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the concentration of medium in accordance with sample size.

Principle And Interpretation

EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna and Perry (3). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (1) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (6). EC Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of faecal coliforms is required. Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods.

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:

Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (8).

Gas formation at 44.5°C or 45.5°C (and 37°C) *Escherichia coli*, possibly also other coliforms.

Gas formation at 37°C Coliform bacteria without *Escherichia coli*-

Type of specimen

Clinical - faeces ; Food samples; Water sample.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,9,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.For identification, organisms must be in pure culture.
2.Morphological, biochemical and/or serological tests should be performed for final identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured, clear solution without any precipitate

Reaction
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

Organism	Inoculum (CFU)	Growth	Gas
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	positive reaction
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	fair to good	negative reaction
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10^4	inhibited	

Key *- Corresponding WDCM Numbers ; # - Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Fishbein M. and Surkiewicz B. F., 1964, Appl. Microbiol., 12:127.
3. Hajna A. A. and Perry C. A., 1943, Am. J. Public Health, 33:550.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
7. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
8. Ray B., 1986, J. Food Prot., 49:651. 6. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 04 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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Acetamide Broth (Twin Pack)

M148I

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

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	2.000
Part B	-
Sodium chloride	0.200
Potassium dihydrogen phosphate	1.000
Magnesium sulphate anhydrous	0.200
Iron sulphate	0.0005
Sodium molybdate	0.005
Final pH (at 25°C)	7.0±0.5

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.4 grams of part B in 1000 ml distilled water. Add 2 grams of Part A. Heat if necessary to dissolve the medium completely. Dispense in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

A wide variety of pathogenic microorganisms can be transmitted to humans through use of natural fresh and marine recreational waters contaminated by waste water (1, 2). *Pseudomonas aeruginosa* is one of the organisms that are capable of growth in water at very low concentrations of nutrients. While the primary indicators of water quality are *Escherichia coli* and *Enterococci* , the enumeration of *Pseudomonas aeruginosa* in recreational waters may be useful in cases of discharge of pulp and paper wastes and effluents from textile finishing plants into receiving waters. One of the unique properties of *P. aeruginosa* is its ability to produce ammonia from acetamide.

Acetamide Broth, formulated as per DRAFT prEN 12780:1999 is recommended for the confirmation of non-fermentative gram-negative *Pseudomonas aeruginosa* (3). Organisms growing in this medium metabolize acetamide by process of deamination (acrylamidase activity) (4, 5). This ability is shown by *Ps. aeruginosa* , *Ps. acidovorans* Group III (*Achromobacter xylosoxidans*) and *Alcaligens odorans* (6).

Acetamide in the medium serves as a sole source of nitrogen and carbon. Magnesium sulphate, sodium molybdate and iron sulphate are the sources of ions that stimulate metabolism. Phosphate serves as a buffering agent.

The test water samples are filtered through sterile cellulose ester membrane filters. These filters are aseptically placed on Pseudomonas Agar Base (M085) containing Cetrinix Supplement (FD029). These plates with filters are incubated at 35- 37°C for 24-48 hours. Pyocyanin-producing colonies are counted as confirmed *Ps.aeruginosa* . Non-pyocyanin- producing fluorescent colonies are counted as presumptive *Ps.aeruginosa* . These presumptive *Ps.aeruginosa* colonies are confirmed by using Acetamide Broth (M148I)(7). Production of ammonia from acetamide can be detected by the addition of Nesslers reagent (R010).

Quality Control

Appearance

Part A : Colourless deliquescent crystals Part B : Off white to white homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless clear solution

Reaction

Reaction of complete medium (mixture of 0.2% w/v Part A and 0.14% w/vof Part B) at 25°C. pH : 7.0±0.5

pH
6.50-7.50

Cultural Response
M148I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Deamination
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	positive, yellow to brick red colour formation on addition of Nessler's reagent (R010)
Stenotrophomonas maltophilia ATCC 13637	50-100	good-luxuriant	negative, no colour formation on addition of Nessler's reagent R010)

Storage and Shelf Life
Store below 30ºC in tightly closed container and the prepared medium at 2-8º C. Use before expiry date on the label.

Reference
1. Cabelli V. J., 1980, U. S. Environmental Protection Agency, Research Triangle Park, N.C.
2. Dufour A. P., 1984, U. S. Environmental Protection Agency, Research Triangle Park, N.C
3. Directive of Council of the European Union, Draft prEN 12780:1999
4. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:351.
5. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:401.
6. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.
7. International Organisation for Standardization(ISO),2006,Draft ISO/DIS,16266

Revision : 3 / 2015

Cooked M Medium (R.C. Medium)

M149

Intended use

Recommended for cultivation of aerobes and anaerobes, especially pathogenic Clostridia from clinical, food and water samples. This can also be used as a maintenance medium for stock cultures.

Composition**

Ingredients	Gms / Litre
HMH peptone B #	98.000
Proteose peptone	20.000
Dextrose(Glucose)	2.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef heart, solids

Directions

Suspend 12.5 grams in 100 ml purified/distilled water (or suspend 1.25 grams in 10 ml distilled water in test tubes). Mix thoroughly and allow to stand for 15 minutes until all the particles are thoroughly wetted. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Clostridium is a large genus of gram-positive spore-bearing anaerobes. They are normally present in soil, some are responsible for human and animal diseases and others are associated with food spoilage. They may be saccharolytic, decomposing sugars to form butyric and acetic acids and alcohols. The HMH peptone in Robertson's Medium is reddened and gas is produced. Other proteolytic species attack the amino acids. HMH peptone B in Robertson's medium is blackened and decomposed by *Clostridium* species, giving the culture a foul odour. The mesophilic spore-forming anaerobes are of primary importance in the spoilage of low acid foods packed in sealed containers, because of their high heat resistance, their ability to grow in the absence of oxygen and a growth range which covers the temperature of normal storage of canned and other processed foods including the refrigerated storage of cured meats.

Cooked M-Medium was originally developed by Robertson (1) for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped M-Medium (2), which supports the growth of many spore forming and non-spore forming strict anaerobes. It has the ability to initiate growth of bacteria from very small inocula and to maintain the viability of cultures over long period. Mixed cultures of bacteria survive in Cooked M-Medium without displacing the slower-growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic organisms. It is used for cultivation and maintenance of Clostridia and for determining proteolytic activity of anaerobes (2,3). FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods (4).

Cooked M-Medium contains HMH peptone B, which provide amino acids and other nutrients. It also contains glutathione, a reducing substance that permits the growth of obligate anaerobes. The sulfhydryl groups, which impart reducing effect, are more available in denatured protein and hence cooked meat is added in the medium. The addition of dextrose allows rapid and heavy growth of anaerobic bacteria in a short time and leads to more rapid identification of important anaerobes. Growth in this medium is indicated by 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. Inoculation should be made near the bottom of the tube in the meat particles for anaerobic cultures. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium. For the isolation of *Clostridium* from food, use a stomacher to prepare 10% suspension of the food in Peptone Water (M028) diluent. Make dilutions and plate, both suspensions and dilutions on Willis and Hobbs Medium Base (M1375), Tryptose Sulphite

Cycloserine (T.C.S.) Agar Base (M837). Place a metronidazole disc on the inoculum. Incubate anaerobically at 37°C overnight. To count the clostridia, pour the plates with the dilutions on Perfringens Agar Base (O.P.S.P.) (M579). Incubate duplicate plates aerobically and anaerobically to distinguish between clostridia and other organisms. Add some of the suspension to two tubes of Cooked Medium. Heat one tube for 10 min at 80°C and incubate as above. Growth of clostridia is visualized as turbidity or gas bubbles. This medium can be further tested for presence of *Clostridium* (5).

Type of specimen

Clinical samples - Faeces, wounds, tissue, and pus; Food and dairy samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1.Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Brown coloured granules

Colour and Clarity of prepared medium
Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.

Reaction
Reaction of 12.5% w/v aqueous suspension at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth
Clostridium botulinum ATCC 25763	50-100	luxuriant
Clostridium perfringens ATCC 12924	50-100	luxuriant
Clostridium sporogenes ATCC 11437	50-100	luxuriant
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant

Key :(*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Robertson, 1916, J. Pathol. Bacteriol., 20:327.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. MacFaddin J. F., 1985, Media for Isolation - Cultivation - Identification - Maintenance of Medical bacteria, Vol. I, Williams & Wilkins, Baltimore.
4. U. S. Food and Drug Administration, 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va.
5. Collins C. H., Lyne P. M., Grange J. M., 1985, 7th Ed., Microbiological Methods.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

Revision :04/2022

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, Plot No. C-40, Road No.21Y, MIDC,Wagle Industrial Area, Thane (W) -400604, MS, India
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Lysine Decarboxylase Broth without Peptone

M376I

Lysine Decarboxylase Broth w/o Peptone are used for differentiating *Salmonella* Arizonae from the Bethesda Ballerup group of *Enterobacteriaceae* .

Composition**

Ingredients	Gms / Litre
L-Lysine hydrochloride	5.000
Yeast extract	3.000
Dextrose	1.000
Bromocresol purple	0.015
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.01 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amount into screw-capped test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medum in an upright position and overlay with 2-3 ml of sterile mineral oil.

Principle And Interpretation

Decarboxylase media were first described by Moeller (1-3) for detecting lysine and ornithine decarboxylase and arginine dihydrolase. Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (4). Falkows Medium was further modified by Taylor (5) by deleting peptone from the formulation (M376I), thus eliminating false positives caused by *Citrobacter freundii* and its paracolons. Taylor's modification has same advantage of Falkow's formulation over Moeller; it does not require the special conditions of anaerobic culture and low pH.

During the initial stages of incubation, fermentation of dextrose by the organisms, with acid production results in a colour change of the indicator to yellow. On further incubation, if L-Lysine is decarboxylated to cadaverine, there will be an alkaline reaction and the indicator colour will then revert back to purple. If the colour remains yellow, the decarboxylase reaction is negative.

Yeast extract provide essential growth nutrients. Dextrose is the fermentable carbohydrate and bromo cresol purple is the pH indicator. Dextrose non-utilizers will not show any change in the medium colour. Use light inocula and do not read the tests under 24 hours incubation as some organisms require longer incubation time of upto 4 days.

Inoculate 25 grams of the test sample into Buffered Peptone Water (M614S). After incubation at 35-37°C for 16-20 hours, inoculate into RVS Broth (M1491) and Fluid Selenite Cystine Broth (M1533I) and incubate at 35-37°C for 24-48 hours. From the second enrichment, streak a loopful on Brilliant Green Agar Base w/ phosphates (M971S). Presumptive *Salmonella* so isolated on M971S are further confirmed by performing biochemical testing using the following media i.e. Nutrient Agar, pH 7.0 (M561A), Triple Sugar Iron Agar (M021S), Urea Agar Base, Christensen (M112I), Lysine Decarboxylase Broth w/o peptone (M376I), VP test, Indole test.

Quality Control

Appearance
Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear solution without any precipitate

Reaction
Reaction of 0.9% w/v aqueous soloution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response

M376I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Lysine decarboxylation
Citrobacter freundii ATCC 8090	50-100	variable reaction
Escherichia coli ATCC 25922	50-100	variable reaction
Enterobacter aerogenes ATCC 13048	50-100	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883	50-100	positive reaction, purple colour
Proteus mirabilis ATCC 25933	50-100	negative reaction, yellow colour
Proteus vulgaris ATCC 13315	50-100	negative reaction, yellow colour
Salmonella Arizonae ATCC13314	50-100	Positive reaction, purple colour
Salmonella Paratyphi A ATCC 9150	50-100	negative reaction, yellow colour
Salmonella Typhi ATCC 6539	50-100	positive reaction, purple colour
Serratia marcescens ATCC 8100	50-100	positive reaction, purple colour
Shigella dysenteriae ATCC 13313	50-100	negative reaction, yellow colour

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:102.
2. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:259.
3. Moeller V., 1955, Acta. Pathol. Microbiol. Scand., 36:158.
4. Falkow, 1958, Am. J. Clin. Pathol., 29:598.
Taylor W. I., 1961, Appl. Microbiol., 9:487.

Revision : 2 / 2015

Giolitti Cantoni Broth Base

M584I

Giolitti Cantoni Broth Base with addition of potassium tellurite is used for selective enrichment of *Staphylococcus aureus* from suspected food stuffs, in accordance with ISO.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Meat extract	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Tween 80	1.000
Final pH (after sterilization)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.20 grams in 1000 ml distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mmx200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.1 ml of 1% Potassium Tellurite Solution (FD052) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

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

Principle And Interpretation

Giolitti-Cantoni (1) formulated the broth base and Mossel et al (2) recommended it for detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent in 1 gram of sample. It is also recommended by ISO Committee (3) for the examination of meat and meat products.

Mannitol and sodium pyruvate present in the basal medium act as growth stimulants for *Staphylococcus aureus* , aiding in detection of small number of organisms (4). Lithium chloride inhibits gram-negative lactose fermenting bacilli (5). Potassium tellurite and glycine inhibit gram-positive bacilli. Addition of sterile paraffin wax to the inoculated medium inhibits *Micrococci* due to creation of anaerobic conditions. Potassium tellurite concentration must be reduced as per the weight of test sample (0.1 - 0.01 gram). The medium should be inoculated as soon as it has been cooled after sterilization, otherwise absorbed oxygen should be expelled by placing the tubes in free-flowing steam for 15 - 20 minutes.

Inoculate 1 gram of sample or 1 ml of a suitable dilution of a sample into 19 ml of Giolitti-Cantoni Broth tubes in duplicate. Overlay the medium with 5 ml molten sterile paraffin wax and incubate at 37°C for 24-48 hours and examine daily. Blackening of the medium (usually at the bottom) within 48 hours indicates the presence of *Staphylococcus aureus* . The blackened medium, when streaked on Baird Parker Agar (M043), shows black colonies surrounded by clear zones (6).

Quality Control

Appearance

Cream to brownish yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured clear solution without any precipitate.

Reaction

Reaction of 5.52% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Tellurite reduction
Cultural Response			
Escherichia coli ATCC 25922	>=10^3	inhibited	Negative reaction
Micrococcus luteus ATCC 10240	>=10^3	inhibited	Negative reaction
Staphylococcus aureus ATCC 25923	50-100	luxuriant	Positive, blackening of the medium

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1.Giolitti C. and Cantoni C., 1966, J. Appl. Bact., 29:395.
2.Mossel D.A.A.,Harrewijn G.A. and Elzebroek J.M., 1973, UNICEF.
3.International Organization for Standardization (ISO), 2003, Draft ISO 6888-3:2003(E).
4.Baird-Parker, A.C.,1962, J.Appl.Bact.,25:12.
5.Lambin S. and German A., 1961, 'Precis de Microbiologie', pg. 63, Paris Masson.
6.De Waart J., Mossel D.A.A., Ten Broeke R. and Van de Moosdijk A.,1968, J.Appl,Bact. 31:276.

Revision : 2 / 2015

Brucella Selective Medium Base

M822

Intended Use:

Recommended for the isolation and identification of *Brucella* species.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	500.000
Tryptose	10.000
Sodium chloride	5.000
Gelatin	1.000
Dextrose (Glucose)	2.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef heart, infusion from

Directions

Suspend 21.75 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add sterile 10% v/v Sheep blood. Also add rehydrated contents of one vial of Brucella Selective Supplement (FD005). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Brucellosis is a zoonotic disease with a domestic animal reservoir. It is an occupational disease of veterinarians, microbiologists, farmers etc. The route of infections is genital, nasopharyngeal, gastrointestinal, conjunctival, respiratory and through abraded skin (6,7). Brucellosis in humans has a variable incubation period, an insidious or abrupt onset and no pathognomic symptoms or signs. Brucella Agar was designed for cultivating *Brucella* species from diagnostic specimens. With the incorporation of blood or other nutritious substances, it facilitates the cultivation of variety of fastidious anaerobic organisms (2). However, Brucella Medium is supplemented with antibiotics to prevent overgrowth of other accompanying organisms. Brucella Agar Base w/ 1.0 % Dextrose was originally developed by Jones and Morgan (5) for preparations of serum-dextrose-antibiotic medium used for the isolation and cultivation of *Brucella* species.

The medium contains HM infusion B and tryptose, which facilitates cultivation of variety of fastidious anaerobic organisms; by providing essential nutrients. Gelatin serves as a source of nutrients. Glucose serves as source of energy. Addition of antibiotics (as FD) makes the medium highly selective for *Brucella* species. Ethyl violet and circulin, which were recommended initially, are no longer used (1).

Type of specimen

Clinical samples: faeces

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. All presumptive anaerobic organisms must be identified by confirmatory test

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured, clear to slightly opalescent gel forms in Petri plates On addition of 10% v/v sterile sheep blood cherry red coloured opalescent gel forms in Petri plates

Reaction
Reaction of 4.35% w/v aqeuous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed in presence of 10% Carbon dioxide (CO2) atmosphere with added sterile 10%v/v sheep blood and Brucella Selective Supplement(FD005),after an incubation at 35-37°C for 24-48 hours

Organism	Growth
Brucella melitensis ATCC 4309	luxuriant
Brucella suis ATCC 4314	luxuriant
Escherichia coli ATCC 25922 (00013*)	inhibited
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	inhibited

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Alton G. G. and Jones L. M., 1967, Lab Technique in Brucellosis, WHO, Geneva.
2. Atlas R. M., 1997, Handbook of Microbiological Media, 2nd Edi., Parks L.C. (Ed.), CRC Press, New York.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Jones Lois M. and Brinley Morgan W. J., 1958, Bull. Wld. Hlth. Org., 19:200-203
6. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
7. Young E. J., 1983, Human Brucellosis, Rev. Infect. Dis., 5:821-842

Revision : 02/2020

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
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EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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Modified Semisolid Rappaport Vassiliadis Medium Base (MSRC) M1428I

Intended Use

Recommended for selective enrichment and isolation of *Salmonella* from food stuffs and environmental samples from the food production area . The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017 .

Composition**

Ingredients	Gms / Litre
Biopeptone #	4.600
Acicase ##	4.600
Sodium chloride	7.300
Potassium dihydrogen phosphate	1.500
Magnesium chloride, hexahydrate	40.00
Malachite green oxalate	0.040
Agar	2.700
Final pH (after sterilization)	5.10- 5.40

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of animal and plant tissue

Equivalent to Acid hydrolysate of casein

Directions

Suspend 39.47 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 47-50°C and aseptically add 1 vial of rehydrated content of IMRV/RV Selective Supplement (FD193). Mix well and dispense into sterile Petri plates.

Note: The motility of *Salmonella* can be drastically reduced when the agar surface becomes too dry. Hence the plates should be well dried before use. If visible moisture occurs on the lid of the plates or the surface of agar, it must be removed. While incubation, incubate the plates aerobically in an upright position for no longer than 24 hours at 42°C.

Principle And Interpretation

Semisolid Rappaport Vassiliadis Medium Base is based on the formulation described by DeSmedt et al (1) for the detection of motile *Salmonella* species from food and environmental specimens. Modified Semisolid Rappaport Vassiliadis Medium Base is recommended by ISO 6579 (2) for detection of *Salmonella* from foodstuffs and the area of food production and food handling. This medium detects more *Salmonella* positive samples than the routinely used enrichment procedures (2, 3, 4).

Bio peptone and Acicase provides the nitrogenous and carbonaceous substances, long chain amino acids, vitamins and other essential growth nutrients. The motility of other microorganisms is largely inhibited by the selective agents (magnesium chloride, malachite green and novobiocin). Sodium chloride maintains osmotic balance. Phosphate buffers the medium.

The working of medium is based on the ability of *Salmonella* species to migrate in the selective medium competing with the other motile organisms, thus producing opaque halos of growth. The motile bacteria will show a halo or zone of growth originating from inoculation spot.

Type of specimen

Food and animal feeding samples, environmental samples in the area of food production and food handling. Samples from primary production stage such as animal faeces, dust and swabs.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The medium is intended for the detection of motile *Salmonella* and is not appropriate for the detection of non-motile *Salmonella* strains.

Quality Control

Appearance
Light yellow to light blue homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.27% Agar gel.

Colour and Clarity of prepared medium
Blue coloured clear to slightly opalescent semisolid gel forms in Petri plates.

Reaction
Reaction of 3.95% w/v aqueous solution at 25°C. pH : 5.10-5.40

pH
5.10-5.40

Cultural Response

Cultural characteristics observed after an incubation at 41.5°C for 24 hours with added IMRV/RV Selective Supplement (FD193)when one drop of culture is inoculated in the centre of the medium plate.(Motility is checked by inoculating a drop of culture in the centre of the medium plate).

Organism	Inoculum (CFU)	Growth	Motility
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	Positive reaction, grey-white turbid zone
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	Positive reaction, grey-white turbid zone
Escherichia coli ATCC 25922 (00013)*	50-100	none-poor	Negative reaction, no turbid zone
Enterococcus faecalis ATCC 29212 (00087)*	>=10^4	inhibited	-

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in tightly closed container. Use freshly prepared medium. Use before expiry period on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. De Smedt J.M., Balderdijk R., Rappold H. and Lautenschlaeger D., 1986, J. Food Prot., 49:510.
2. International Organization for Standardization 6579-1:2017(E), Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 00 / 2019

PLET Agar Base

M1446

PLET Agar Base medium is recommended for the selective isolation and cultivation of *Bacillus anthracis* .

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	500.000
Tryptose	10.000
Sodium chloride	5.000
EDTA	0.300
Thallous acetate	0.040
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.34 grams in 990 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Anthracis Selective Supplement (FD185). Mix well and dispense as desired.

Principle And Interpretation

Anthrax is an infectious disease caused by spores of the bacterium *Bacillus anthracis* .

In human anthrax, the bacillus is usually demonstrable in material from a malignant pustule, sometimes in sputum from pulmonary anthrax and also in the blood in the septicemic stage of all forms of the infections. Man is relatively resistant to anthrax and laboratory workers are rarely infected. However great care should be taken to avoid escape of the long surviving spores into laboratory environment and all the procedures should be carried out in safety cabinet. Anthrax cannot spread directly from human to human but anthrax spores can be transported by human clothings, shoes etc. In humans, anthrax is caused by exposure to dead infected animals, consumptions of infected animal tissue or exposure to light density anthrax spores from animal wool, fur, hide, etc.

PLET Agar Base originally formulated by Knisley (1) is the best selective medium for cultivation of *B.anthracis* (2, 3, 4) from suspected environmental specimens, animal products or clinical specimens, inhibiting *Bacillus cereus* .

Beef heart infusion from solids and tryptose provide the carbonaceous and nitrogenous compounds necessary for growth whereas sodium chloride provides the osmotic equilibrium. Thallous acetate and Polymyxin (FD185) are inhibitory agents allowing growth of *B.anthracis* while inhibiting contaminants. Lysozyme (FD185) specifically suppresses the growth of gram-negative contaminants. The suspected specimen may be used directly for streaking or heat-treated or alcohol-treated specimens can be used for streaking. On incubation at 37°C for 24 hours colonies develop from 30-100% of the *B.anthracis* spores that would grow on non-selective Heart Infusion Agar (M169), being smaller and smoother than on the later medium. PLET Agar Base inhibits growth of most strains of *B.cereus*, *B.subtilis* , other *Bacillus* species, *Enterobacteriaceae* and *Pseudomonas* species. Some strains of *B. cereus* from soil form colonies but they are smaller than those of B. anthracis, minute after 24 hours and moderately sized after 48 hours. Colonies of *B.anthracis* appear in 36-40 hours after incubation at 37°C. Roughly circular, creamy- white colonies with a ground-glass texture are further subcultured on blood agar plates for identification. Capsule production can be seen directly or on blood agar plates (4).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.03% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1446: Cultural characteristics observed with added Anthracis Selective Supplement (FD185), after an incubation at 35-37°C for 36-40 hours.

Organism	Growth
Bacillus anthracis ATCC 14578	luxuriant
Bacillus cereus ATCC 10876	inhibited

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1.Knisely R. F. 1966, J. Bacteriol, 92:784-786.
2.Norris J. R., Berkley C. W., Logan N. A., and ODonnell A. G., 1981, In M. P. Starr et al (Ed) The Prokaryotes: a Handbook on Habitats, Isolation and Identification of Bacteria, Vol. 2, Springer-Verlag, Berlin.
3.Parry J. M., Turnbull P. C. B. and Gibson J. R., 1983, A Colour Atlas of Bacillus species. Wolfe Medical Publications, London, United Kingdom.
4.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C. ,,

Revision : 1 / 2011

Buffered Peptone Water

M1494I

Intended use

Buffered Peptone Water is used as pre-enrichment medium for increasing the recovery of injured *Salmonella* species from foods prior to selective enrichment and isolation. The composition and performance criteria of this medium are as per the applications laid down in ISO 6579-2017.

Composition**

Ingredients	Gms / Litre
Tryptone #	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate.12H2O	9.000
Potassium dihydrogen phosphate	1.500
FinalpH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Enzymatic digest of casein

Directions

Suspend 20.07 grams(equivalent weight of dehydrated medium) in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Microorganisms that are subjected to environmental stresses may become structurally or metabolically damaged or injured. These microorganisms are unable to replicate in selective environments. Therefore these injured organisms must be resuscitated or permitted to repair the damage by incubation in an appropriate, non-selective environment (7). Edel and Kampelmacher (2) noted that sub-lethal injury to Salmonellae may occur in many food preservation processes. Enriching injured cells in Lactose Broth (pH 6.9) may be further detrimental to their recovery (1). Pre-enrichment in Buffered Peptone Water (M1494I) at 35°C for 18-24 hours results in repair of injured cells (6). The buffering system prevents bacterial damage due to change in the pH of the l dcht l - Qdbdmskx HRN bnl l hssdd g`r `krn qdbnl l dmcdc sghr oqd,dmqfbgl dms l dcht l enq sgd cdsdbshmmne *Enterobacteriaceae* from food stuffs and other materials (3).
Inoculate 10 grams specimen in 50 ml of Buffered Peptone Water (M1494I) and incubate at 35°C for 18 hours. Transfer 10 ml from this medium to 100 ml of Mueller Kauffman Tetrathionate Novobiocin Broth Base (M1496I) and Rappaport Vassiliadis Soya Broth (RVS Broth) (M1491) and incubate at 43°C for 24-48 hours and then subculture on selective media like XLD Agar, Modified (M031I). Examine the plates for colonies of *Salmonella* species.

Type of specimen

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variations some strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.(Recovery is observed on XLD Agar, M031I)

Organism	Inoculum (CFU)	Growth	Recovery
Salmonella Enteritidis ATCC13076 (00030*)	50-100	luxuriant	>=50%
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	>=50%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	50-100	fair-good	30-40%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=50%

Key :* Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Angelotti R., 1963, “Microbiological Quality of Foods”, Academic Press, New York.
2. Edel W. and Kampelmacher E. H., 1973, Bull. Wld. Hlth. Org., 48: 167.
3. International Organization for Standardization (ISO), 2017, Draft ISO/DIS, 6579.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Sadovski A. Y., 1977, J. Food Technol., 12.85.
7. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 04/2018

King's Medium B Base w/ 1.5% Agar

M1544F

Kings Medium B Base w/ 1.5% Agar is recommended for the non-selective isolation, cultivation and pigment production of *Pseudomonas* species in accordance with FDA BAM, 1998

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate	1.500
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.00 grams of dehydrated medium in 1000 ml distilled water containing 10 ml of glycerol. Heat to boiling to dissolve the medium completely. Mix well. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically pour into sterile Petri plates.

Principle And Interpretation

Pseudomonas aeruginosa is known to produce two types of pigments, pyocyanin and fluorescein which is a characteristic property and aids in its isolation from from clinical and food samples. An additional pigment entitled pyorubin was reported by King(1). Pyocyanin is green , fluorescein is fluorescent yellow and pyorubin is reddish brown in colour. Some strains produce all the three pigments while the others produce one or two. Kings Medium B Base w/ 1.5% agar, recommended by FDA BAM is particularly suited for fluorescein production(2). This medium can be used as a general medium for the non-selective isolation and pigment production of *Pseudomonas* species from foods, cosmetics etc (3). This media contain proteose peptone, which provides carbonaceous and nitrogenous compounds for the growth of bacteria. Glycerol serves as a source of energy and also as an enhancer in pigment production. Magnesium sulphate also enhances pigment production. Pigments and/ or their derivatives produced by *Pseudomonas* species play a role as siderophores in the iron uptake systems of bacteria, and hence, their production is markedly enhanced under conditions of iron deficiency. The production of pigments especially non-fluorescent blue pigment, pyocyanin is readily demonstrated by culturing on Kings Medium B Base w/ 1.5% Agar, which contains no added iron (4). The addition of dipotassium phosphate increases the phosphorus content of the medium thereby enhancing production of fluorescent pigment.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.8% w/v aqueous solution (containing 1.0 %v/v glycerol) at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Pigment production
Cultural Response				
Pseudomonas aeruginosa ATCC 17934	50-100	good-luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 9027	50-100	good-luxuriant	>=70%	greenish yellow
Burkholderia cepacia ATCC 25609	50-100	good-luxuriant	>=70%	no pigment

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1.King, E. O, M. K Ward, and D. E Raney. 1954. J. Lab and Clin. Med 44: 301-307.
2.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3.Ann, G, and Matthysse. 1998. The Genus Agraobacterium. The Prokaryotes 3 ed.
4.Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin -Madison, Department of Bacteriology.

Revision : 1/ 2015

Cetrimide Agar Base (w 1.3% Agar)

M1742

Intemded Use:

Recommended for the selective isolation of *Pseudomonas aeruginosa* from various materials.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.7 grams in 1000 ml purified / distilled water containing 10 ml glycerin/glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, rehydrated contents of 1 vial of Nalidixic Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cetrimide Agar Base w / 1.3% Agar is recommended as a selective medium for isolation of *Pseudomonas aeruginosa*. It is similar in composition as cited in various pharmacopoeias (1,2,3,4) except that the concentration of agar in this medium is 1.3%.

The original formula was described by King et al (5). It can also be used for determining the ability of an organism to produce fluorescein and pyocyanain. Cetrimide (N-acetyl-N-N,N-trimethylammaonium bromide) in the medium acts as a selective agent inhibiting bacterias other than *Pseudomonas aeruginosa*. It is a quarternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. Magnesium chloride and potassium sulphate incorporated in the medium enhances the production of pigment pyocyanin, which is a blue-green pigment, diffusing into the medium. This improves detection of *Pseudomonas* on this medium. Presence of magnesium ions can also neutralize EDTA, if present in the sample. Gelatin peptone provides the essential nutrients for growth of *Pseudomonas*, while glycerin/glycerol serves as slow and continuous carbon source for the growing cell.

King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas* (5). Cetrimide agar developed by Lowburry (6) is a modification of Tech Agar (Medium A) with addition of 0.1% cetrimide for selective isolation of *P. aeruginosa*. Later, due to the availability of the highly purified cetrimide, its concentration in the medium was decreased (7). The incubation was carried out at 37^0C for a period of 18-24 hours (8). *P. aeruginosa* can be identified due to their characteristic production of pyocyanin, a blue, water soluble, nonfluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape like odour of aminocetophenone (9).

For the isolation of *P. aeruginosa*, plates of cetrimide agar should be inoculated from non-seelctive medium such as Brain Heart infusion Broth (M210) or Soyabean Casein Digest Medium (M011).If the count is high,the test sample can be directly inoculated onto Cetrimide Agar. *P. aeruginosa* colonies may appear blue, blue-green or nonpigmented. Colonies exhibiting fluorescence at 250 nm and a blue green pigmentation are considered as presumptive positive. *P. aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for short time. Fluorescence reappears after the plates are re-incubated. Goto and Enomoto recommended that addition of nalidixic acid aids in inhibiting the growth of accompanying flora (10).

Type of specimen

Clinical samples - pus, urine

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some strains of *Pseudomonas* other than *aeruginosa* species may show poor growth as cetrimide is highly toxic.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel

Colour and Clarity of prepared medium
Light amber coloured, opalescent gel with a slight precipitate forms in Petri plates

Reaction
Reaction of 4.47% w/v aqueous solution containing 1.0% glycerol at 25°C . pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Nalidixic Selective Supplement (FD130) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	Luxuriant(with yellow green pigment)	>=50 %
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	Luxuriant(with yellow green pigment)	>=50 %
Pseudomonas aeruginosa ATCC 25668 (00114*)	50-100	Luxuriant(with yellow green pigment)	<=0 %
Escherichia coli ATCC 25922 (00013*)	>=10^4	Inhibited	
Proteus mirabilis ATCC 29906 (00023*)	>=10^4	Inhibited	
Stenotrophomonas maltophilia ATCC 13637	>=10^4	Inhibited	
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	>=10^4	Inhibited	
Escherichia coli ATCC 8739 (00012*)	>=10^4	Inhibited	
Salmonella Typhimurium *ATCC 14028* (00031*)	>=10^4	Inhibited	

Escherichia coli NCTC 9002	>=10^4	Inhibited
Staphylococcus aureus NCIMB 9518	>=10^4	Inhibited
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	>=10^4	Inhibited

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

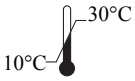
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

1.British Pharmacopoeia, 2019, The Stationery office British Pharmacopoeia.
2.European Pharmacopoeia, 2019, European Dept. for the Quality of Medicines.
3.Japanese Pharmacopoeia, 2016.
4.The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention. Rockville, MD.
5.King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
6.Lowbury, 1951, J.Clin.Path., 4:66.
7.Lowbury and Collins ,1955, J.Clin. Pathol., 8:47.
8.Brown and Lowbury ,1965. .J. Clin. Pathol., 18: 752.
9.Murray, P.R, Baron.J.H., Pfaller M.A., Jorgensen, J.H and YolkenR.H (Ed.) 2003, Manual of Clinical Microbiology,8th Ed., American Society for Microbiology, Washington, D.C.
10.Goto, S. and Enomoto, S.,1970. Japan. J. Microbiol., 14; 65.
11.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
12.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :03 / 2022

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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2,3,5-Triphenyltetrazolium chloride MB188

For Molecular Biology

Product Identifier

CAS No.	:	298-96-4
EC No.	:	206-071-6
Molecular Formula	:	$C_{19}H_{15}N_4Cl$
Molecular Weight	:	334.80
HS Code	:	2933 99 90
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	White to yellow crystals or powder
Solubility	:	33.3 mg soluble in 1 mL of water
DNases	:	None detected
RNases	:	None detected
FTIR	:	Matches with the standard pattern
Melting range	:	235 - 245°C
Assay (AT/NT)	:	min. 99.00%

Safety Information

Hazard Pictogram(s) :

Signal Word	:	Warning
Hazard Statement(s)	:	H315- H319- H335
Precautionary Statement(s)	:	P261- P305+P351+P338
UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	XF8100000
WGK	:	3

Cetrimide Agar

MH024

Intended use

Recommended for the selective isolation of *Pseudomonas aeruginosa* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone #	20.000
Magnesium chloride	1.400
Dipotassium sulphate	10.000
Cetrimide	0.300
Agar	13.600
pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Directions

Suspend 45.3 grams in 1000 ml purified/distilled water containing 10 ml glycerin/glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cetrimide Agar was described by King et al (6). This media formulation is in accordance with the harmonized method of USP/EP/BP/JP/IP (1,2,3,5,9). It is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products. This medium is also used for microbial limit testing for non- sterile products. Lowburry first reported the use of cetrimide as an agent for selective isolation of Pseudomonas (7). This medium is also used for determining the ability of an organism to produce fluorescein and pyocyanin. Cetrimide (N-acetyl-N,N,N-trimethylammonium bromide) is incorporated in the medium to inhibit bacteria other than *Pseudomonas aeruginosa.* This compound a cationic detergent acts as a quaternary ammonium compound, which causes nitrogen and phosphorus to be released from bacterial cells other than *Pseudomonas aeruginosa.* Magnesium chloride and potassium sulphate incorporated in the medium enhances the production of pigment pyocyanin, which is a blue-green pigment, diffusing into the medium. This improves detection of *Pseudomonas* on this medium. Presence of magnesium ions can also neutralizes EDTA, if present in the sample. Gelatin peptone provides the essential nutrients for growth of *Pseudomonas*, while glycerin serves as slow and continuous carbon source for the growing cell.

For the isolation of Pseudomonas aeruginosa, plates of Cetrimide Agar should be inoculated from non-selective medium such as Soybean Casein Digest Medium (MH011). If the count is high the test sample can be directly inoculated onto this medium. *Pseudomonas aeruginosa* colonies may appear pigmented greenish (under uv light also).

Type of specimen

Pharmaceutical samples: Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1,2,3,5,9).

For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (4,8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.
2. Further biochemical tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.36% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel with a slight precipitate forms in Petri plates

pH

7.00-7.40

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100 cfu (at least 100 cfu) (at 30-35°C for >= 72 hours).

Cultural Response

Cultural characteristics observed after incubation at 30-35 °C for 18-72 hours. Recovery rate is considered as 100%for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth promoting						
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=18 hrs
Inhibitory						
Escherichia coli ATCC 8739 (00012*)	>=10^3	inhibited	0	0 %	30 -35 °C	>=72 hrs
Additional Microbiological testing						
Pseudomonas aeruginosa ATCC 27853(00025*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668 (00114*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

Salmonella Typhimurium *ATCC 14028* (00031*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Proteus mirabilis ATCC 29906 (00023)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,8).

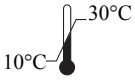
Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017 European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
5. Japanese Pharmacopoeia, 2016
6. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
7. Lowbury E J L., 1951, J.Clin.Path., 4:66.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. , 11th Ed., 2015, Manual of Clinical Microbiology.
9. The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention.Rockville, MD.

Revision : 04/ 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Nessler's Reagent

R010

Nessler's Reagent is used to detect production of ammonia and ammonia salts.

Composition**

Ingredients	
Mercuric chloride	10.0 gm
Potassium iodide	7.0 gm
Sodium hydroxide	16.0 gm
Water (ammonia free)	100.0 ml
Final pH (at 25°C)	13.2±0.05

**Formula adjusted, standardized to suit performance parameters

Directions

Emulsify a 24 hours old culture of organism to be tested for urease test in 0.5 ml substrate in a test tube containing 2% urea. Place the tube in a water bath at 37°C for 3 hours. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes. Read the results after 3 - 5 minutes after adding the Nessler's Reagent. Both negative and control tubes must be absolutely colourless. When isolated colonies are to be examined, the volume of substrate is reduced to 0.3 ml and only one drop of Nessler's reagent is added.

For detecting NH3 production in L-arginine breakdown : Remove a loopful from a 4 day L-arginine culture and place into 0.5 ml of ammonia free distilled water. Add 1 drop of Nessler's reagent. Run the same check on the control.

Principle And Interpretation

Bacteria, particularly those growing naturally in an environment exposed to urine may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH3) production by means of a suitable pH indicator. An alternative method is to test for the production of ammonia from urea by means of Nessler's reagent (1) and/or to detect NH3 production due to L-arginine breakdown (2, 3).

Quality Control

Appearance
Pale yellow coloured solution.

Clarity
Clear with no insoluble particles.Note : On storage of the reagent, precipitate may develop. This will not affect the performance criteria of thereagent.

Reaction
Reaction of the solution at 25°C.

pH
13.05-13.25

Test
Emulsify a 24 hour old culture of organism to be tested for urease test, in 0.5 ml substrate containing 2% urea.Place the tube in a waterbath at 37°C for 3 hours. Remove tube and add 0.1 ml of Nessler's reagent.Read the results after 3-5 minutes.

Results
A positive reaction for presence of ammonia is a colour ranging from a pale yellow to a dark brown precipitate.

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Reference

1) Mackie and MacCartney,1989,Practical Medical Microbiology, Collee J.G.,Duguid J.p.,Fraser A.G.and Marmion B.p. (Eds.),13th ed., Churchill Livingstone, edinburgh.
2) Kauffmann F. and Moller U., 1955, Acta Pathol. Microbial. Scand.,36:173
3) MacFaddin J.,1980,Biochemical Tests for identification of Medical Bacteria,2nd ed. Williams and Wilkins, Baltimore.

Revision : 1 / 2015

CE

Material Safety Data Sheet

Name of the Product : **Gridded Cellulose Nitrate Membrane, Sterile**
Diameter : 47 mm
Pore Size : 0.45 micron

Code No. : **SF97D**

Section 1 : **Chemical Identification**

Code No.	: SF97D
Name of the Product	: Gridded Cellulose Nitrate Membrane, Sterile Diameter : 47 mm Pore Size : 0.45 micron
Produced by	: HiMedia Laboratories Pvt. Ltd.
Address	: 23, Vadhani Indl. Estate, LBS Marg, Mumbai 400 086, India.
Tel. No.	: 2500 0970, 2500 1607 Fax No. 022 2500 2468

Section 2 : **Gridded Cellulose Nitrate Membrane, Sterile**

Section 3 : **Hazards Identification**
Hazard : Not classified as hazardous.

Section 4 : **First - Aid Measures**
No specific measures necessary.

Section 5 : **Fire Fighting Measures**
Not combustible.

Section 6 : **Accidental Release Measures**
No specific measures necessary.

Section 7 : **Handling and Storage**
Handling - Refer to Section 8
Storage - Store below 30°C

Section 8 : **Exposure Controls / Personal Protection**
Not applicable

Section 9 : **Physical and Chemical Properties**
Appearance : Gridded Cellulose Nitrate Membrane

Section 10 : **Stability and Reactivity**
Stability : Product is stable if stored as per the conditions specified under storage of Section No. 7.

Section 11 : **Toxicological Information**
Non toxic.

Section 12 : **Ecological Information**
Data not available

Section 13 : **Disposal Considerations**

No special disposal method required except that it be in accordance with current and local authority regulation.

Section 14 : **Transport Information**

UN No. : Not applicable.

Section 15 : **Regulatory Information**

Risk Phrases : Not applicable

Safety Phrases : Not applicable

Section 16 : **Other Information**

The information contained in this data sheet represents the best information currently available to us. However, no warranty is made with respect to its completeness and we assume no liability resulting from its use. The information is offered solely for user's obligation to investigate and determine the suitability of the information for their particular purpose.

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Dulbecco's Phosphate Buffered Saline

Without Calcium, Magnesium and Phenol red

Product Code: TS1006

Product Description :

All media used in tissue culture have a basis of a synthetic mixture of inorganic salts known as a physiological or balanced salt solution (BSS). All the physiological salt solutions have been derived from the salt solution originally described by Sydney Ringer (1885). The first balanced salt solution to be developed specifically for supporting the metabolism of mammalian cells was Tyrode's solution. Since then many modifications have been done to obtain better buffering salt solutions and to prevent calcium precipitation.

The function of a salt solution is:

- To maintain the medium within physiological pH range.
- To maintain intracellular and extra cellular osmotic balance.
- Modified with a carbohydrate, such as glucose serves as an energy source

TS1006, Dulbecco's Phosphate Buffered Saline without calcium, magnesium and phenol red is most commonly used for tissue disaggregation and monolayer dispersal since presence of Calcium and Magnesium ions may hinder the trypsin activity.

Composition :

Ingredients	mg/L
INORGANIC SALTS	
Disodium hydrogen phosphate	1150.000
Potassium chloride	200.000
Potassium phosphate, monobasic	200.000
Sodium chloride	8000.000

Directions :

1. Suspend 9.6gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water. Stir until dissolved.
2. Adjust the pH to 0.2-0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration.
3. Make up the final volume to 1000ml with tissue culture grade water.
4. Sterilize the solution immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
5. Aseptically dispense the desired amount of sterile solution into sterile containers.
6.Store the liquid solution at ambient temperature.

Material required but not provided :

Tissue culture grade water (TCL010)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)

Quality Control:

Appearance
Off-white to Creamish white

Solubility
Clear solution at 9.6 gms/L

pH without sodium bicarbonate
7.20 -7.80

Osmolality without sodium bicarbonate
275.00 -315.00

Toxicity test
Passes

Endotoxin content
NMT 1EU/ml

Storage and Shelf Life:

1. All the powdered salt mixtures and prepared salt solutions should be stored at ambient temperature. Use before the expiry date. In spite of above recommended storage condition certain powdered salts may show some signs of deterioration /degradation in certain instances. This can be

indicated by change in colour, change in appearance and presence of particulate matter and haziness after dissolution.
2. Preparation of concentrated solutions is not recommended as salt complexes having low solubility may precipitate in concentrated solutions.
3. If desired, sterile supplements can be added to the sterile solution observing all sterility precautions. Shelf life of the solution will depend on the nature of supplements added to the solution.

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