Phenyl Alanine Agar Slant

M281

Intended Use:

Recommended for differentiation of *Proteus* and *Providencia* group of organisms from other members of *Enterobacteriaceae* on the basis of their ability to form phenyl pyruvic acid from phenylalanine.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Sodium chloride	5.000
DL-Phenylalanine	2.000
Disodium hydrogen phosphate	1.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Allow the tubed medium to cool in a slanting position.

Principle And Interpretation

The ability of *Proteus* species to convert phenylalanine to phenylpyruvic acid is an important reaction in the differentiation of *Enterobacteriaceae* (3,7). Based on this criterion, Buttiaux developed Phenylalanine Agar for differentiation of *Proteus* and *Providencia* group from other members of *Enterobacteriaceae* (1,6) by the ability of organism in the genera within *Proteus* to deaminate phenylalanine. Phenylalanine Agar is the modification of the medium originally developed by Ewing et al (2).

Yeast extract in the medium supports the growth of the organisms. Sodium chloride maintains osmotic equilibrium. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid. A recommended technique is to inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 0.2 ml of 10% ferric chloride solution so that the solution floods all over the growth. The addition of (0.2 ml 3-5 drops) of a 10% aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 ml of concentrated HCl per 100 ml of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly (6,7).

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

A recommended technique is to inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 0.2 ml of 10% ferric chloride solution so that the solution floods all over the growth. The addition of (0.2 ml 3-5 drops) of a 10%aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 ml of concentrated HCl per 100 ml of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly (6,7).
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. Some organism may show poor growth due to nutritional variation.

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 amber coloured slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 12-16 hours

Organism	Inoculum (CFU)	Growth	Phenylalanine deaminase
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	negative reaction
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Proteus vulgaris ATCC 13315	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Providencia alcalifaciens ATCC 9886	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Buttiaux R., Osteux R., Fresnoy R. and Moriamez J., 1954, Ann. Inst. Pasteur Lille., 87:375.

2. Ewing W. H., Davis B. R. and Reavis R. W., 1957, Public Health Lab., 15:153.

3. Henrikson S. D., 1950, J. Bacteriol., 60:225.

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. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.

7. Singer J. and Volcani B. E., 1955, J. Bacteriol., 69:303.

Revision : 1 / 2011

EE Broth, Mossel

M287

Intended Use:

Recommended for selective enrichment of *Enterobacteriaceae* in the bacteriological examination of food.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate	6.450
Potassium dihydrogen phosphate	2.000
Bile, purified#	20.000
Brilliant green	0.0135
Final pH (at 25°C)	7.2±0.2

Equivalent to Ox bile, purified
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.46 grams in 1000 ml purified / distilled water. Dispense in tubes or flasks as desired. Stopper with cotton plugs or loose fitting caps. Heat in free flowing steam or boiling water for 30 minutes. Cool to 45- 50°C. Avoid overheating of the medium. DO NOT AUTOCLAVE.

Principle And Interpretation

The family *Enterobacteriaceae* consists of *Salmonella, Shigella* and other enteric pathogens. These organisms find entry into the food system through faecally contaminated water. Majority of these organisms may be eliminated under the stringent food processing parameters. But some of these organisms may become sublethally injured during the changes in pH, exposure to steam or heat and other unfavourable conditions (6). Therefore the important aspect of food monitoring depends upon the identification and enumeration of these injured cells, after resuscitation. EE Broth, Mossel, formulated by Mossel et. al. (4) is recommended as an enrichment medium for *Enterobacteriaceae* in the biological examination of foods (4) and animal feed stuffs (10).

Peptone and dextrose provide the essential nutrients required for the growth of most of the members of *Enterobacteriaceae.* Brilliant green and Bile, purified, purified inhibit growth of gram-positive bacteria. Lactose-negative, anaerogenic lactose-positive or late lactose-fermenting *Enterobacteriaceae* are often missed by the standard coli-aerogenes test. To overcome this problem, lactose is replaced by dextrose in these media. Phosphates form the buffering system of the medium.The cells damaged while drying or low pH are resuscitated in well-aerated Tryptone Soya Broth (M011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (5), animal feeds (7) and semi-preserved foods (8). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (M581).

Subcultures must be made onto lactose differential media such as MacConkey Agar (M081), Deoxycholate Citrate Agar (M065) or Brilliant Green Agar (M016) for the detection of lactose negative or delayed lactose fermenters. This is used to inoculate MPN tubes prepared using EE Broth. Inoculate a loopful from these tubes onto Violet Red Bile Glucose Agar (M581) after an initial incubation at 35-37°C for 24 hours. Typical pink colonies from M581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (1). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (M287).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).
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. Avoid overheating of the medium as media is heat sensitive.

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 greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Emerald green coloured, clear solution without any precipitate
pH of 4.35% w/v aqueous solution at 25°C .

pH
7.00-7.40

Cultural Response
Cultural response was observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Acid
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 9027(00026*)	50 -100	luxuriant	-
*Staphylococcus aureus subsp. aureus ATCC 6538 (*00032*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	positive reaction, yellow colour
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	-
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	luxuriant	positive reaction, yellow colour
Proteus mirabilis ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour

Salmonella Enteritidis *ATCC*13076 (00030*)	50 -100	luxuriant	variable reaction
Shigella boydii ATCC 12030	50 -100	luxuriant	negative reaction
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=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 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 Organization for Standardization (ISO), 1993, Draft ISO/DIS 7402.

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. Mossel D. A. A., Vissar M. and Cornellisen A. M. R., 1963, J. Appl.Bacteriol., 26(3):444.

5. Mossel D. A. A. and Ratto M. A., 1970, Appl. Microbiol., 20:273.

6. Mossel D. A. A., and Harrewijn G. A., 1972, Alimenta II, 29-30

7. Mossel D. A. A., Shennan J. L. and Clare V., 1973, J. Sci. Fd. Agric., 24: 499.

8. Mossel D. A. A. and Ratto M. A., 1973, J. Food Technol., 8: 97.

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. Van Schothurst M. et al, 1966, Vet Med., 13(3):273.

Revision : 03 / 2019

HIMEDIA®

Technical Data

Soyabean Casein Digest Agar (Tryptone Soya Agar) (Casein Soyabean Digest Agar)

M290

Intended use

Soyabean Casein Digest Agar is a general purpose medium used for cultivation of a wide variety of microorganisms from clinical and non-clinical samples and for sterility testing in pharmaceutical procedures.

Composition**

Ingredients	Gms / Litre
Tryptone #	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of casein

Directions

Suspend 40 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, aseptically add 5% v/v defibrinated blood in previously cooled medium to 45-50°C for cultivation. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Soyabean Casein Digest Agar is a widely used medium, which supports the growth of wide variety of organisms even that of fastidious ones such as *Neisseria, Listeria*, and *Brucella* etc. The medium with addition of blood provides perfectly defined haemolysis zones, while preventing the lysis of erythrocytes due to its sodium chloride content. It has been frequently used in the health industry to produce antigens, toxins etc. It's simple and inhibitor-free composition makes it suitable for the detection of antimicrobial agents in the food and other products. Tryptone Soya Agar is recommended by various pharmacopoeias as sterility testing medium (1,2).

Tryptone Soya Agar conforms as per USP (1) and is used in microbial limit test and antimicrobial preservative - effective test. Gunn et al (3) used this medium for the growth of fastidious organisms and study of haemolytic reaction after addition of 5%v/v blood. The combination of tryptone and soya peptone makes this media nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Sodium chloride maintains the osmotic balance.

Soyabean Casein Digest Agar does not contains X and V growth factors. It can be conveniently used in determining the requirements of these growth factors by isolates of *Haemophilus* by the addition of X-factor (DD020), V-factor (DD021), and X+V factor discs (DD022) factor to inoculated TSA plates (4).

Type of specimen

Pharmaceutical samples, Clinical samples- urine, faeces, abscess etc.

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, handling and processing as per pharmacopeias. 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.This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Further biochemical and serological 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

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of 5-7%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

pH of 4.0% w/v aqueous solution at 25°C .

pH

7.10-7.50

Cultural response

Cultural characteristics was observed after an incubation for Bacterial at 30-35°C 18-24 hours and for Fungal at 30-35°C <=5days.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Observed Lot value (CFU) w/blood	Recovery w/ blood	Haemolysis
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	35 -100	>=70 %	35 -100	>=70%	beta
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	beta
Escherichia coli ATCC 25922 (00013)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 8739 (00012)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 11775 (00090)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
*Escherichia coli NCTC 13167 (00179)**	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli NCTC 9002	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa ATCC 10145 (00024)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Salmonella Abony *NCTC 6017* (00029)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Micrococcus luteus ATCC 9341	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Streptococcus pneumoniae ATCC 6305	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Salmonella Typhimurium *ATCC 14028* (00031)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

Enterococcus faecalis ATCC 29212 (00087)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
# Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	25 -70	50-70%			-
Clostridium perfringenes ATCC 13124 (00007)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

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 20-30°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 (5,6).

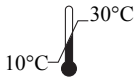
Reference

1.The United States Pharmacopoeia , 2019, The United States Pharmacopoeial Convention Inc., Rockville, MD.
2.Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
3.Gunn B. A., Ohashi D K., Gaydos C. A., Holt E. S., 1977, J. Clin. Microbiol., 5(6) : 650.
4.Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey and 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 : 03/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Tinsdale Agar Base

M314

Tinsdale Agar Base with supplement is used for selective isolation and differentiation of *Corynebacterium diphtheriae* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Sodium chloride	5.000
L-Cystine	0.240
Sodium thiosulphate	0.430
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.67 grams in 1000 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 and aseptically add Diphtheria Virulence Supplement (FD073, Part A and Part B). Mix well and pour into sterile Petri plates.

Principle And Interpretation

The Corynebacteria are gram-positive, non-sporulating, non-motile rods. They are often club-shaped and frequently banded or beaded with irregularly stained granules. These bacteria are generally aerobic or facultative, but microaerophilic species do occur. *Corynebacterium diphtheriae* produces a powerful exotoxin that causes diphtheria in humans. In nature, *C. diphtheriae* occurs in nasopharyngeal area of infected persons or healthy carriers.

The three biotypes of *C. diphtheriae* are *mitis* , *intermedius* and *gravis* (6). The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea (2). Tinsdale Agar Base Medium was developed by Tinsdale (1) for the selective isolation and differentiation of *C. diphtheriae* from diphtheroids. This medium was modified by Billings (2), which improved the recovery and differential qualities of *C. diphtheriae* . The present medium is according to the modified Billings Medium. Moore and Parsons (3) confirmed the halo formation as a characteristic property of *C. diphtheriae* with the exception of *C. ulcerans* , which forms colony with similar features as *C. diphtheriae* .

Peptic digest of animal tissue provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

C. diphtheriae forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as an ideal medium for the routine cultivation and isolation of *C. diphtheriae* . They also confirmed the stability of halo formation on clear medium and its specificity for *C. diphtheriae* and *C. ulcerans* . *C. ulcerans* found in nasopharynx form colonies same as *C. diphtheriae* and require further biochemical confirmation (4).

Do not incubate the plates in 5-10% CO2 as it retards the development of characteristic halos (5). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae* (6). *C. ulcerans* , *C. pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (6).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (6).

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 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M314: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Diptheria Virulence Supplement (FD073, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Corynebacterium diphtheriae type gravis	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type intermedius	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type mitis	50-100	good-luxuriant	>=50%	brown-black with halo
Klebsiella pneumoniae ATCC 13883	>=10^3	inhibited	0 %	
Streptococcus pyogenes ATCC 19615	50-100	good	40-50%	black pin point, without halo

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. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. 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
6. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.

Revision : 1 / 2011

Lactobacillus MRS Broth (MRS Broth)

M369

Intended Use

Recommended for cultivation of Lactobacilli from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM Peptone B#	10.000
Yeast extract	5.000
Dextrose(Glucose)	20.000
Polysorbate 80 (Tween 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Extract

Directions

Suspend 55.15 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes, bottles or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (3), foods (2), faeces (4,5) and other sources (6).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms.

Type of specimen

Clinical samples - faeces, swab from oral cavity; Food and dairy 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 (2,7,8).
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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
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 theexpiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 5.51% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer. (with 5% CO2)

Organism	Inoculum (CFU)	Growth
Lactobacillus fermentum ATCC 9338	50-100	luxuriant
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus saki ATCC 15521 (00015*)	50-100	luxuriant
Lactobacillus lactis ATCC 19435 (00016*)	50-100	luxuriant
Pediococcus pentosaceas ATCC 33316 (00158*)	50-100	luxuriant

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated 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 (4,5).

Reference

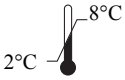
1. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.
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. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.

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.
8.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 : 04/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Malonate Broth

M382

Malonate Broth is recommended for the differentiation of *Enterobacter* and *Escherichia* on the basis of malonate utilization.

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	2.000
Dipotassium phosphate	0.600
Monopotassium phosphate	0.400
Sodium chloride	2.000
Sodium malonate	3.000
Bromothymol blue	0.025
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Dissolve 8.02 grams in 1000 ml distilled water. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid the addition of carbon and nitrogen from other sources.

Principle And Interpretation

Leifson developed a synthetic liquid medium, which differentiated *Aerobacter* (now *Enterobacter*) from *Escherichia* species based on their ability to utilize malonate (1) where *Enterobacter* utilizes malonate and *Escherichia* does not.

An organism that can simultaneously utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces alkalinity due to the formation of sodium hydroxide (2). The alkali changes the color of the bromothymol blue indicator in the medium to light blue and finally to prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Also some malonate-positive organisms produce only a slight alkalinity that causes the results to be difficult to interpret. Therefore these tubes should be compared with an un-inoculated malonate tube (2).

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

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

Reaction
Reaction of 0.8% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M382: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours .

Organism	Inoculum (CFU)	Growth	Malonate Utilization
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction, dark blue colour
Escherichia coli ATCC 25922	50-100	poor-fair	negative reaction
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	positive reaction, dark blue colour

Salmonella Arizonae ATCC 13314	50-100	luxuriant	positive reaction, dark blue colour
Salmonella Typhimurium ATCC 14028	50-100	fair-good	negative reaction

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. Leifson, 1933, J. Bact., 25:329.
2. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore

Revision : 2 / 2015

Decarboxylase Broth Base, Moeller(Moeller Decarboxylase Broth Base)

M393

Intended Use:

Recommended to differentiate bacteria on the basis of their ability to decarboxylate the amino acids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B	5.000
Dextrose (Glucose)	0.500
Bromocresol purple	0.010
Cresol red	0.005
Pyridoxal	0.005
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.52 grams in 1000 ml purified / distilled water. Add 10 gm. of L-Lysine, L-Arginine, L-Ornithine or other L-amino acids. When using DL-amino acids, use 2% concentration. Heat if necessary to dissolve the medium completely. When L-Ornithine is added,readjustment of the pH is required. Dispense in 5 ml amount in screw-capped tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.

Principle And Interpretation

Moeller Decarboxylase Broth Base is used for differentiating gram-negative enteric bacilli on the basis of their ability to decarboxylate amino acids. Moeller introduced the Decarboxylase Broth for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase (8). Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale (3) and Gale and Epps (4). Production of ornithine decarboxylase is a helpful criterion in differentiating *Klebsiella* and *Enterobacter* species. *Klebsiella* are nonmotile and do not produce ornithine decarboxylase while *Enterobacter* are motile and produce ornithine decarboxylase except *Enterobacter agglomerans* (7).

This medium contains HM peptone B and peptone which provides nitrogenous and cabonaceous compounds, long chain amino acids and other essential nutrients for the growth of bacteria. Dextrose is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced stimulates decarboxylase enzyme. Decarboxylation of lysine yields cadaverine while putrescine is produced due to ornithine decarboxylation. Arginine is first hydrolyzed to ornithine which is then decarboxylated to form putrescine. Formation of these amines increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Each isolate to be tested should also be inoculated into Moeller Decarboxylase Broth Base medium tube lacking the amino acid.

Inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium which makes the test invalid.

Type of specimen

Clinical samples - Blood; Food and dairy samples; Water 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 (1,9,10).
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 guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations :

1.Some fastidious organisms may show delayed reaction.

2. Overlaying with mineral oil is essential for appropraite 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

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured, clear solution without any precipitate in tubes

Reaction

Reaction of 1.05% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH

5.80-6.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for upto 4 days with addition of appropriate amino acids and overlaying with sterile mineral oil.

Organism	Inoculum (CFU)	Arginine decarboxylation	Ornithine decarboxylation	Lysine decarboxylation
Citrobacter freundii ATCC 8090	50-100	variable reaction	variable reaction	negative reaction, yellow colour
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
Escherichia coli ATCC 25922 (00013*)	50-100	variable reaction	variable reaction	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	negative reaction, yellow colour	negative reaction, yellow colour	positive reaction, purple colour
Proteus mirabilis ATCC 25933	50-100	negative reaction, yellow colour	positive reaction, purple colour	negative reaction, yellow colour
Proteus vulgaris ATCC 13315	50-100	negative reaction, yellow colour	negative reaction, yellow colour	negative reaction, yellow colour
Salmonella Paratyphi A *ATCC 9150*	50-100	delayed positive reaction/positive reaction,purple colour	positive reaction, purple colour	negative reaction, yellow colour

Salmonella Typhi *ATCC 6539*	50-100	delayed positive reaction / negative reaction	negative reaction, yellow colour	positive reaction, purple colour
Serratia marcescens ATCC 8100	50-100	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
Shigella dysenteriae ATCC 13313	50-100	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
Shigella flexneri ATCC 12022 (00126*)	50-100	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
Shigella sonnei ATCC 25931	50-100	variable reaction	positive reaction, purple colour	negative reaction, yellow colour

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.
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 Inc., Washington, D.C.
3. Gale G. F., 1940, Biochem. J., 34:392.
4. Gale and Epps, 1943, Nature, 152:327.
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. MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.
8. Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36:158.
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. 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

IVD — In vitro diagnostic medical device

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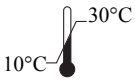

Storage temperature

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Thayer Martin Medium Base

M413

Thayer Martin Medium Base used for selective isolation of Gonococci from pathological specimens.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Starch	1.000
Sodium chloride	5.000
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.0 grams in 450 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 45°C. Aseptically add 50 ml of sterile lysed blood and rehydrated contents of one vial of Vitamino Growth Supplement (FD025) and V.C.N Supplement (FD023) or V.C.N.T Supplement (FD024). If desired GC Supplement with Antibiotics (FD021) can be used as a single supplement. Mix well before pouring into sterile Petri plates. If Hemoglobin (FD022) is used suspend 21.0 grams of Thayer Martin Medium Base in 250 ml distilled water.Heat to boiling to dissolve the medium completely. Prepare 250 ml of 2% hemoglobin solution. Sterilize separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C. Mix both and add the supplements as above.

Principle And Interpretation

Carpenter and Morton reported an improved medium to isolate Gonococci in 24 hours (1). Later on the efficiency of GC medium supplemented with haemoglobin and yeast concentrate was demonstrated for isolating gonococci (2). Subsequently Thayer and Martin Medium was developed for the primary isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis* from specimens containing mixed flora collected from throat, vagina, rectum an d urethra (3, 4). Thayer and Martin (4) used Vancomycin, Colistin and Nystatin. Martin and Lester (5) used an additional antibiotic Trimethoprim to make the medium selective.

Special peptone provides nutrients to the organisms while starch neutralizes the toxic fatty acids if present in the agar. Haemoglobin provides the X factor whereas the V factor (N.A.D.) is provided by the added supplement. Supplement (FD025) also supplies vitamins, amino acids, coenzymes etc. which enhances the growth of pathogenic *Neisseria* . Vancomycin and colistin inhibits gram-positive and gram-negative bacteria respectively (6). Nystatin inhibits fungi. This medium may inhibit *Haemophilus* species. Some strains of *Capnocytophaga* species may grow on this medium when inoculated with oropharyngeal specimens

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium
Basal Medium : Yellow coloured clear to slightly opalescent gel. After addition of haemoglobin or sterile lysed blood and supplements: chocolate coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response

M413: Cultural characteristics observed with added sterile lysed blood/Haemoglobin solution (FD022), Vitamino Growth Supplement (FD025) and V.C.N. Supplement (FD023)/V.C.N.T. Supplement (FD024) after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Neisseria gonorrhoeae ATCC 19424	50-100	good-luxuriant	>=50%	small, grayish-white to colourless, mucoid
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=50%	medium to large, blue-gray, mucoid
Proteus mirabilis ATCC 25933	>=10^3	inhibited	0%	

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. Carpenter and Morton, 1947, Proc. N.Y. State Assoc. Public Hlth. Labs., 27:58.
2. Carpenter et al, 1949, Am. J. Syphil. Gonorrh. Vener. Dis., 33:164.
3. Martin, Billings, Hackney and Thayer, 1967, Public Hlth. Rep., 82:361.
4. Thayer J. and Martin J.E. Jr., 1966, Public Health Rep., 81:559.
5. Martin J.E. Jr. and Lester A., 1971, HSMHA Hlth. Service Rep., 86(1):30.
6. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

Revision : 1 / 2011

Yeast Extract Agar

M456

Yeast Extract Agar is a highly nutritive medium recommended for plate count of microorganisms in water.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Yeast extract	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Yeast Extract Agar is formulated according to the formula described by Windle Taylor (1) for the plate count of microorganisms in water. Water can contain a large number of microorganisms, particularly coming from the earth and vegetation.

Yeast extract and peptic digest of animal tissue provide nitrogenous compounds, vitamin B complex and other growth nutrients. From the water sample, make a decimal dilution bank with Ringer Solution (M525) and take aliquots to 2 parallel series of plates. Pour the molten, cooled (45°C) Yeast Extract Agar and homogenize with sample. Incubate one of the series of plates at 35°C for 24 hours and the other series of plates at 20-22°C for 3 days. Separate counts are made of the organisms forming visible colonies after 24 hours at 35°C and the organisms forming colonies after 3 days at 20-22°C (2). Select the plates containing 30-300 colonies.

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 2.3% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M456: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%

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.Taylor W. E., 1958, The Examination of Waters and Water Supplies, 7th Ed., Churchill Ltd, London, pg. 394, 778.
2.Dept. of Health and Social Security, 1982, report No.71: HMSO, London, 54.

Revision : 02 2015

Leptospira Medium Base, Korthof, Modified

M457

Leptospira Medium is used for isolation, cultivation and maintenance of *Leptospira* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	0.800
Sodium chloride	1.400
Sodium bicarbonate	0.020
Potassium chloride	0.040
Calcium chloride	0.040
Monopotassium hydrogen phosphate	0.240
Disodium hydrogen phosphate	0.880
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

1)Preparation of Base:

Suspend 3.42 grams of M457 in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in 100 ml amounts in flasks. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 55°C.

2) Preparation of Haemoglobin Solution:

To the rabbit blood clot, after removing serum, add equal volume of distilled water. Freeze and thaw repeatedly to haemolyse the corpuscles. Sterilize by Seitz or millipore filtration.

3) Complete Medium:

To 100 ml sterile base, add sterile 8 ml inactivated blood serum and 0.8 ml sterile haemoglobin solution. Mix thoroughly. Distribute if desired in 2-3 ml amount in sterile screw capped Bijou bottles/tubes. Test for sterility by incubating at 37°C.

Principle And Interpretation

Leptospirosis is an acute febrile disease caused by members of the genus *Leptospira* (1,2). Direct culture of blood is the most reliable way to detect *Leptospira* during the first week of illness. After the first week of illness and for several months thereafter, leptospires may be isolated by direct culture of undiluted urine specimens. By autopsy, leptospires may be isolated from kidney and liver tissues as well as from blood and urine. Leptospira Medium Base, Korthof, Modified is formulated as described by Korthof (3, 4) for cultivation and maintenance of *Leptospira* species.

Peptic digest of animal tissue provide amino acids and other nitrogenous substances to support bacterial growth. Haemoglobin solution and inactivated blood serum provide additional sources of nutrients to the Leptospires. The salts supply essential nutrients for the growth of the organisms. Phosphates form buffering system while sodium chloride maintains osmotic equilibrium and also provides essential ions.

All cultures are incubated at room temperature in the dark for up to 6 weeks. The organisms grow below the surface. Material collected from a few centimeters below the surface of broth cultures should be examined weekly for the presence of growth using a direct wet preparation under dark field illumination. Letpospires will exhibit corkscrew like motility (1).

Examine the tubes for growth every 5-7 days. Growth occurs as a ringed area (disc) 1-3 cm below the surface of the medium. The absence of a ringed area of growth doesnt necessarily mean leptospires are not present. Remove a small amount of growth from the disc area and examine microscopically (gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa's stain to show rod forms (3).

Quality Control

Appearance
Off-white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellowish brown coloured, clear to slightly opalescent solution after addition of serum and haemogloin

Reaction
Reaction of 0.342% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M457: Cultural characteristics observed with added inactivated blood serum and sterile haemoglobin solution, after an incubation at 30°C for upto 2-7days.

Organism	Growth
Leptospira interrogans sero.grippotyhosa	luxuriant
Leptospira interrogans sero. Australis	luxuriant
Leptospira interrogans sero. Canicola	luxuriant

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.Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
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.Korthof G., 1932, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.
4.Rechcigl M. Jr. (Ed.), 1978, Handbook Series in Nutrition and Food, Vol. III, CRC Press.

Revision : 1 / 2011

Bile Esculin Azide Agar

M493

Intended Use:

Recommended for selective isolation and presumptive identification of faecal Streptococci from clinical and non-clinical specimen.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
HM peptone B #	5.000
Proteose peptone	3.000
Bile ##	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.1±0.2

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 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. Mix well and pour into sterile Petri plates. **Caution:** Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (10). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (13). Bile Esculin Agar was originally formulated by Swan (15) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (2) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other Enterobacteriaceae genera (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg (6). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (11). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation. All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (11).

Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Clinical sample- Faecal specimen, Food samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (14).
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 nutritional variations, certain 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

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.

Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

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

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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8.Isenberg, 1970, Clin. Lab. Forum, July.
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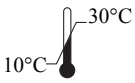
Revision :04/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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MacConkey Broth (Double strength) w/ Neutral Red

M539

MacConkey Broth (Double strength) w/ Neutral Red is recommended for the primary isolation of coliforms from large samples such as water and wastewater.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	40.000
Lactose	20.000
Bile salts	10.000
Sodium chloride	10.000
Neutral red	0.150
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.15 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute into test tubes with inverted Durham tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes before inoculation.

Principle And Interpretation

MacConkey Broth is widely used as a differential medium for detection and enumeration of coliforms from a wide variety of clinical, food and water samples. Identification is based on colour change of the medium due to the presence of the indicator neutral red (1, 2). Peptic digest of animal tissue provides necessary nitrogen source. Lactose serves as the fermentable carbohydrate source. Sodium chloride maintains the osmotic balance of the cells. The selective action of these media is attributed to the presence of bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on these media and are differentiated by their ability to ferment lactose. The colour change of the medium shown by lactose-fermenters is due to production of acid from lactose and a subsequent colour change of the indicator dye when the pH of the media falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* do not alter the appearance of the media.

MacConkey Broth (Double Strength) w/ Neutral Red M539 is recommended for the primary isolation of coliforms form large samples such as water and wastewater. The medium turns pink in case of lactose fermentors and yellow in case of lactose- non-fermenters, due to neutral red. MacConkey Broth Double Strength w/Neutral Red M539 has the same composition in double strength to that of MacConkey Broth (M007), which contains neutral red as an indicator and is considered as a standard medium for the primary isolation as well as presumptive identification of coliform-aerogenes group of organisms in food and water.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

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

Reaction
Reaction of 8.02% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Acid	Gas
Cultural Response				
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction	positive reaction
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction	positive reaction
Salmonella Choleraesuis ATCC 12011	50-100	fair - good	negative reaction	negative reaction
Staphylococcus aureus ATCC 25923	>=10^3	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.MacConkey, 1900, The Lancet, ii:20.
2.MacConkey, 1905, J. Hyg., 5:333.

Revision : 2 / 2015

Violet Red Bile Glucose Agar w/o Lactose

M581

Intended Use:

Recommended for detection and enumeration of *Enterobacteriaceae* in raw food and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Glucose (Dextrose)	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.53 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Agar, a modification of MacConkey original formulation (1) is used for the enumeration of coli-aerogenes bacterial group.

Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (2). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (5-7).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (8).

Type of specimen

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

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 (11-13).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(14). 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. Over incubation may result in reverting of reaction.
2. Further biochemical tests must be carried out on colonies of pure culture 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
Light yellow to pinkish beige homogeneous free flowing powder

Gelling
Firm,comparable with 1.2% Agar gel.

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

Reaction
Reaction of 3.85% w/v of aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics was observed after an incubation at 35-37°C for 18-24 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	good-luxuriant	>=50 %	pink-red
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink-red with bile precipitate
Escherichia coli NCTC 9002	50 -100	luxuriant	>=50 %	pink-red with bile precipitate
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	good-luxuriant	>=50 %	pink to red
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	>=50 %	light pink
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	good-luxuriant	>=50 %	pink-red
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.*aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	

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. 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 (9,10).

Reference

1. MacConkey A., 1905, J. Hyg., 5, 333-379.
2. Mossel D. A. A., Eclderink I., Koopmans M. and Van Rossem F., 1978, Lab. practice, 27 No. 12: 1049.
3. Corry J. E. L., Curtis G. D. W. and Baird R. M., (Ed.), 1995, Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, Elsevier, Amsterdam.
4. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 7402.

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Revision : 05/2022

IVD — In vitro diagnostic medical device

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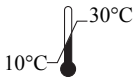

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Giolitti-Cantoni Broth Base

M584

Intended Use:

Recommended for selective enrichment of *Staphylococcus aureus* from food.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
HM peptone B #	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 54.2 grams in 1000 ml purified/distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mm x 200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.3 ml of 3.5% Potassium Tellurite Solution (FD047) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

Principle And Interpretation

Giolitti-Cantoni Broth Base is a fluid medium employed for the recovery of low number of Staphylococci from foodstuffs as described by Giolitti and Cantoni (2). Giolitti- Cantoni Broth was also recommended by Mossel et.al. for detecting *Staphylococcus aureus* in dried milk, baby food and other food products (9). This medium was recommended as an enrichment medium by the International Dairy Federation (IDF) and APHA for detecting *S.aureus* in dried milk and other foods stating that the organism should be absent in 1 gram of sample (4,8). ISO committee has also recommended this medium for examination of meat and meat products (3).

Giolitti-Cantoni Broth Base contains tryptone, yeast extract and HM peptone B as sources of carbon, nitrogen, vitamins and minerals and B-complex vitamins. Mannitol and sodium pyruvate in the basal medium act as growth stimulants for *S. aureus*.

Lithium chloride inhibits gram-negative lactose fermenting bacilli. Potassium tellurite and glycine inhibit gram-positive bacilli (7). 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).

Type of specimen

Food samples

Specimen Collection and Handling

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 *S.aureus*. The blackened medium, when streaked on Baird Parker Agar (M043), shows black colonies surrounded by clear zones (1). 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. 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.

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 brownish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear solution without any precipitate

Reaction

Reaction of 5.42% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural characteristics observed with added 3.5% PotassiumTellurite Solution (FD047), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Tellurite reduction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant	positive, blackening at the bottom of the tubes or general blackening of the medium
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Micrococcus luteus ATCC 10240	>=10^4	inhibited	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	good-luxuriant	positive, blackening at the bottom of the tubes or general blackening of the medium
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibited	
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	poor-fair	variable reaction

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

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Revision : 03 /2021

Slanetz and Bartley Medium

M612

Intended Use

Recommended for detection and enumeration of faecal Streptococci by membrane filtration technique.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Yeast extract	5.000
Dextrose (Glucose)	2.000
Disodium hydrogen phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Slanetz and Bartley Medium was originally devised by Slanetz and Bartley (9) for the detection and enumeration of Enterococci by membrane filtration technique. It can be also used as a direct plating medium (2,7). The medium is highly selective for Enterococci.

Tryptose and yeast extract in the medium provide the necessary nitrogen, carbon, vitamins and minerals required for the growth of organisms. Sodium azide has inhibitory effect on gram-negative organisms. Triphenyl Tetrazolium Chloride is reduced to the insoluble formazan inside the bacterial cell forming dark red-coloured colonies. When the medium is incubated at higher temperature (44-45°C), all red or maroon colonies can be considered as presumptive Enterococci (6,10). The Department of Health (3) has recommended this medium to be used for enumeration of Enterococci in water supplies. Water is filtered through a membrane filter which is then placed on the surface of the Slanetz and Bartley Medium plates and incubated at 35°C for 4 hours and then at 44-45°C for 44-48 hours. Red or maroon colonies are counted as Enterococci. The preliminary incubation at 35°C helps for the recovery of stressed organisms. Not all the species reduce TTC, hence pale colonies also should be considered.

Food samples are homogenized and so diluted with physiological saline to give 15-150 colonies on each Petri plate. Homogenates or dilutions are spread on agar surface and incubated at 35°C for 48 hours. Pink or dark red colonies with a narrow whitish border are counted (7).

Type of specimen

Food; Water samples

Specimen Collection and Handling:

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

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. Further biochemical testing is required for identification of 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
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 4.65% 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 44-45°C for 44-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087)*	50-100	good-luxuriant	>=50%	red or maroon
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	0%	

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 (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. Burkwall M.K. and Hartman P.A., 1964, Appl. Microbiol., 12:18.
3. Department of Health and Social Security, 1982, Report 71, HMSO, London.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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6. Mead G.C., 1966, Proc. Soc. Wat. Treat. Exam., 15:207.
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8. 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.
9. Slanetz L. W. and Bartley C.H., 1957, J. Bact., 74:591.
10. Taylor E.W. and Burman N.P., 1964, J. Appl. Bact., 27:294.

Revision : 03 / 2019

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Buffered Peptone Water

M614S

Buffered Peptone Water is used for pre-enrichment of injured *Salmonella* species from foods prior to selective enrichment and isolation.It is recommended by BIS committee under the specifications IS:5887(Part III)-1999.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Disodium phosphate.12H2O	9.000
Monopotassium phosphate	1.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.07 grams of dehydrated medium in 1000 ml distilled water. Dispense in 50 ml amounts. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes.

Principle And Interpretation

Edel and Kampelmacher (1) noted that sublethal injury to Salmonellae may occur in many food preservation processes. Enriching injured cells in Lactose broth (M1003S) (pH 6.9) may be further detrimental to their recovery (2). Pre-enrichment in Buffered Peptone Water at 35°C for 18-24 hours results in repair of injured cells (3). Recently ISO committee has also recommended this pre-enrichment medium for the detection of *Enterobacteriaceae* (4). Present formulation is recommended by BIS as a non-selective pre-enrichment medium as well as a diluent for detection of *Salmonella* (5).

Inoculate the test sample in Buffered peptone water and incubate at 35 - 37°C for 16 - 20 hours. Transfer the culture to selective enrichment media, Modified Rappaport Vassiliadis Medium (M1137I) and Fluid Selenite Cystine Broth (M025I). Incubate M1137I at 42°C and M025I at 35 - 37°C for 24 hours. Subculture on selective plating media. Examine the plates for colonies of *Salmonella* species.

Quality Control

Appearance
Cream to yellow coloured 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
M614S: Cultural characteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth
Salmonella Typhimurium ATCC 14028	50-100	luxuriant
Salmonella Typhi ATCC 19430	50-100	luxuriant
Salmonella Enteritidis ATCC 13076	50-100	luxuriant

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.Edel W. and Kampelmacher E.H., 1973, Bull. Wld. Hlth. Org., 48:167.
2.Angelotti R., 1963, “Microbiological Quality of Foods”, Academic Press, New York.
3.Sadovski A.Y., 1977, J. Fd. Technol., 12:85.
4.International Organization for Standardization (ISO), 1993, Draft ISO/DIS, 6579.
5.Bureau of Indian Standards, IS : 5887 (Part 3) 1999.

Revision : 2 / 2015

Alkaline Peptone Water

M618

Intended use

Recommended for enrichment of *Vibrio* species from food, water and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	10.000
Final pH (at 25°C)	8.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Clinical materials containing small numbers of *Vibrio* should be inoculated into an enrichment medium prior to plating onto a selective medium, such as TCBS Agar (M189). Alkaline Peptone Water is a suitable enrichment broth for this purpose (1-3).

The relatively high pH of the medium (approximately 8.4) provides a favorable environment for the growth of *Vibrio*'s.

This medium is recommended by APHA (4) for enrichment of *Vibrio* species from seafood, infectious materials and other clinical specimens such as faeces (5).

Peptone provides nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients. Sodium chloride maintains osmotic equilibrium.

Add 10 grams of seafood to 90 ml of Alkaline Peptone Water and incubate for upto 18-20 hours at 37°C. Prolonged incubation will result in growth of the suppressed contaminating organisms to develop (6). Growth in tubes is indicated by turbidity compared to an un-inoculated tube (control). Growth from the enrichment broth is used for plating on selective media. For biochemical identification a pure culture is recommended.

Type of specimen

Clinical samples: faeces; Food samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,7).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
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. Certain strains of *Vibrio* species requiring higher sodium chloride concentration may show poor growth.
2. Further recovery from this enriched broth onto selective media is required.
3. Biochemical characterization is carried out from pure isolates 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

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

Reaction

Reaction of 2% w/v aqueous solution at 25°C. pH : 8.4±0.2

pH

8.20-8.60

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Vibrio cholerae ATCC 15748	50-100	luxuriant
Vibrio parahaemolyticus ATCC 17802 (00037*)	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 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 (3,7).

Reference

1.Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.

2.Gilligan, Janda, Karmali and Miller, 1992, Cumitech 12A, Laboratory Diagnosis of Bacterial Diarrhea, Coord. Ed., Nolte, American Society for Microbiology, Washington, D.C.

3.Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, American Society for Microbiology, 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.Cruikshank R., 1968, Medical Microbiol., 11th Ed., Livingstone Ltd., London

6.Finegold S. M. and Martin W. J., 1982, W. J. Bailey and Scotts Diagnostic Microbiol, 6th Ed., C.V. Mosby Co., St. Louis, p. 242

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.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
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Lactobacillus MRS Agar(MRS Agar) M641I

Intended use

Recommended for the isolation and enumeration of lactic acid bacteria from meat and meat products.The composition and performance criteria of this medium are as per the specifications laid down in ISO 1995, Draft ISO/DIS 13721.

Composition**

Ingredients	Gms / Litre
HM extract B#	8.000
Peptone	10.000
Yeast extract	5.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate, heptahydrate	0.200
Manganese sulphate, tetrahydrate	0.050
Dipotassium phosphate	2.000
Glucose, anhydrous	20.000
Polysorbate 80 (Tween 80)	1.000
Agar	12.000
Final pH (at 25°C)	5.7±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 65.13 grams (the equivalent weight of dehydrated medium per litre) in 1000 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli MRS medium is based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (1), dairy products (6), foods (8), faeces (7) and other sources (5). Lactobacillus MRS Agar is recommended by ISO Committee (2).

Peptone and HM extract B supplies nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Yeast extract provides vitamin B complex and glucose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophillic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate (5). Lactobacilli isolated on MRS Agar should be further confirmed biochemically

Type of specimen

Clinical samples: faeces; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,8,9).

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. Biochemical identification required for confirmation of *lactobacillus* 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

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 6.51% w/v aqueous solution at 25°C. pH : 5.7±0.2

pH

5.50-5.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (longer if neccesary) (with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus acidophilus ATCC 4356 (00098)*	50-100	luxuriant	>=50%
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	luxuriant	>=50%
Lactococcus lactis subsp. lactis ATCC 19435 (00016*)	50-100	luxuriant	>=50%
Lactococcus sakei ATCC 15521 (00015*)	50-100	luxuriant	>=50%
Pediococcus damnosus ATCC 29358	50-100	luxuriant	>=50%
Pediococcus pentosaceus ATCC 33316 (00158*)	50-100	luxuriant	<=10%
Bifidobacterium bifidum# ATCC 11863	50-100	luxuriant	<=10%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibition	0%
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibition	0%

Key : # Growth under anaerobic conditions for 72 hours, *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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.

2.International Organization for Standardization (ISO), 1995, Draft ISO/DIS, 13721

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.MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.

6.Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.

7.Sabine and Vaselekos, 1965, Nature, 206:960.

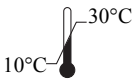
8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

9.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 Marking



Storage temperature

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