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Technical Datasheet

Dulbecco's Modified Eagle Medium (DMEM), High glucose

With L-Glutamine, 4.5gms Glucose per litre, 25mM HEPES buffer and Sodium bicarbonate Without Sodium pyruvate

Product Code: AL067A

Product Description

Dulbecco's Modified Eagle Medium (DMEM) is one of the most widely used modification of Eagle's medium. DMEM is a modification of Basal Medium Eagle (BME) that contains four-fold concentration of amino acids and vitamins. Additionally, the formulation also includes glycine, serine and ferric nitrate. The original formulation contains 1000mgs/L of glucose and was originally used to culture embryonic mouse cells.

DMEM high glucose is a further modification of original DMEM and contains 4500mgs/L of glucose. The additional glucose has proved to be useful in cultivating various other cell lines including primary cultures of mouse and chicken cells as well as various normal and transformed cell lines.

AL067A is DMEM with L-glutamine, 4.5gms per litre glucose, 25mM HEPES buffer and sodium bicarbonate. It does not contain sodium pyruvate. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37ºC prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition:

Ingredients	mg/L
INORGANIC SALTS	
Calcium chloride dihydrate	265.000
Ferric nitrate nonahydrate	0.100
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium bicarbonate	3700.000
Sodium chloride	4400.000
Sodium dihydrogen phosphate anhydrous	109.000
AMINO ACIDS	
Glycine	30.000
L-Arginine hydrochloride	84.000
L-Cystine dihydrochloride	62.570
L-Glutamine	584.000
L-Histidine hydrochloride monohydrate	42.000
L-Isoleucine	105.000
L-Leucine	105.000
L-Lysine hydrochloride	146.000
L-Methionine	30.000
L-Phenylalanine	66.000
L-Serine	42.000
L-Threonine	95.000
L-Tryptophan	16.000
L-Tyrosine disodium salt	103.790
L-Valine	94.000
VITAMINS	
Choline chloride	4.000
D-Ca-Pantothenate	4.000
Folic acid	4.000
Nicotinamide	4.000
Pyridoxal hydrochloride	4.000
Riboflavin	0.400
Thiamine hydrochloride	4.000
i-Inositol	7.200
OTHERS	
D-Glucose	4500.000
HEPES Buffer	5958.000
Phenol red sodium salt	15.900

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Quality Control:

Appearance
Orangish red colored, clear solution.

pH
7.00 -7.60

Osmolality in mOsm/Kg H_2O
310.00 -350.00

Sterility
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response
The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin Content
NMT 1EU/ml

Storage and Shelf Life:

Store at 2-8°C away from bright light.
Shelf life is 12 months
Use before expiry date given on the product label.

Technical Data

Clostridium Difficile Supplement

FD010

An antibiotic supplement recommended for the selective isolation of *Clostridium difficile*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
D-Cycloserine	250mg
Cefoxitin	8mg

Directions:

Rehydrate the contents of one vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add along with 7% v/v defibrinated horse blood to 500 ml sterile, molten, cooled (45-50°C) Clostridium Difficile Agar Base M836 / Clostridium Difficile HiVeg™ Agar Base MV836 / Clostridium Brazier Agar Base M1803 . Mix well and pour into sterile petri plates. Sheep blood may be used in place of horse blood but some strains of the organism will show a slightly reduced growth.

Storage and Shelf Life

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

Revision : 02/2021

*** Not For Medicinal Use**

IVD — In vitro diagnostic medical device

CE Marking

2°C – 8°C — Storage temperature

Do not use if package is damaged

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23 Vadhani Industrial Estate,
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EC REP

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Technical Data

Haemoglobin Powder

FD022

An enrichment supplement recommended for isolation of *Neisseria*.

Composition

Per bottle

Ingredients	Concentration
Haemoglobin powder	50G / 100G

Directions:

A specially prepared powder whose 2% w/v aqueous solution is autoclavable. The aqueous solution is chocolate brown, opaque and contains flocculent dispersible precipitate. It is used for 500 ml medium preparation of GC Agar Base M434 - 5 gms / GC HiVeg™ Agar Base MV434 - 5 gms / Thayer Martin Medium Base M413 - 5 gms / Thayer Martin HiVeg™ Medium Base MV413 - 5 gms / Chocolate No. 2 Agar Base M1548 - 5 gms / Chocolate No. 2 HiVeg™ Agar Base MV1548 - 5 gms / Tellurite Blood Agar Base M1260 - 10 gms / Chocolate Agar Base M103 - 10 gms / Chocolate HiVeg™ Agar Base MV103 - 10 gms / Modified Protease Agar M1606 - 10 gms / Transgrow Medium Base M1149 - 2 gms.

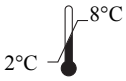
Storage and Shelf Life

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

Revision : 03 / 2021

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Technical Data

Potassium Tellurite 1%

FD052

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

Recommended for the selective isolation of Staphylococci and Corynebacteria.

Composition

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

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

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

Storage and Shelf Life

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

Revision : 2 / 2017

Technical Data

Diphtheria Virulence Supplement (Part A & Part B) FD073

A selective supplement recommended for the isolation and presumptive identification of *Corynebacterium diphtheriae* .

Composition

Per vial sufficient for 1000 ml medium

Ingredients	Concentration
Part A	
Horse serum	100ml
Part B	
Potassium tellurite	1ml

Directions:

Warm up the refrigerated contents of Part B vial and aseptically add 29 ml sterile distilled water. Mix thoroughly. Aseptically add warmed up (to 50°C) contents of Part A and B vials to sterile, molten, cooled (45-50°C) Tinsdale Agar Base M314 / Tinsdale HiVeg™ Agar Base MV314 as required.

For 10 ml of M314 : 1.0 ml of Part A and 0.3 ml of Part B, is recommended.

Mix well and pour into sterile petri plates.

Storage and Shelf Life

Store Part A at -20°C & Part B at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

Nutrient Agar

M001

Intended use

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 28.0 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. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - faeces, urine ; 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 (3,4,7).
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. 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.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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 2.8% 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 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good-luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	good-luxuriant	>=70%
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=70%

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 (5,6).

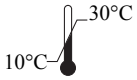
Reference

1.Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
3.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4.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.
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.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
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 : 06/2022

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

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.
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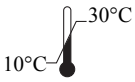
Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE Marking



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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.
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Revision : 1 / 2011

PNY Medium

M835

Intended Use:

Recommended for cultivation and isolation of *Lactobacillus* species.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	5.000
Dextrose (Glucose)	5.000
Potassium dihydrogen phosphate	0.500
Dipotassium hydrogen phosphate	0.500
Magnesium sulphate	0.250
Manganese sulphate	0.010
Ferrous sulphate	0.010
Sodium chloride	0.010
Zinc sulphate	0.001
Copper sulphate	0.001
Cobalt sulphate	0.001
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.28 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.

Principle And Interpretation

Lactobacilli grow in a variety of habitats, wherever high levels of soluble carbohydrate, protein background products, vitamins and a low oxygen tension occur (1). These sites include the oral cavity, the intestinal tract (8,2), the vagina (5), food products (6) and dairy products (7). PNY Medium is formulated for isolation and cultivation of *Lactobacillus* species. Peptone and yeast extract provide amino acids, other nitrogenous nutrients, vitamin B complex etc. Dextrose is the fermentable carbohydrate. The phosphates form buffering system while sodium chloride maintains osmotic equilibrium. Other salts supply essential nutrients for the growth of the organisms.

Type of specimen

Clinical samples - Swabs from oral cavity, Faeces, etc.; 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 (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Further biochemical and serological tests must be carried out for complete identification.
2. Individual organisms differ in growth due to nutritional variations.

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 coloured homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.1% 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 18-24 hours in presence of 3-5% CO2.

Organism	Inoculum (CFU)	Growth
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus leichmannii ATCC 4797	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant

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 (3,4).

Reference

1. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H., (Eds.), The Prokaryotes, 2nd Edi, 1992, Springer-Verlag
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6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/2022

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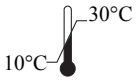	Storage temperature
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Clostridium Difficile Agar Base

M836

Intended Use:

Recommended for selective isolation of *Clostridium difficile* from food and certain pathological specimens.

Composition**

Ingredients	Gms / Litre
Proteose peptone	40.000
Disodium hydrogen phosphate	5.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.100
Sodium chloride	2.000
Fructose	6.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.55 grams in 500 ml purified / distilled water. Heat gently to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Clostridium Difficile Supplement (FD010) together with 7% (v/v) defibrinated Horse blood or Sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The spectrum of disease caused by *Clostridium difficile* (a pathogenic *Clostridium* affecting the bowel) ranges from pseudomembranous colitis (PMC) through antibiotic associated colitis (AAC). It also includes chronic inflammatory bowel diseases, post-operative diarrhoea and non-antibiotic associated diarrhoea (1). Smith and King (6) first reported the presence of *C.difficile* in human infections. George et al (2) recommended the use of a fructose-containing medium with egg yolk for the isolation of *C.difficile* from faecal specimens. The medium was made inhibitory to the accompanying flora by the addition of the selective agents namely, D-cycloserine and cefoxitin.

This medium does not contain neutral red indicator, as in the original formulation, as it is recommended for use with sheep or horse blood (2). Clostridium Difficile Agar Base is used for the primary isolation of *C.difficile*from faecal specimens.The medium composition is designed so as to obtain luxuriant growth of *C.difficile*. The selective agents D-cycloserine and cefoxitin used in the medium inhibit the growth of majority of *Enterobacteriaceae* and also *Enterococcus faecalis,* Staphylococci, gram-negative anaerobic bacilli and *Clostridium* species other than C. difficile, which may be found abundantly in faecal samples. Addition of 7% v/v horse blood to the base increases the recovery of C. difficile and also increases its colony size.

Spread a part of the faecal sample on the medium to obtain isolated colonies. Incubate the plates anaerobically at 37°C for 18 - 48 hours. C. difficile forms grayish white, irregular, raised and opaque colonies, 4-6 mm in diameter, after 48 hours.

Typical gram stain morphology of C. difficile may not be seen in colonies taken from this medium due to the presence of antibiotics. Subculture on Blood Agar (M073) to obtain characteristic morphology. *C.difficile* colonies will not exhibit the typical fluorescence and colour of colony on this medium whereas other Clostridia can give fluorescence. Therefore, for complete identification and confirmation, other tests like gram staining, morphology, biochemicals, specific cytotoxin and clinical observation should be carried out.

Type of specimen

Clinical samples - Stool sample; Food samples.

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition 7% v/v defibrinated horse blood: Cherry red coloured, opaque gel forms in Petri plates.

Reaction
Reaction of 6.91% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement(FD010) and 7% v/v defibrinated horse blood, after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white
Shigella flexneri ATCC 12022	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=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.
Product performance is best if used within stated expiry period.

Disposal

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

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
2. George W. L., Sutter V. L., Citron D., and Finegold S. M., 1979, J.Clin. Microbiol., 9:214
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6. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.

Revision :02 / 2019

IVD — In vitro diagnostic medical device

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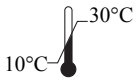

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HiCrome™ Vibrio Agar

M1682

Intended use

HiCrome™ Vibrio Agar is recommended for the isolation, and selective chromogenic differentiation of *Vibrio* species from seafood.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	25.000
Sodium thiosulphate	5.000
Sodium citrate	6.000
Sodium cholate	1.000
Chromogenic mixture	5.500
Agar	15.000
Final pH (at 25°C)	8.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Vibrio' s have played a significant role in human history. Outbreaks of cholera, caused by *Vibrio cholerae*, can be traced back in time to early recorded descriptions of enteric infections. The *Vibrio*'s have also received the attention of marine microbiologists who observed that the readily cultured bacterial population in near-shore waters and those associated with fish and shell fish were predominantly *Vibrio* species (1). *Vibrio* species are mainly responsible for causing cholera and food poisoning in humans. *Vibrio cholerae* causes cholerae due to the intake of contaminated food such as raw oysters. *Vibrio parahaemolyticus* is a major cause of food borne infections, causing food poisoning (2). Since *Vibrio* species naturally occur in sea water, worth special mention is their need for sodium chloride, although some species can grow with minimum sodium chloride concentration (1). The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Peptone Water (3). However accompanying sucrose-fermenting bacteria pose a problem in the identification of *Vibrio* species on TCBS Agar. On HiCrome™ Vibrio Agar, the colour development by *Vibrio* species in not affected by the presence of colonies of other bacteria. This is because, the amount of colour developed depends on the reaction of the bacterial beta-galactosidase with the substrate contained in the media (4).

Peptone provides carbonaceous, nitrogeneous and essential nutrients to the organisms. High concentration of sodium chloride in addition to maintaining the osmotic equilibrium also has an inhibitory action on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are used in the formulation because they can inhibit the growth of gram positive and some gram negative bacteria, but not members of *Enterobacteriaceae*. The proprietary chromogenic mixture incorporated in the medium helps in the chromogenic differentiation of *Vibrio cholerae* and *Vibrio parahaemolyticus* . The high (alkaline) pH of the medium helps in selective isolation of *Vibrio* species.

Type of specimen

Food samples

Specimen Collection and Handling

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

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

Warning and Precautions

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

Limitations

Not applicable

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to light tan 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 6.75% w/v aqueous solution at 25°C. pH : 8.5±0.2

pH

8.30-8.70

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923 (00034*)	>=10^3	inhibited	0%	
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	purple
*Vibrio parahaemolyticus ATCC 17802 (*00037*)	50-100	good-luxuriant	>=50%	bluish green

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store below 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 (6,7).

Reference

1.Thompson et al (ed.). 2006. The Biology of Vibrios, ASM Press, chapter 1, pg 3.
2.Alcamo. E.I, 2001. Fundamentals of Microbiology, 6th ed, Jones and Bartlett Publishers, Inc. pg 254, 244.
3.Clesceri, Greenberg and Eaton (ed.). 1998. Standard Method for the examination of Water and Waste water, 20th ed. American Public Health Association, Washington, D. C.
4.Kudo. H. Y et al, 2001. Improved Method for Detection of ! Vibrio parahaemolyticus @ in Seafood. ASM. Vol 67, 12,Npg 5819-5823.
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6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Isenberg, H. . Clinical icrobiology Procedures Handb0ook. 2nd Edition.

Revision : 02/2018

Barritt Reagent B (for VP test)

R030

The reagent is used in Voges-Proskauer test for detection of acetoin production by bacterial culture.

Composition**

Ingredients

Potassium hydroxide	40.000 gm
Distilled water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

Grow test culture in MR-VP Medium (M070). Add 0.2 ml (2 drops) of Reagent A and 0.2 ml (2 drops) of Reagent B (R030) for 10 ml medium. Shake tubes gently for 30 seconds to 1 minute to expose the medium to atmospheric oxygen in order to oxidize the acetoin (acetylmethylcarbinol) so as to obtain a colour reaction. Allow tube to stand at least 10 to 15 minutes.

Principle And Interpretation

VP test is helpful in identifying members of the family Enterobacteriaceae. Initially all enterics will give a positive MR reaction if tested. However, after further incubation, required by the test procedure (2-5 days), MR - positive organisms continue toproduce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintain an acidic environmentin the medium (pH 4.2 or less).MR- negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methylcarbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above). In the presence of atmospheric oxygen and alkali(potassium hydroxide), the neutral end products, acetoin and 2, 3-butanediol, are oxidized to diacetyl, and a-Naphthol serves as a catalyst to produce a red colour complex.

Quality Control

Appearance

Colourless solution.

Clarity

Clear solution without any precipitate.Note : On storage of the reagent, precipitate may develop. This will not affect the performance criteria of thereagent.

Cultural Response

R030: Biochemical identification was carried out by adding Barritt Reagent (Part A) (R029) and Barritt Reagent (Part B) (R030) in 24-48 hours old cultures grown in MR-VP Medium (M070).

Organism	Growth	VP Test
Enterobacter aerogenes ATCC 13048	Luxuriant	Positive(Red colour formation)
Escherichia coli ATCC 25922	Luxuriant	Negative(No red colour formation)
Klebsiella pneumoniae ATCC 13883	Luxuriant	Positive(Red colour formation)

Storage and Shelf Life

Store at 10-30°C in tightly closed container. Use before expiry period on the label.

Reference

1.Color Atlas and Textbook of Diagnostic Microbiology,4th edition ,Elmer W. Koneman. Stephen D.Allen. ,William M. Janda., Paul C. Schreckenberger.,Washington C.Winn.

Revision : 1 / 2015