PLET Agar Base, Modified

M1451

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

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	500.000
Tryptose	10.000
Sodium chloride	5.000
EDTA	0.350
Thallous acetate	0.040
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.40 grams in 990 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Anthracis Selective Supplement (FD185). Mix well and dispense as desired.

Principle And Interpretation

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

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

PLET Agar Base originally formulated by Knisley (1) is the best selective medium for cultivation of *B. anthracis* (2, 3, 4) from suspected environmental specimens, animal products or clinical specimens, inhibiting *Bacillus cereus* .PLET Agar Base, Modified Medium is similar to base except rhat it contains increased concentration of EDTA, which helps in inhibiting *Staphylococcus aureus* . Beef heart infusion from solids and tryptose provide the carbonaceous and nitrogenous compounds necessary for growth whereas sodium chloride provides the osmotic equilibrium. Thallous acetate and Polymyxin (FD185) are inhibitory agents allowing growth of *B.anthracis* while inhibiting contaminants. Lysozyme (FD185) specifically suppresses the growth of gram-negative contaminants. The suspected specimen may be used directly for streaking or heat-treated or alcohol-treated specimens can be used for streaking. On incubation at 37°C for 24 hours colonies develop from 30-100% of the *B.anthracis* spores that would grow on non-selective Heart Infusion Agar (M169), being smaller and smoother than on the later medium. PLET Agar Base, Modified inhibits growth of most strains of *B.cereus, B. subtilis* , other *Bacillus* species, *Enterobacteriaceae* and *Pseudomonas* species. Some strains of *B.cereus* from soil form colonies but they are smaller than those of *B.anthracis* , minute after 24 hours and moderately sized after 48 hours. Colonies of *B.anthracis* appear in 36-40 hours after incubation at 37°C. Roughly circular, creamy- white colonies with a ground-glass texture are further subcultured on blood agar plates for identification. Capsule production can be seen directly or on blood agar plates (4).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

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

Reaction
Reaction of 4.04% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1451: Cultural characteristics observed after an incubation at 35-37°C for 36-40 hours

Organism	Growth
Bacillus anthracis ATCC 14578	luxuriant
Bacillus cereus ATCC 10876	inhibited
Staphylococcus aureus ATCC 25923	inhibited

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

Reference
1.Knisely R.F. 1966, J. Bacteriol, 92:784-786.
2.Norris J. R., Berkley C.W., Logan N.A., and ODonnell A.G., 1981. In M. P. Starr et al (ed) The Prokaryotes : a Handbook on Habitats, Isolation and Identification of Bacteria, Vol. 2, Springer Verlag, Berlin.
3.Parry J.M., Turnbull P.C.B. and Gibson J.R., 1983, A Colour Atlas of Bacillus species. Wolfe Medical Publications, London, United Kingdom.
4.Murray et al, 1999, Manual of Clinical Microbiology, 7th Edition, ASM Press, Washington, D.C.

Revision : 1 / 2011

Rappaport Vassiliadis Soya Broth (RVS Broth)

M1491

Rappaport Vassiliadis Soya Broth (RVS Broth) is recommended as a selective enrichment medium for the Salmonellae species from the food and animal feeding stuffs.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	4.500
Sodium chloride	8.000
Potassium dihydrogen phosphate	0.600
Dipotassium phosphate	0.400
Magnesium chloride. hexahydrate	29.000
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.11 grams of dehydrated medium in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C for 15 mins.

Principle And Interpretation

Rappaport Vassiliadis Soya Broth is designed according to the revised formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (2). Addition of magnesium chloride to the medium was reported by Peterz et al (3). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

Salmonella generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of *Salmonella* . Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of Papaic digest of soyabean meal provide essential growth nutrients and enhance the growth of *Salmonella* (4). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enrich *Salmonella* . The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella* .This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of Salmonellae in milk samples. The enriched culture of Rappaport Vasiliadis Soya Broth (M1491) can be further subcultured and isolated on Brilliant Green Agar (M016) or Deoxycholate Citrate Agar (M065), Xylose Lysine Deoxycholate Agar (M031).

Quality Control

Appearance
Light yellow to light blue homogeneous free flowing powder

Colour and Clarity of prepared medium
Greenish blue clear to slightly opalescent with a slight precipitate.

Reaction
Reaction of 2.77% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH
5.00-5.40

Cultural Response
Cultural response was observed after an incubation at 30-35°C for 18-24 hours Recovery is carried out using Xylose Lysine Deoxycholate Agar (M031) after enrichment.

Cultural Response

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of colony
Cultural Response					
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	>=35	>=70 %	red with black centers
Salmonella Abony NCTC 6017	50 -100	luxuriant	>=35	>=70 %	red with black centers
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%	
Escherichia coli ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellow
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellow
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	>=35	>=70 %	red with black centre
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	>=35	>=70 %	red with black centre
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%	
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0	0%	
E.coli +S.Typhimurium (mixed culture)					
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	>=35	>=70 %	red with black centre

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.Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2.Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3.Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523 4.McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171

Revision : 1 / 2011

Mueller Kauffman Tetrathionate Novobiocin Broth Base

M1496I

Mueller Kauffman Tetrathionate Novobiocin Broth Base is used for improved enrichment and isolation of Salmonellae.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.300
Casein enzymic hydrolysate	8.600
Ox bile	4.750
Sodium chloride	2.600
Calcium carbonate	38.700
Sodium thiosulphate, pentahydrate	47.800
Brilliant green	0.0095
Final pH (at 25°C)	8.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 89.42 grams of dehydrated medium in 1000 ml distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C and just before use aseptically add 20 ml of iodine solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water) along with rehydrated contents of 1 vial of MKTT Novobiocin Supplement (FD203). Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

Note: Due to presence of calcium carbonate, the prepared media forms opalescent solution with white precipitate.

Principle And Interpretation

The examination of various types of food products for *Salmonella* requires methods different from those used in clinical laboratories. The need for such method is due to the generally low numbers of Salmonellae in foods and the frequently poor physiological state of these pathogens following exposure to stressful conditions during food processing or storage. Injured *Salmonella* are resuscitated in non-selective broth medium, which facilitates detection of sublethally injured *Salmonella* . The ideal pre-enrichment broth should provide for the repair of cell damage, dilute toxic or inhibitory substances and nutritive enough to favour growth of *Salmonella* . In the analysis of food for *Salmonella* , pre-enrichment cultures are usually incubated at 35-37°C for 18-24 hours and then a portion is sub cultured to one or more selective enrichment broths. Normally 1 ml of pre-enrichment culture is inoculated to 9 ml of selective enrichment broth. Selective enrichment media contains selective ingredients that allow the proliferation of *Salmonella* and inhibit the growth of competing non-salmonella microorganisms. Lactose Broth (M1003) is recommended by BAM for pre-enrichment of *Salmonella* from food. Selective enrichment is done in Tetrathionate Broth and Rappaport Vassiliadis Medium. For the detection of foodborne *Salmonella* , various modifications of Tetrathionate Broth have generally found wider applications (7).

Mueller (1) recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella* . Kauffman (2) modified the formula to include ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* species. The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products and from poultry and poultry products (3). It is also a recommended selective broth for isolating *Salmonella* from animal feces and sewage-polluted water (4). Selectivity is conferred by tetrathionate (from the reaction of thiosulphate and iodine). Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes (5).

Mueller Kauffman Tetrathionate Novobiocin Broth Base contains casein enzymic hydrolysate and peptic digest of animal tissue as sources of carbon, nitrogen, vitamins and minerals. Ox bile and added brilliant green are selective agents, which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffer. Sodium chloride maintains osmotic equilibrium. Sodium thiosulphate is a source of sulfur. The tetrathionate (S_4O_6) anions constitute the principle selective agent in these enrichment media. If desired, 4 mg of novobiocin per litre of broth can be added to suppress *Proteus* species

(6).Add approximately 10 grams of sample to 100 ml of broth. Shake well and place the flask in a 45°C water bath for 15 minutes. Remove the flasks and place in an incubator or water bath at 43°C. Several studies have shown increased recovery of *Salmonella* following incubation of selective enrichment at 43°C (8). After an incubation for 18-24 hours and 48 hours, subculture on Brilliant Green Agar, Modified (M016). This medium is not suitable for the growth of *Salmonella* Typhi, *Salmonella* Sendai, and *Salmonella* Pullorum etc.

The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than 7 days.

Organisms other than Salmonellae, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium. Therefore, confirmatory tests should be carried out on all presumptive Salmonella colonies that are recovered.

Quality Control

Appearance
Cream to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light green coloured opalescent solution forms with heavy white precipitate

Reaction
Reaction of 8.93% w/v aqueous solution at 25°C. pH : 8.2±0.2

pH
8.00-8.40

Cultural Response
M1496I: Cultural characteristics observed after an incubation at 43°C for 18-48 hours with added 20ml iodine solution and MKTT Novobiocin Supplement (FD203), when subcultured on Soyabean Casein Digest Agar (M290).

Organism	Inoculum	Recovery
Escherichia coli ATCC 25922	50-100	none-poor
Proteus vulgaris ATCC 13315	50-100	none-poor
Shigella flexneri ATCC 12022	>=10^3	inhibited
Salmonella Enteritidis ATCC 13076	50-100	excellent
Salmonella Enteritidis ATCC 13076	50-100	
Salmonella Paratyphi A ATCC 9150	50-100	excellent
Salmonella Paratyphi B ATCC 8759	50-100	excellent
Salmonella Typhi ATCC 6539	>=10^3	inhibited
Salmonella Typhimurium ATCC 14028	50-100	excellent

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.Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.
2.Kauffman F., 1935, Ztschr. F. Hyg., 117:26.
3.International Organization for Standardization, 1974, (Draft International Standard ISO/DIS 3565), Geneva, Switzerland.
4.Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England.
5.Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333.
6.Jeffries L., 1959, J. Clin. Pathol., 12:568.
7.Speck M. L., (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., American Public Health Association, Washington, D.C.
8.DAoust J. Y., 1989, Salmonella in Food borne Bacterial pathogens, (Eds.) Doyle M. P., 327, Marcel Dekker, New York.

Revision : 2 / 2015

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M-BCG Yeast and Mould Agar

M1504

M-BCG Yeast and Mould Agar is used for the detection of fungi in routine analysis of beverages using membrane filter technique.

Composition**

Ingredients	Gms / Litre
Yeast extract	9.000
Dextrose	50.000
Biopeptone	10.000
Magnesium sulphate	2.100
Potassium phosphate	2.000
Diastase	0.050
Thiamine hydrochloride	0.050
Bromocresol green	0.026
Agar	15.000
Final pH (at 25°C)	4.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 8.82 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 12 - 15 lbs pressure (118 - 121°C) for 10 minutes.

Principle And Interpretation

The microbiology of beverages will vary greatly depending upon the method of processing and the means of preservation. High microbial populations often indicate poor quality in raw material, unsanitary equipments or opportunity for growth in the food at some stage in the process. Heat processed beverages will be free of aciduric microorganism but may yield low numbers of viable spore forming bacteria when cultured on non-selective media. Bacteria cannot grow in the high acid environment and therefore direct microscopic count for yeast, bacteria or moulds may provide a clue to the conditions of sanitization during processing. Heat resistant spores may be present in low numbers. Because of their slow growth and poor competitive ability, yeast and moulds often manifest themselves on or in foods in which the environment is less favourable for bacterial growth.

M-BCG (Bromocresol Green) Yeast and Mould Agar is used for the detection of fungi in routine analysis of beverages using membrane filter technique (1).

This medium is used for enrichment of yeasts and moulds from populations containing bacteria.

The medium is highly nutritious for the growth of yeasts and moulds. Biopeptone and yeast extract provide nitrogenous compounds and vitamin B complex. Thiamine is also a B vitamin in the medium. Dextrose acts as the energy source. Diastase is a mixture of amylolytic enzymes. Bromocresol green is the pH indicator, which is green at acidic pH (pH 4.0) while blue at pH 5.6. Potassium phosphate helps in maintaining buffering action in the medium. The low pH inhibits bacterial growth. The membrane filter is directly placed on the agar surface of M-BCG Yeast and Mould Agar and incubated at 30-35°C for 48 hours.

Quality Control

Appearance
Cream to light green homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Green coloured opalescent gel forms in Petri plates

Reaction
Reaction of 8.82% w/v aqueous solution at 25°C. pH : 4.6±0.2

pH
4.40-4.80

Cultural Response
M1504: Cultural characteristics observed after an incubation at 25 - 30°C for 48 - 72 hours.

Organism	Inoculum (CFU)	Growth
Cultural Response		
**Aspergillus brasiliensis ATCC 16404*	50-100	good-luxuriant
Candida albicans ATCC 10231	50-100	good-luxuriant
Saccharomyces cerevisiae ATCC 9763	50-100	good-luxuriant

*Key : * - Formerly known as Aspergillus niger*

Storage and Shelf Life
Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

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

Revision : 2 / 2015

Eugonic LT 100 Broth Base w/o Tween 80

M1517

Intended Use:

Recommended for the enrichment and detection of mesophilic aerobic bacteria present in cosmetic products. The composition Eugonic and performance criteria of the medium are as per the specifications laid down in ISO 21149.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	4.000
L-Cystine	0.700
Sodium sulphite	0.200
Glucose	5.500
Egg lecithin	1.000
Tritox X-100	1.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.4 grams in 1000 ml purified/distilled water containing 5 grams of Polysorbate 80 (Tween 80). Heat to boiling to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Eugonic LT 100 Broth Base was developed by Pelczar and Vera (1) for cultivation of fastidious organisms like *Brucella*. Eugon media were developed to obtain eugonic (luxuriant) growth of fastidious microorganisms like *Brucella* which areotherwise difficult to cultivate (2). The unenriched medium supports rapid growth of lactobacilli associated with cured meat products, dairy products and other foods. Eugonic media is quite similar to Tryptone Soya Agar (M290) but more bacterial propagation is expected on Eugonic media. Organisms like *Bordetella* and *Neisseria* grow luxuriantly in Eugon Media because large amount of sulfur and carbon sources have been added in the formula. Eugonic LT 100 Broth Base can be used for growth of a variety of fastidious microorganisms like *Neisseria, Francisella* and *Brucella*. The composition of the medium is as per ISO (3) for the detection of mesophilic aerobic bacteria from cosmetic products.

Tryptone and soya peptone provide the nitrogen, vitamins and amino acids, which supports the growth of fastidious microbial species. The high concentration of glucose is the energy source for rapid growth of bacteria. L-Cystine and sodium sulphite are added to stimulate growth. Sodium chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity (4). Lecithin and polysorbate 80 in Eugonic LT 100 Medium w/o Tween 80 neutralize antimicrobial agents hence this medium can be used as a neutralizing diluent.

Type of specimen

Clinical samples - urine, faeces, Cosmetic samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,5).
For cosmetic samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6)
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 fastidious organisms may not grow due to nutritional variation.
2. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured, Clear to slightly opalescent solution.

Reaction
Reaction of 3.24% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours (fungal cultures incubated at 25-30°C for 2-7 days).

Organism	Inoculum (CFU)	Growth
Bacillus pumilus ATCC 14884	50-100	good
Candida albicans ATCC 26790	50-100	good
Lactobacillus fermentum ATCC 9338	50-100	good
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant (under 3-5% CO2)
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant (under 3-5% CO2)
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	50-100	good-luxuriant
Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	50-100	good
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	good
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	good
Escherichia coli ATCC 8739 (00012*)	50-100	good
Candida albicans ATCC 10231 (00054*)	50-100	good
Neisseria meningitidis ATCC 13090	50-100	good

* 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 (3,4).

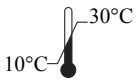
Reference

1.Frank H. A., 1955, J. Bacteriol., 70:269.
2.ISO 21149 (2006) Cosmetics-Microbiology- Enumeration and detection of aerobic mesophilic bacteria
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. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
6.Pelczar and Vera J., 1949, Milk Plant Monthly 38:30

Revision : 05/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature


Do not use if package is damaged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HiCrome™ Listeria Ottaviani-Agosti Agar Base

M1540I

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes*. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017

Composition**

ISO 11290 Specification - Agar Listeria according to Ottaviani and Agosti

Ingredients	Gms / Litre
Enzymatic digest of animal tissues	18.000
Enzymatic digest of Casein	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Glucose	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate (anhydrous)	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate (anhydrous)	2.500
5-Bromo-4 chloro-3-indolyl-β–D-glucopyranoside	0.050
Agar	12.00 - 18.00
Final pH (after sterilization)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

M1540I - HiCrome™ Listeria Ottaviani-Agosti Agar Base

Ingredients	Gms / Litre
HM Peptone #	18.000
Tryptone ##	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Glucose(Dextrose)	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate	2.500
5-Bromo-4 chloro-3-indolyl-β–D-glucopyranoside	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

Key : # - Equivalent to Enzymatic digest of animal tissues, ## - Equivalent to Enzymatic digest of casein

Supplements to be added after autoclaving	Gms / Litre
I	
Nalidixic acid sodium salt	0.020
Ceftazidime	0.020
Polymyxin B sulfate	76 700 IU
Cycloheximide OR	0.050
Amphotericin B	0.010
II	
L-α- phosphatidylinositol	2.00

FD212A - 2 vials	mg / vial
Nalidixic acid sodium salt	10.000
Ceftazidime	10.000
Polymyxin B sulfate	38350 IU
Amphotericin B	5.000

(FD214) - 2 vials	
L.mono Enrichment Supplement I	1g

Directions

Suspend 36.02 grams in 465 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of OA Listeria Selective Supplement (FD212A). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L. monocytogenes* and L.innocua have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). The media is based on the formulation of Ottoviani and Agosti (3, 4) for the selective and differential isolation of Listeria monocytogenes from food and animal feeds which is adopted by ISO Committee (5,6).
HM peptone, tryptone and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium pyruvate provide essential growth nutrients. Glucose (Dextrose) is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212A) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate (5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside) which produces blue to green coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Type of specimen

Clinical samples - Blood samples ; Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).

For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
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. Some strains of *L.monocytogenes* exposed to stress condition particularly acid stress may show a very weak halo (or even no halo).
2. Further biochemical tests must be carried out to differentiate between *L.monocytogenes* and *L. ivanovii*, since both shows opaque halo of PIPLC activity
3. Some organisms other than *Listeria* spp. may also produce blue colonies on this medium, so biochemical characterization is required for differentiation.

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, opalescent gel forms in Petri plates

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

pH
7.00-7.40

Cultural Response
Cultural characteristics observed with added sterile OA Listeria Selective supplement (FD212A) and L.mono Enrichment supplement I (FD214) after an incubation for 48 ± 4 hours at 37° ± 1°C.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
Productivity					
Listeria monocytogenes ATCC 13932 (00021*)	50-100	luxuriant	>=50%	Blue-green	positive, opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Listeria monocytogenes ATCC 35152 (00109*)	50-100	luxuriant	>=50%	Blue-green	positive, opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Specificity					
Listeria innocua ATCC 33090 (00017*)	50-100	luxuriant		Blue-green	negative
Selectivity					
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%		
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 19433 (00009)*	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 29212 (00087)*	>=10^4	inhibited	0%		
Additional Microbiological Testing					
Listeria ivanovii ATCC 19119	50-100	luxuriant	>=50%	greenish-blue	positive, opaque halo around the colony exhibiting phophatidyl -inositol specific phospholipase acivity

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°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 (1,2).

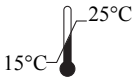
Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3
4. Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p. 6, A.D.R.I.A. Quimper, France, 16-18 June 1997.
5. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017
6. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2 , Enumeration method ; ISO 11290-2:2017

Revision : 00 / 2020

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature


Do not use if package is damaged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Tryptone Bile Glucuronic Agar (TBX Agar)

M1591

Tryptone Bile Glucuronic Agar is selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs and animal feed and water.

Composition**

Ingredients	Gms / Litre
Bile salt mixture	1.500
Enzymatic digest of casein	20.000
X-ß-D-glucoronic acid	0.075
Dimethyl sulfoxide	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.6 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 45-50°C, mix gently and pour in sterile Petri plates.

Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (4). Tryptone Bile Glucoronic Agar contains the enzyme ß -D- glucorinodase which differentiates most *E.coli* species from other coliforms. *E.coli* absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (1).The enzyme ß-glucorinodase splits the bond between the chromophere 5-bromo-4-chloro-3-indolyl and the ß-D-glucoronide. *E.coli* colonies are blue green coloured (2,3). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

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
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.66% 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°C for 18- 24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
Citrobacter freundii ATCC 8090	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	blue-green
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	

Storage and Shelf Life

Store dehydrated and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Frampton E W, Restaino L, Blaszko L.1988. Eavaluation of ß-glucoridase substrate 5-bromo-4-chloro3-indolyl-B-D-glucuonide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. J. Food Protection 51:402-404.
2.Killian M. and Bolow P 1976 Rapid diagnosis of Enetrobacteriacea I. Detection of bacterial glycosidases. Acta Rattol. Microbiol Scand Sct B 84245:251.
3.Ley A N, Bowers R J, Wolfe S 1988 Indocyl –B-D-glcuaoride, a novel chromogenic coli reagent for the detection and enumeration of Escherichia coli in environmental samples. Canadian Journal of Microbiology 34:690-693.
4.International Standard ISO 16649-2: 1999. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid.

Revision : 02 / 2015

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.
5. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
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

Neo Enrichment Broth Base

M1733

Neo Enrichment Broth Base is a selective enrichment broth for *Listeria* species from food samples.

Composition**

Ingredients	Gms / Litre
Peptone special	28.000
Carbohydrate mix	6.000
Salt mix	10.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22 grams in 500 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Neo Enrichment Selective Supplement (FD249). Mix well and dispense in sterile test tubes.

Warning : Salt mix of this medium contains harmful substance. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Principle And Interpretation

Neo Enrichment Broth Base is a medium developed for the selective enrichment and isolation of *Listeria* species from food samples.

Recovery of *Listeria* species can be achieved in 24 hours using Neo Enrichment Broth. This allows the early detection of *Listeria* species as primary and secondary enrichment steps are avoided, which are time consuming. Neo Enrichment Broth Base therefore, is a single enrichment medium, which eliminates the need of secondary enrichment and the recovery levels of Listeria species at 24 hours are comparable to the ISO enrichment method (1).

This medium contains peptone special, mixture of salts and carbohydrates to give optimal recovery and growth of *Listeria* species from food samples after 24 hours. *Listeria monocytogenes* hydrolyses esculin (which is available in carbohydrate mix) to form esculetin and dextrose. Esculetin reacts with ammonium ferric citrate (which is available in salt mix) producing blackening. The medium is rendered selective by addition of selective supplement.

For the enrichment, 25 grams of food sample is added to 225 ml of Neo Enrichment Broth in a stomacher bag. Homogenize the material if required (1). Incubation is carried out at 30°C for 24 hrs and the sample is subcultured on suitable agar medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent solution having a bluish tinge

Reaction
Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M1733: Cultural characteristics observed with added Neo Enrichment Selective Supplement (FD249),after an incubation at 35-37°C for 24 hours

Organism	Inoculum (CFU)	Growth	Esculin hydrolysis

Cultural Response

Escherichia coli ATCC 25922	>=10^3	inhibited	
Listeria monocytogenes ATCC 19111	50-100	good-luxuriant	positive, reddish brown colouration of medium
Listeria monocytogenes ATCC 19112	50-100	good-luxuriant	positive, reddish brown colouration of medium
Listeria monocytogenes ATCC 19117	50-100	good-luxuriant	positive, reddish brown colouration of medium
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. ISO 11290 - 1 : Microbiology of food and animal feeding stuffs horizontal method for the detection and enumeration of Listeria monocytogenes, 1996.

Revision : 2 / 2015

Cronobacter Selective Broth (CSB) M1786I

Intended Use

Recommended for screening *Cronobacter* (formerly *Enterobacter sakazakii*) from food. The composition and performance of this media are as per specifications laid down in ISO 22964:2017(E).

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM Extract #	3.000
Sodium chloride	5.000
Bromocresol purple	0.040
Sucrose	10.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Directions

Suspend 28.04 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add the contents of 1 vial of Vancomycin supplement (FD233). Mix well and dispense 10ml into sterile test tubes.

Principle And Interpretation

Cronobacter (formerly *Enterobacter sakazakii*) are gram-negative rod-shaped *Enterobacteriaceae* that have been implicated in outbreaks of disease causing sepsis, meningitis and necrotising enterocolitis (1). *Cronobacter* species have also been isolated from powdered infant formula as high tolerance to desiccation provides a competitive advantage in dry environments increasing the risk of contamination (2).

Cronobacter Screening Broth was specifically designed by Iversenetal (3). Cronobacter Selective Broth is recommended by ISO Committee for the isolation of *Cronobacter* species from food samples (4). Peptone and HM extract provide carbonaceous, nitrogenous and growth nutrients. Sodium chloride maintains osmotic equilibrium. Sucrose is the fermentable carbohydrate and bromocresol purple is the indicator. Sucrose is fermented by *Cronobacter*. Consequently the broth turns yellow after incubation.

Type of specimen

Food samples

Specimen Collection and Handling:

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

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

Warning and Precautions :

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

Limitations :

Due to variable nutritional requirements, some strains mayshow poor growth on this medium.

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

Purple coloured clear solution forms in tubes.

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 with added Vancomycin Supplement (FD233), after an incubation at 41.5 ± 1°C for 24±2 hours.The broth is recovered on HiCrome™ Cronobacter Isolation Agar (CCI Agar) (M2062I) and incubated at 41.5±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Colour of medium	Colour of Colony on M2062I
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant	yellow colour	blue-green
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good-luxuriant	yellow colour	blue-green
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	none-poor	purple	-
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50-100	none-poor	purple	-
Mixed cultures				
Cronobacter sakazakii ATCC 29544 (00214)* + *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*)	50-100	good-Luxuriant	yellow	blue-green
Cronobacter sakazakii ATCC 29544 (00214)* + *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*)	50-100	good-Luxuriant	yellow	blue-green
Cronobacter muytjensii ATCC 51329 (00213*) + *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*)	50-100	good-Luxuriant	yellow	blue-green
Cronobacter muytjensii ATCC 51329 (00213*) + *Staphylococcus aureus subsp. aureus* ATCC 6538 (00032*)	50-100	good-Luxuriant	yellow	blue-green

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. 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 (6,7).

Reference

1. Mullane et al. 2007. Minerva Pediatr. 59.137-148.
2. Lai.2001.Medicine.80.113-122.
3. Iversen et al.2008.Appl.Environ.Microbiol.74, 2550-2552.
4. International Organization for Standardization. Microbiology of the food chain- Horizontal method for the detection of Cronobacter spp. Draft ISO/ TS 22964, 2017 (E).
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03 / 2017

Mueller Hinton Agar, 2% Glucose with Methylene blue

M1825

Mueller Hinton Agar, 2% Glucose with Methylene blue is recommended for testing performing Antifungal Disk Diffusion Susceptibility of yeasts.

Composition**

Ingredients	Gms / Litre
Beef infusion from	300.000
Casein Acid Hydrolysate	17.500
Starch	1.500
Glucose	20.000
Methylene blue	0.0005
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Mix well before pouring.

The performance of this batch has been tested and standardized as per the current CLSI (formerly NCCLS) document M44-A2 in Method for Antifungal Disk Diffusion susceptibilty Testing of yeasts

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species (1). Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for the diffusion of antifungal agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (2).

When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue to a final concentration of 5µg/ml enhances zone edge definition.

Kirby-Bauer et al recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (3). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Haemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae* . Similarly Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for antifungal susceptibity testing of discs.

Beef infusion and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. Glucose serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition.

Technique:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.

2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 106 - 5 x 106 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Mueller Hinton Agar, Modified (as per CLSI for antifungal) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.

2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the Petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.

3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart. (Not more than 12 discs should be placed on a 150-mm plate or not more than 5 discs on a 100-mm plate

4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.

5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Quality Control

Appearance
Light yellow to yellow may have slight blue tinge homogeneous free flowing powder

Gelling
Firm, comparable with 1.7% agar gel.

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

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH
7.20-7.40

Cultural response
A luxuriant growth of test organisms was observed on Mueller Hinton Agar, Modified (as per CLSI for antifungal) in 24-48 hours at 33-37°C along with inhibition zones with respective antibiotic concentrations

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Amphotericin-B AP(100units)	Amphotericin-B AP(20 mcg)	Amphotericin-B AP(50 mcg)
Cultural response						
Candida albicans ATCC 90028	50-100	Luxuriant	>=70%	10 -17 mm	10 -15 mm	31 -42 mm
Candida parapsilosis ATCC 22019	50-100	luxuriant	>=70%	11 -20 mm	10 -17 mm	28 -37 mm
Candida tropicalis ATCC 750	50-100	luxuriant	>=70%	8 -12 mm	8 -10 mm	13 -17 mm
Candida krusei ATCC 6258		luxuriant	>=70%	9 -14 mm	8 -12 mm	16 -25 mm
Candida albicans ATCC 10231	50-100	luxuriant	>=70%	10 -18 mm	10 -16 mm	30 -40 mm
Saccharomyces cerevisiae ATCC9763	50-100	luxuriant	>=70%	11 -18 mm	8 -12 mm	29 -38 mm

Storage and Shelf Life

Store dehydrated powder below 30°C and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2.Method for Antifungal Disk Diffusion Susceptibilty Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.
3.Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
4.Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.

Revision : 1 / 2011

CE

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Technical Data

HiCrom™ Selective Salmonella Agar Base

M1842

Intended Use:

Recommended for the selective isolation of *Salmonella* species from food and clinical samples

Composition**

Ingredients	Gms / Litre
HI powder #	12.000
Yeast hydrolysate	5.000
Tryptose	5.000
Sodium cholate	3.000
Sodium taurocholate	5.000
Sodium deoxycholate	1.000
Chromogenic mixture	8.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Heart Infusion powder

Directions

Suspend 54.00 grams in 1000 ml purified/ distilled water. Gently heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Aseptically add the rehydrated contents of one vial of HiCrome™ Selective Salmonella Agar Supplement (FD274). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella species have been isolated from humans and almost all animals throughout the world. They cause many types of infections from mild, self-limiting gastroenteritis to life threatening typhoid fever. *Salmonella* Typhi and *Salmonella* Paratyphi A & B cause gastroenteritis, bacteremia and enteric fever, *Salmonella* Choleraesuis causes gastroenteritis and enteric fever, especially in children. *Salmonella* Typhimurium is the most frequently isolated serotype of *Salmonella* . *Salmonella* species are the major cause of food poisoning (3).

Various chromogenic media are available for the differentiation of *Salmonella* species. The original media formulated by Rambach (4) differentiates *Salmonella* based on propylene glycol utilization and presence of a chromogenic indicator. However HiCrome™ Selective Salmonella Agar Base uses chromogenic mixture for identification and differentiation of *Salmonella* species. Sodium cholate, Sodium taurocholate and Sodium deoxycholate in the medium helps to restrict the growth of other organisms. Besides the selective supplement added to the medium inhibits competing microorganisms.

HI powder, yeast hydrolysate and tryptose in the medium provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Due to the presence of chromogenic mix in the medium *Salmonella* are easily distinguishable and forms purple coloured colonies while some *Enterobacteriaceae* like *Klebsiella* and *Enterobacter* forms blue to dark blue coloured colonies.

Conventional method employes the H2S production property for *Salmonella* detection which is also exhibited by other non *Salmonella* species such as *Citrobacter, Proteus,* etc. Hence further biochemical confirmation is required for further identification.

This medium is specially employed for food samples where the sample is initially enriched in Salmonella Selective Enrichment Broth (M1843) and then isolated on HiCrome™ Selective Salmonella Agar Base. *Salmonella* species give purple coloured colonies due to the enzyme specificity.

Type of specimen

Clinical- stool samples, blood; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2) .
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

In Vitro diagnostic 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. Being highly selective, some strains may show poor growth. 2. Most of the *Salmonella* strains shows purple colonies except few. 3. Final confirmation of suspected colonies must be carried out by serological and biochemical tests.

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 beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.5 % Agar gel.

Colour and Clarity of prepared medium
Whitish cream coloured, opaque gel forms in Petri plates

Reaction
Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed with added HiCrome Selective Salmonella Agar Supplement (FD274), after an incubation at 35-37°C for 22-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^3	inhibited	0%	
Klebsiella pneumoniae ATCC 13883 (00097*)	50 -100	good	40 -50 %	blue
Salmonella Typhimurium *ATCC 14028* (00031*)	50 -100	good-luxuriant	>=50 %	purple
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	>=50 %	purple
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0 -0 %	

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 15-25°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 (1,2).

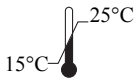
Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3.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.
4.Rambach A., 1990, Appl. Environ. Microbiol., 56:301.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 03 / 2020

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Iron Sulphite Agar Modified

M1852I

Intended Use:

Recommended for the enumeration of sulfite – reducing bacteria growing under anaerobic conditions. The composition and performance criteria of this medium are as per the specifications laid down in ISO 15213:2003

Composition**

Ingredients	Gms / Litre
Casitose ▲	15.000
Soya peptone	5.000
Yeast extract	5.000
Disodium disulfite	1.000
Ferric ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

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

Directions

Suspend 42 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense into sterile tubes or pour into sterile Petri plates.

Principle And Interpretation

Iron Sulphite Agar, Modified is recommended by ISO for the enumeration of sulphite reducing bacteria.(3). Most Clostridia possess sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Casitose and soya peptone provides carbon, nitrogen ompounds, vitamins, minerals and amino acids necessary for the growth of organism. Yeast extract serves as a rich reservoir of vitamins especially B-complex vitamins. Ferric citrate ammonium citrate and Disodium sulfite serves as are H_2S indicators, wherein *Clostridium perfringens* reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Agar is the solidifying agent.

Enumeration with this medium can be performed using either tubes or plates. In case of tubes distribute 20-25 ml of the medium in tubes and inoculate 1 ml of test sample or 1 ml of serial dilutions of 10-1 and 10-2 in molten state. Allow to solidify, and pour 2-3 ml of the same medium in each tube to overlay. In case of Petri plates, transfer 1 ml of test sample or initial dilution. Further dilution can be carried out and 1 ml of each dilution (10-1 and 10-2) is transferred to an empty Petri plate. Cool the medium to 44-47°C and pour 15-20 ml of the medium to the Petri plate containing the inoculum. Mix the inoculum and allow the medium to solidify. Overlay the medium with 5-10 ml of the same medium.

After solidification , incubate the medium at 36-38°C for 24-48 hours. If thermophilic bacteria are suspected, a second of tubes is incubated at 49-51°C for 24-48 hours. After incubation, black coloured colonies, possibly surrounded by a black zone are counted as sulphite reducing bacteria.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. Further biochemical and serological testing is required 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

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed under anaerobic conditions, after an incubation at 36-38°C for 24-48 hours.

Organism	Inoculum	Growth	Recovery	Colour of colony
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	black
Clostridium butyricum ATCC 13732	50-100	luxuriant	>=50%	black
*Clostridium sporogenes ATCC 19404 (00008)**	50-100	luxuriant	>=50%	black
*Clostridium perfringens ATCC 13124 (00007)**	50-100	luxuriant	>=50%	black
*Clostridium perfringens ATCC 12916 (00080)**	50-100	luxuriant	>=50%	black
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
*Escherichia coli ATCC 25922 (00013)**	50-100	good	40-50%	no blackening
*Escherichia coli ATCC 8739 (00012)**	50-100	good	40-50%	no blackening

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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of sulphite reducing bacteria growing under anaerobic conditions, ISO 15213.

Revision : 03 / 2019

HiCrome™ Chromogenic Coliform Agar (CCA) M1991I

Intended Use

Recommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition**

Ingredients	Gms / Litre
Tryptone #	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, $2H_2O$	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexamine ammonium salt, monohydrate	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Enzymatic digest of casein

Directions

Suspend 30.92 grams(the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiChromogenic Coliform Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water samples (1). The medium contains three chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxyl-β-D-galactopyranoside to form pink to red coloured colonies (3). The enzyme ß-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl-β-D-glucuronic acid (2). Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (3).

The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples - Water and wastewater

Specimen Collection and Handling

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

Warning and Precautions :

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

Limitations :

1.Further biochemical testing is required for identification of microorganism.
2. Certain variations in colour may be observed .

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 opalescent gel forms in Petri plates

Reaction
Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 34-38°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony#
*Citrobacter freundii ATCC 43864 (00006)**	50-100	luxuriant	>=70 %	pale pink to pink
$ Klebsiella aerogenes ATCC 13048 (00175)*	50-100	luxuriant	>=70%	pale pink to pink
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	>=70%	dark blue to violet
Escherichia coli ATCC 8739 (00012)*	50-100	luxuriant	>=70%	dark blue to violet
Enterococcus faecalis ATCC 19433 (00009)*	>=10^3	inhibited	0%	
Pseudomonas aeruginosa ATCC 10145 (00024)*	50-100	luxuriant	>=70%	colourless

Key * : Corresponding WDCM numbers # : either on plate or membrane
$ - Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period 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.International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I-Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2.Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
3.Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.

4. 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.

5..Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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 : 02 / 2018

Technical Data

HiCrome™ Cronobacter Isolation Agar(CCI Agar) M2062I

Intended Use

Recommended for the isolation and identification of *Cronobacter sakazakii* from food products.The composition and performance of this media are as per specifications laid down in in ISO /TS 22964: 2017. It can also be used for clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone#	7.000
Yeast extract	3.000
Sodium chloride	5.000
Sodium deoxycholate	0.250
5-Bromo-4-chloro-3-indolyl α–D-glucopyranoside	1.500
Ammonium iron(III) citrate	1.000
Sodium thiosulfate	1.000
Agar	15.000
Final pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Tryptic digest of casein

Directions

Suspend 32.4 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

Enterobacter species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human faeces. **Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (1). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of **C.sakazakii* from food samples (2). The chromogenic substrate (5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside) is cleaved specifically by **C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colourless colonies. Tryptone and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Sodium deoxycholate inhibits the accompanying gram-positive flora.

Key: *: Formerly known as *Enterobacter sakazakii*

Type of specimen

Clinical samples- blood, urine , cerebrospinal fluid ; Food samples.

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3).
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

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

Limitations

1. Slight variation in colour may be observed depending on enzyme production by organism and substrate utilization from the medium.
2. Some species may show poor growth due to nutritional variations.
3. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.24% 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 41.5±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant	>=50%	blue-green
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good-luxuriant	>=50%	blue-green
Enterobacter cloacae ATCC 13047 (00083*)	50-100	good-luxuriant	>=50%	colourless without green or blue green colour
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

Storage and Shelf Life

Store between 15-25°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.

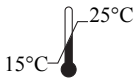
Disposal

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 (2,3).

Reference

1. International Organization for Standardization. Microbiology of the food chain- Horizontal method for the detection of Cronobacter spp. Draft ISO/ TS 22964, 20176 (E).
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. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 01/ 2020

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Cetrimide Agar

MH024

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Directions

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

Principle And Interpretation

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

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

Type of specimen

Pharmaceutical samples: Clinical samples

Specimen Collection and Handling

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

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

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

Warning and Precautions:

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.36% Agar gel

Colour and Clarity of prepared medium

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

pH

7.00-7.40

Growth Promotion Test

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

Growth promoting properties

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

Inhibitory properties

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

Cultural Response

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

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

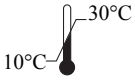
Reference

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

Revision : 04/ 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
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EC REP

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HiMedia CellCulture
Enabling Breakthroughs

Product Information

HiViral™ Transport Kit (S)

With Viral Transport Medium in self-standing / conical bottom tube, One Sterile Nasopharyngeal Nylon Flocked Swab with Breakpoint and One Sterile Oropharyngeal Viscose Swab with Breakpoint

Product Code: MS2760S

Intended Use: For collection and transportation of potentially infectious samples containing Viruses, Mycoplasma and Ureaplasma (other than Cytomegalovirus and Chlamydia) from the collection site to the laboratory.

Introduction:

HiMedia's HiViral™ Transport Kit (S) is a specially designed transport system to collect and transport viruses in an active form to the laboratory for isolation. It is designed to maintain the viability and the virulence of the viral sample. It contains 3.0ml viral transport medium in self-standing or conical bottom tube.

HiViral™ Transport Medium is made of Hanks Balanced Salt Solution and contains a protective protein, antibiotics to control microbial and fungal contamination and buffers to control the pH. Phenol red is used as a pH indicator. The medium also contains a cryoprotectant which helps in preserving the viruses if specimens are frozen for prolonged storage.

The nasopharyngeal nylon flocked swab has a short perpendicular ultra-flexible plastic shaft that is designed for better patient comfort. This plastic shaft is attached with soft nylon strands that results in efficient collection and release of particulate matter. It yields significantly more sample which helps in maximizing the sensitivity of serological and molecular detection assays. This swab has a molded breakpoint which allows the swab to be broken in to the tube. The oropharyngeal viscose swab is a tipped swab on a polypropylene shaft, which can be used for collecting throat samples. It has a breakpoint.

Kit Contents:

1. HiViral™ Transport Medium in self-standing or conical bottom tubes (AL167) - 1No.
2. Sterile nasopharyngeal nylon flocked swab with breakpoint (PW1172) - 1No
3. Sterile oropharyngeal viscose swab with breakpoint (PW043B) - 1No

Procedure:

A. Collection of Samples

For a complete diagnostic analysis of viral diseases, it is important that the infectivity of the viruses is preserved after sample collection. The infectivity of viruses' decreases over time and the decay rate is generally a function of temperature. Stability of samples is enhanced by cooling therefore samples should be kept at 2-8°C. The probability of a successful isolation is more if the samples are processed immediately after collection and the viral load in the sample is more. Viral load is maximum if the samples are collected immediately after the onset of clinical symptoms and before the administration of antiviral medications.

B. Directions:

1. Cut open the pouch to remove the swab.
2. Specimen can be collected with the swab in the following manner.

Nasopharyngeal swab

Insert dry swab in to nostril and back to the nasopharynx. Leave in place for a few seconds. Slowly withdraw the swab with a rotating motion.

Oropharyngeal swab

Ask patient to open his/her mouth. Swab the back of the throat near the tonsils thoroughly.

3. Break the swab near the break point and insert swab into the tube containing viral transport medium and close the cap tightly.
4. Label the sample correctly with the name of the patient and time and date of collection.
5. Transport the samples immediately to the laboratory for processing.

Transportation of the Samples:

Samples should be transported to the laboratory as soon as possible.
Samples can be refrigerated at 2-8^{0}C after collection or can be transported at 2-8^{0}C on wet ice within 48 hours. If a long delay is expected in transit and processing, samples should be transported on dry ice and should be frozen at -70^{0}C.

Precautions:

1. Isolation of viruses will largely depend on proper specimen collection, timing of sample collection and processing of samples.
2. Specimen collection should be done in the acute phase of illness.
3. Do not use the product if (1) there is change in the color of the medium,(2) there is evidence of leakage,(3) there are other signs of deterioration.
4. To maintain infectivity of viruses it is important that temperature be properly maintained for sample collection to processing.
5. Avoid repeated freeze-thaw of collected samples.
6. It is recommended to refer to standard procedures and published protocols for sample collection and processing.

Quality control:

Appearance
Orange coloured clear solution

pH at 25°C
7.00-7.60

Osmolality in mOsm/Kg H_2O
600mOsm* - 700mOsm*

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

Storage and shelf life:

Store at 15-30°C.
Use before expiry date given on the product label.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Consult instructions for use
	Do not use if package is damaged
	Reg. Off : 23, Vadhani Ind Est., LBS Marg, Mumbai-400086, India. Works : B-4-5-6 / MIDC, Palkhed, Dindori, Nashik- 422202 Maharashtra, India www.himedialabs.com
EC REP	CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
	Single use. Not intended to be reprocessed and/ or used on another patient

Revision 01/2021

Dey-Engley (D/E) Neutralizing Broth (without Bromo cresol purple)

MU1062

Dey-Engley (D/E)(without Bromo cresol purple) is used in disinfectant testing where neutralization of antiseptics and disinfectants is important for determining its bactericidal activity in accordance with United States Pharmacopoeia

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose	10.000
Sodium thioglycollate	1.000
Sodium thiosulphate	6.000
Sodium bisulphite	2.500
Lecithin	7.000
Polysorbate 80	5.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Dey-Engley (D/E) Neutralizing Broth (without Bromo cresol purple) is formulated as per United States Pharmacopoeia (1). It neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. Sodium thioglycollate, sodium thiosulphate, sodium bisulphite, soya lecithin and polysorbate 80 act as neutralizing components.

For testing disinfectants, prepare two sets of test tubes, one containing 9 ml Dey-Engley Neutralizing Broth (MU1062) and other with 9 ml Dey-Engley Neutralizing Broth Base. Add 1 ml of disinfectant under test. Mix well and allow it to stand for 15 minutes. Inoculate 0.1 ml of 1:100,000 dilution of overnight broth cultures and incubate at 30-35°C for 48 hours Growth in Neutralizing Broth and no growth in Neutralizing Broth Base indicates neutralization of disinfectant. To check bactericidal activity, both broth tubes are inoculated on D/E Neutralizing Agar (M186). Positive growth from negative tubes of Neutralizing Broth Base indicates bacteriostatic substance while negative growth indicates a bactericidal disinfectant. All positive tubes should show growth on Dey-Engley Neutralizing Agar. The control disinfectants used in test procedure are 2% chlorine, 2% formaldehyde, 1% glutaraldehyde, 2% iodine, 2% phenol, 1/750 quaternary ammonium compounds, 1/1000 mercurials etc.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured opalescent solution

Growth Promotion Test
As per United States Pharmacopoeia

Cultural Response
MU1062: Cultural characteristics observed after an incubation at i)For bacteria at 30-35°C for <=3 days i)For fungi at 20-25°C for <=5days.

Organism	Inoculum (CFU)	Growth
Bacillus subtilis ATCC 6633	50-100	luxuriant
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant
Salmonella Typhimurium ATCC 14028	50-100	luxuriant
Escherichia coli ATCC 8739	50-100	luxuriant
Staphylococcus aureus ATCC 6538	50-100	luxuriant
**Aspergillus brasiliensis ATCC 16404*	50-100	luxuriant
Candida albicans ATCC 10231	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. The United States Pharmacopoeia 2011, The US Pharmacopoeial Convention Inc., Rockville, MD

Revision : 2 / 2015

Product Information

PCT0314 L-Lysine, Plant Culture Tested

Product Number		Packing
PCT0314	:	10G
PCT0314	:	25G

Product Information

Product Code	:	PCT0314
Product Name	:	L-Lysine, Plant Culture Tested
Synonym	:	(S)-2,6-Diaminocaproic acid
Molecular Formula	:	$C_6H_{14}N_2O_2$
Molecular Weight	:	146.19
CAS No.	:	56-87-1
EC No.	:	200-294-2
HS Code	:	2922 41 00
Shelf Life	:	4 years

Technical Specification

Appearance	:	White to yellow crystals or powder or chunks
Solubility	:	33.3 mg soluble in 1 mL of water
Cultural response	:	Cultures conditions - Incubation period (5wks), Relative humidity (60±2%), Temperature (25±2ºC), Photoperiod Day: Night in hours (16:8)
Shoot culture	:	No structural deformity observed, actively growing shoots, no toxicity to shoots
Callus culture	:	No necrotic tissues, actively growing callus, no toxicity to callus
FTIR (KBr disc)	:	Matches with the standard pattern
Specific rotation	:	+21.20° to +27.20° (c = 2 in 6 N HCl)
Melting range	:	210 - 220°C (dec.)
Water (K.F.)	:	<= 12.00%
Assay (NT, anhydrous basis)	:	98.00 - 103.00%

Risk and Safety Information

WGK	:	1
RTECS	:	OL5540000
Storage Temperature(°C)	:	Store below 30°C

Transport Information

Marine Pollutant	:	No
ADR/RID	:	Not Dangerous Goods
IMDG	:	Not Dangerous Goods
IATA	:	Not Dangerous Goods

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Fax:+91-022-6147 1920 Gram : STERILITY . email : info@himedialabs.com. Visit us at our website: www.himedialabs.com

Bile, Dried, Purified

RM010

Principle And Interpretation

Bile, Dried, Purified ,is obtained from purified fresh bile dessicated under controlled conditions of temperature that helps to ensure a uniform and reproducible product. It is a greenish yellow coloured, homogeneous, fine powder having characteristic bile like odour with partly bitter, partly sweet and disagreeable taste. It is freely soluble in distilled water. The aqueous solution is clear, yellow coloured and produces foam if shaken strongly. The solution remains clear after autoclaving without developing any precipitate, or scum on surface of the liquid.It is used as a selective inhibitory agent. It is equivalant to Ox Bile, Dried, Purified.

Quality Control

Apperance

Greenish yellow, homogeneous, free flowing powder having bile like odour.

Solubility

Freely soluble in distilled water.

Clarity

1% w/v aqueous solution is clear and free from extraneous matter.

Reaction

Reaction of 1% w/v aqueous solution at 25 °C

pH

6.50- 7.50

Cultural Response

Cultural response after an incubation at 35-37°C for 18-48 hours by preparing Brilliant Green Bile Broth 2% (M121) using Bile,Dried, Purified as an ingredient.

Cultural Response

Organism	Growth	Gas
Cultural Response		
Bacillus cereus ATCC 10876	Inhibited	-
Escherichia coli ATCC 25922	Good-luxuriant	Positive reaction
Enterobacter aerogenes ATCC 13048	Good-luxuriant	Positive reaction
Enterococcus faecalis ATCC 29212	None-poor	Negative reaction
Staphylococcus aureus ATCC 25923	Inhibited	-

Chemical Analysis

Cholic acid Content(on Dried basis)	>= 45.0%
Loss on drying	<= 6.0%

Storage and Shelf Life

Store below 30°C. Use before expiry date on the label.

D-(+)-Mannose RM104

Product Identifier

CAS No.	:	3458-28-4
EC No.	:	222-392-4
Molecular Formula	:	$C_6H_{12}O_6$
Molecular Weight	:	180.16
HS Code	:	2940 00 00
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	White to off-white crystals or powder
Solubility	:	Clear colorless solution at 5g in 100ml of water.
Specific rotation	:	+13.50° to +15.00° (c = 4% in water at 20°C)
Melting range	:	133 - 140°C
Assay (HPLC)	:	min. 98.00%

Safety Information

UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	Not available
WGK	:	3

HiMedia CellCulture
Enabling Breakthroughs

Product Information

Fetal Bovine Serum

Origin: South America, EU Approved
Heat inactivated
Sterile filtered

Product Code: RM9955

Product Description:

Fetal bovine serum (FBS) is a ubiquitously used essential supplement in cell culture media. FBS is a cocktail of proteins, vitamins, carbohydrates, lipids, hormones, growth factors, minerals and trace elements and is used as an universal growth supplement effective for most types of human and animal (including insect) cells. The major functions of serum in culture media are to provide:
(i) Hormonal factors stimulating cell growth and proliferation and promoting differentiated functions.
(ii) Transport proteins carrying hormones (e.g. transcortin), minerals and trace elements and lipids (e.g. lipoproteins)
(iii) Attachment and spreading factors, acting as germination points for cell attachment.
(iv) Stabilising and detoxifying factors needed to maintain pH or to inhibit proteases either directly, such as α-antitrypsin or α2-macroglobulin, or indirectly, by acting as an unspecific sink for proteases and other (toxic) molecules.

RM9955 is heat inactivated Fetal bovine serum. Heat inactivation is done to destroy heat labile components such as complement that can lead to complement mediated cell lysis. Complement proteins, antibodies and enzymes present in the serum are inactivated by heat inactivation.

Applications of Heat inactivated Serum:

- Suitable for immunoassays, enzyme assays and cytotoxicity assays
- For culture of insect cells

***Note**: Heat inactivation process can be detrimental to the growth promoting capacity of serum. When heat inactivation of serum is done, along with the complement certain amino acids, vitamins and growth factors are subjected to temperatures that could cause degradation. Hence it is recommended that researcher should experimentally determine and document the reasons for using heat inactivated serum.*

RM9955 is sourced in countries approved for import into the European Union by European Commission. Currently this includes Central and South America, USA, Canada, Australia, New Zealand and South Africa. This serum is collected and processed in facilities registered and inspected by the competent authority in the country of origin. EU approved serum can be freely moved between EU member countries and many other countries outside of Europe where the USDA or FDA regulations are not required.

Directions for Thawing of Serum:

Thawing of the sera should be done as quickly as possible in order to minimize the period of time during which elevated salt concentration prevail in the thawed liquid.

1. Remove the bottles from the freezer and allow them to acclimatize at room temperature for 10 minutes and keep in 2-8°C overnight in refrigerator.

   Note: Do not place the serum in the water bath or incubator. Avoid exposing serum to elevated temperatures as this can lead to degradation of heat labile nutrients.

2. Swirl the bottle of serum frequently during thawing to disperse the released salts and proteins uniformly in the liquid.

Note on Cryoprecipitate:

We advise our users to follow the recommended thawing procedure. Proper thawing with periodic agitation is crucial to a serum's optimum performance. If bottle of serum is not frequently swirled during thawing, the released proteins and salts tend to form crystalline or flocculent precipitates. These cryoprecipitates are not detrimental to the performance of serum but might affect serum's appearance and consistency.

Slight turbidity or small amount of flocculent material is normal in most serum products and will not affect its performance in any manner. Filtering serum to remove cryoprecipitate is not recommended and could result in loss of nutrients.

Quality Control:

Physical and Chemical analysis:

pH	: 6.8 - 8.2
Osmolality	: 280 – 340 mOsm/KgH_2O
Endotoxin	: Value EU/ml
Hemoglobin	: < 20mg/dl
Identity	: Typical

Protein:

Total protein	: 3.0 - 4.5 g/dl
Albumin	: value g/dl
α-Globulin	: value g/dl
β-Globulin	: value g/dl
γ-Globulin	: value g/dl
IgG	: NMT 250µg/ml

Sterility Testing:

Aerobic bacteria	: Not detected
Anaerobic bacteria	: Not detected
Fungi	: Not detected
Mycoplasma	: Not detected

Virus testing:

Bovine Virus Diarrhea Virus (BVD-V)	: Not detected
Bovine Herpes Virus 1 (BHV-1)	: Not detected
Parainfluenza Type 3 (PI-3)	: Not detected

Antibody testing:

BVD-1 Antibody titer	: Value
BVD-2 Antibody titer	: Value

Growth promotion and cytotoxicity:

Each lot of serum is tested for growth promotion and cytotoxicity. Growth promotion shows the ability of the serum to support the growth of a cell line using a standardized low inoculum in media with 10% serum over a period of 10 to 14 days.

Storage and Shelf Life:

Store at -10°C to -40°C away from bright light. Shelf life of the product is 48 months. Thawed serum can be stored at 2-8°C up to four weeks.

Multiple freeze thaw cycles should be avoided. Serum should never be stored in frost free freezers. Frost free appliance undergoes intermittent warming cycles to prevent ice deposits and this might lead to multiple thawing of serum.

To avoid multiple free thaw cycles or long periods of refrigeration, we recommend freezing small aliquots which can be thawed and used as required.

Use before expiry date given on the label.

Lactophenol

S015

Lactophenol is used for staining as well as wet mounting of fungi. Lactic acid preserves the fungal structure and clears the tissue while phenol acts as a disinfectant.

Composition**

Ingredients

Lactic acid	100.0ml
Phenol	100.0gm
Glycerol	200.0ml
Distilled water	100.0ml

**Formula adjusted, standardized to suit performance parameters

Directions

i) Place a drop of Lactophenol along with 0.05% cotton blue on a clean and dry slide and place a fungal culture in it.

ii) By using a nichrome inoculating wire, carefully tease the fungal culture into a thin preparation.

iii) Place a coverslip on the preparation. Wait for about 5 minutes and observe first under microscope under low power for screening in low intensity. Then observe under high power.

Quality Control

Appearance

Colourless to amber coloured solution.

Clarity

Clear without any particles.

Microscopic Examination

Fungal staining is carried out. Fungal Spores and hyphae are observed under microscope after staining.

Results

Fungal spores & hyphae : Pale to dark blue.

Storage and Shelf Life

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

Revision : 1 / 2015

Material Safety Data Sheet

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

Code No. : **SF97D**

Section 1 : **Chemical Identification**

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

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

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

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

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

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

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

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

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

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

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

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

Section 13 : **Disposal Considerations**

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

Section 14 : **Transport Information**

UN No. : Not applicable.

Section 15 : **Regulatory Information**

Risk Phrases : Not applicable

Safety Phrases : Not applicable

Section 16 : **Other Information**

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

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