Bile Esculin Azide Agar

M493

Intended Use:

For selective isolation and presumptive identification of faecal Streptococci.

Composition**

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

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.*

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (5) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (6) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (7) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (8).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg (9). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation.

All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (10). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Clinical- Faeces, Food samples

Specimen Collection and Handling:

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

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3. Facklam R., 1972, Appl. Microbiol., 23:1131.
4. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Swan, 1954, J. Clin. Pathol., 7:160.
6. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
7. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. 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.
10. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.

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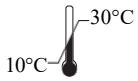

Storage temperature

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Clostridium Difficile Agar Base

M836

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

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

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

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

Type of specimen

Clinical samples - Stool sample; Food samples.

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

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

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

pH
7.20-7.60

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white
Shigella flexneri ATCC 12022	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

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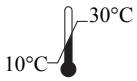

Storage temperature

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Diphtheria Virulence Agar Base

M882

Intended Use:

Recommended for determining toxigenicity of *Corynebacterium diphtheriae.*

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Sodium chloride	2.500
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.5 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 55-60°C. Aseptically add 2 ml sterile KL Virulence Enrichment (FD072) and 0.5 ml sterile 1% Potassium Tellurite (FD052) to a 100 mm Petri plate and quickly add 10 ml of sterile Diphtheria Virulence Agar Base. Before the medium solidifies, place a filter paper strip saturated with potent Diphtheria antitoxin across the diameter of the plate. Allow the strip to sink to the bottom of the plate. Inoculate the plate with heavy inoculum across the strip.

Principle And Interpretation

Corynebacterium diptheriae is a principle human pathogen and owes its pathogenicity to the production of a potent exotoxin active on a variety of tissue including heart muscles and peripheral nerves (2). Toxin diffusing from a streak culture of suspected *C. diphtheriae* is demonstrated by the formation of a white line of precipitate where it meets with diphtheria antitoxin diffusing from a strip of filter paper embedded in the agar. In vitro toxigenicity (virulence) of *C. diphtheriae* was first described by Elek (3). Eleks technique was further improved by King, Frobisher and Parsons (7) by the use of a standardized medium. This medium gave results comparable with animal inoculation test. Also it was found that proteose peptone supported toxin production in addition to maintaining the consistency of results. Hermann et al (4) developed a non-serum based enrichment to overcome the irregularities encountered during the usage of horse, sheep or rabbit serum based enrichments. These non-serum based enrichments consist of Acicase™, tween 80 and glycerol (8).

Upon incubation of the inoculated plate, a line of precipitin is observed for toxigenic strains.

Proteose peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and also for toxin production. Sodium chloride maintains the osmotic balance of the medium. Agar is incorporated as the solidifying agent. Potassium tellurite inhibits most gram-negative bacteria except *Corynebacterium* species, *Streptococcus mitis, Streptococcus salivarius* and Enterococci. *Staphylococcus epidermidis* may exhibit growth. False positive results may also be encountered. Therefore, a positive control has to always be run in parallel (9). *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may also produce line of precipitation (1).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling

For clinical 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 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. False positive results may also be encountered. Hence, a positive control has to always be run in parallel (9)

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

Cultural characteristics observed with added KL Virulence Enrichment (FD072) and 0.5 ml of 1% Potassium tellurite solution (FD052) after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Line of precipitin
Bacillus subtilis subsp. *spizizenii ATCC 6633* (00003*)	>=10^4	inhibited	0%	
Corynebacterium diphtheriae type gravis	50-100	luxuriant	>=50%	positive
Corynebacterium diphtheriae type intermedius	50-100	luxuriant	>=50%	positive
Corynebactrium diphtheriae type mitis	50-100	luxuriant	>=50%	positive
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	none-poor	<=10%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Branson, 1972, Methods in Clinical Bacteriology, Charles C. Thomas,Springfield, III
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology,1996, 14th Edition, Churchill Livingstone
3. Elek S. D., 1948, Br. Med. J., 1:493.
4. Hermann G. J., Moore M. S., and Parsons E. I., 1958, Am. J. Clin. Pathol., 29:181.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. King E. O., Frobisher M. and Parsons E. I., 1949, Am. J. Public Heal th, 39:1314.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. I, Williams and Wilkins, Baltimore.
9. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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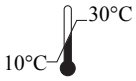

Storage temperature

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Mueller Kauffman Tetrathionate Novobiocin Broth Base M1496I

Intended Use:

Recommended for improved enrichment and isolation of *Salmonellae*. The composition and performance criteria of this media are as per the specification laid down in ISO 6579-1:2017.

ISO 6579-1 Specification - Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth

M1496I - Mueller Kauffman Tetrathionate Novobiocin Broth Base

Composition**

Ingredients	Gms / Litre
Meat extract	4.300
Enzymatic digest of casein	8.600
Ox bile for bacteriological use	4.780
Sodium chloride (NaCl)	2.600
Calcium carbonate ($CaCO_3$)	38.700
Sodium thiosulphate, pentahydrate ($Na_2S_2O_3$ $5H_2O$)	47.800
Brilliant green	0.0096
Final pH (at 25°C)	8.0±0.2

Ingredients	Gms / Litre
HM extract#	4.300
Tryptone###	8.600
Bile##	4.780
Sodium chloride	2.600
Calcium carbonate	38.700
Sodium thiosulphate, pentahydrate	47.800
Brilliant green	0.0096
Final pH (at 25°C)	8.0±0.2

Supplements to be added after autoclaving

	Gms / Litre
Novobiocin sodium salt	0.040
Iodine-iodide solution	20.00ml
Iodine	4.000
Potassium iodide (KI)	5.000

FD203	Gms / Litre
	1 vial
Novobiocin	0.040
Iodine-iodide solution	20.000ml
$ Iodine	4.000
Potassium iodide (KI)	5.000

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract ## Equivalent to Ox bile ### Equivalent to Enzymatic digest of casein

$ To be added but not provided (To be freshly prepared)

Directions

Suspend 89.42 grams (equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C and just before use aseptically add rehydrated contents of 1 vial of MKTT Novobiocin Supplement (FD203) and 20 ml of iodine-iodide solution (20 gram iodine and 25 gram potassium iodide in 100 ml sterile distilled water). 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.*

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, meat products, and from poultry and poultry products (3). ISO committee has also recommended this pre-enrichment medium for the detection of *Salmonella* species from from food stuffs and other materials (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 (1).

Mueller Kauffman Tetrathionate Novobiocin Broth Base contains Tryptone and HM extract as sources of carbon, nitrogen, vitamins and minerals. 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. 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. Method (5).

Type of specimen

ISO 6579-1:2017

Food samples including milk and milk products, in animal feed, in animal faeces, and in environmental samples from the primary production stage.

Specimen Collection and Handling:

Processesing : ISO 6579-1:2017 (4)

Pre-enrichment : Samples (25 grams in 225 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428I) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours .

Isolation : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 37± 1°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

Limitations :

1. The complete medium is unstable and should be used immediately. After incubation, it is permissible to store the selective enrichment medium at 5 °C for a maximum of 72 h.
2. Individual organisms differ in their growth requirement and may show variable growth patterns in the medium
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4. Confirmatory tests should be carried out on all presumptive *Salmonella* colonies that are recovered.

Performance and Evaluation

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

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 : 8.0±0.2

pH

7.80-8.20

Cultural Response

Cultural characteristics observed with added 20ml iodine solution and MKTT Novobiocin Supplement (FD203) after an incubation at 37 ±1°C for 24 ± 3 hours. Further subculture is carried out on XLD Agar, Modified (M031I) and incubated at 37 ±1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Recovery on XLD Agar (M031I)	Colour of colony on XLD Agar (M031I)
Salmonella Enteritidis ATCC 13076 (00030*)+	50-100	>10 colonies	red colonies w/ black centre
Escherichia coli ATCC 8739 (00012*) +	>=10^4		
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4		

Salmonella Typhimurium ATCC 14028 (00031*)+ *Escherichia coli* ATCC 25922 (00013*) + *Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	red colonies w/ black centre

Selectivity

Cultural characteristics observed after an incubation at 37±1°C for 24±3 hours. Further subculture is carried out on Tryptone Soya Agar (M290)and incubated at 37±1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery on Tryptone Soya Agar
Escherichia coli ATCC 8739 (00012*)	>=10^4	partial inhbition	<=100 colonies
Escherichia coli ATCC 25922 (00013*)	>=10^4	partial inhbition	<=100 colonies
Enterococcus faecalis ATCC 29212(00087*)	>=10^4	inhbition - partial inhibition	<10 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhbition - partial inhibition	<10 colonies

* - Corresponding WDCM Numbers

Storage and Shelf Life

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

Product performance is best if used within stated expiry period.

Disposal

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

Reference

1.Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333.
2.Kauffman F., 1935, Ztschr. F. Hyg., 117:26.
3.Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England.
4.Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Detection of Salmonella spp. ISO 6579-1:2017
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05 / 2022

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 are otherwise 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

Cosmetic samples

Specimen Collection and Handling

For cosmetic samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

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

Reference

1.Pelczar and Vera J., 1949, Milk Plant Monthly 38:30
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
3.ISO 21149 (2006) Cosmetics-Microbiology- Enumeration and detection of aerobic mesophilic bacteria
4.Frank H. A., 1955, J. Bacteriol., 70:269.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2023

Bifidobacterium Selective Count Agar Base (BSC Propionate Agar Base)

M1734

Intended Use:

Recommended for enumeration of presumptive Bifidobacteria by colony count technique from milk products.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	1.000
Potassium dihydrogen phosphate	3.000
Dipotassium hydrogen phosphate	4.800
Ammonium sulphate	3.000
Magnesium sulphate heptahydrate	0.200
L-Cysteine HCl monohydrate	0.500
Sodium propionate	15.000
Galactooligosaccharide	10.000
Agar	15.000
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 62.35 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at (115±3°C) for 15 minutes. Cool to 45-50°C. For selective isolation of Bifidobacteria add contents of 2 vials of Mup Selective Supplement (FD250). Mix carefully to avoid the formation of air bubbles and pour into sterile Petri plates or dispense as desired.

Note: This medium being sensitive to heat, excessive heat treatment may therefore indicatively influence the properties of the medium. For more selectivity Glacial acetic acid (1% glacic Selective Supplement B FD251) may also be added. After addition of 1% glacic Selective Supplement (FD251) pH of the medium will shift to the acidic side, which does not affect the performance of the medium.

Principle And Interpretation

Bifidobacteria Selective Count Agar Base is specifically prepared for selective enumeration of Bifidobacteria in fermented milks and fermented milk drinks living together with lactic acid bacteria.
Bifidobacteria Selective Count Agar Base contains highly purified Galactooligosaccharides, which is one of the most excellent Bifidobacteria growth substances. Cysteine hydrochloride helps in creating reduced conditions required for the growth of Bifidobacteria. Tryptone acts as rich nitrogen source.
The antibiotic mupirocin inhibits the growth of most lactic acid bacteria commonly used in fermented and non-fermented dairy products. Freshly prepared culture media not exposed to direct sunlight is recommended (2).
Test Procedure: Before opening the sample container, clean the external surface surrounding of the area from which the test sample is to be taken, in order to remove any material that might contaminate the sample. Weigh 90 gm of diluent in each of the 250 ml pre-sterilized bottles. Close the bottles. Weigh 10 gm of the test sample directly into the bottle with the diluent at 45°C. To dissolve the test sample, swirl slowly to wet the powder. The time between ending the preparation of the primary dilution until addition of culture medium shall not exceed 15 min.. Immediately after solidification of the medium, invert all Petri dishes in the anaerobic culture jar or anaerobic incubator at 37°C for 72 hrs ± 3 hrs. Count the colonies after incubation. Bifidobacterial colonies are recognized by their whitish colour and acetic acid odour. Some of the bifidobacterial strains may appear in different colony size as well as colony appearance on the same plate (2).

Type of specimen

Dairy samples

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Some *Bifidobacterias* are extremely fastidious that may show poor growth due to nutritional variations and selectivity.
2. 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
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of Prepared medium
Yellow coloured opalescent gel forms in Petri plates
Reaction
Reaction of 6.20% w/v aqueous solution at 25°C. pH : 6.3±0.2
pH
6.10-6.50
Cultural Response
Cultural characteristics observed with added Bifido Selective Supplement A under anaerobic conditions, after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Growth with FD250
Bifiobacterium breve ATCC 15100	50-100	luxuriant	Good-luxuriant
Lactococcus lactis ATCC 19435 (00016*)	50-100	good-luxuriant	inhibited
Lactococcus cremoris ATCC 19257	50-100	good-luxuriant	inhibited
Lactobacillus acidophilus ATCC 4356 (00098*)	50-100	good	inhibited

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. ISO/DIS 29981 IDF 220, Milk products- Enumeration of presumptive bifidobacteria- colony count technique at 37°C, 2008.
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.Thitaram, S, Siragusa, Hinton, 2005, Letters in Applied Microbiology, vol 41, 355-360, *Bifidobacterium* selective isolation and enumeration from chiken ceca by an oligosaccharide- antibiotic selective agar medium.
6.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2023

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 :

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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

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 22964: 2017. It can also be used for samples.

Composition

ISO 22964: 2017 Specification - Chromogenic Cronobacter isolation (CCI) agar

Ingredients	Gms / Litre
Tryptic digest of casein	7.000
Yeast extract	3.000
Sodium chloride (NaCl)	5.000
Sodium desoxycholate ($C_{24}H_{39}NaO_4$	0.250
5-Bromo-4-chloro-3-indolyl α–D-glucopyranoside	0.15
Ammonium iron(III) citrate ($C_6H_8O_7FeNH_3$	1.000
Sodium thiosulfate ($N_2S_2O_3$)	1.000
Agar	9.00-18.00
Final pH after sterilization (at 25°C) 7.3±0.2	

M2062I -HiCrome™ Cronobacter Isolation Agar (CCI Agar)

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	0.15
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 (1,2). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of **C.sakazakii* from food samples (3). 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

ISO 22964: 2017: Food products and ingredients intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.

Specimen Collection and Handling:

Processesing : ISO 22964: 2017

Non-selective pre-enrichment in BPW (M1494I) : Samples (10 grams/ 10ml in 90 ml) are preenriched in Buffered Peptone Water and incubated at 34 °C and 38 °C for 18 ± 2 hours.

Selective enrichment (CSB) : 0.1 ml of enriched culture from BPW (M1494I) is then inoculated into Cronobacter Selective Broth (CSB) and incubated at 41,5 °C ± 1 °C for 24 ± 2 hours.

Identification on chromogenic agar (CCI agar) -: 10 microlitre of selectively enriched culture from CSB (M1786I) is then cultured onto CCI Agar (M2062I) and incubated at 41,5 °C ± 1 °C for 24 ± 2h.

Confirmation : Typical colonies are selected from the chromogenic agar, purified on a non-selective agar such as TSA and biochemically characterized.

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. Slight variation in colour may be observed depending on enzyme production by organism and substrate utilization from the medium.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4. 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	Inoculum (CFU)	Colour of Colony
Productivity				
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good	>=50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good	>=50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
Selectivity				
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Specificity				
Enterobacter cloacae ATCC 13047 (00083*)	>=10^4	growth or partial inhibition		Colonies without green or blue green colour

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 (1,4).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983
3. Microbiology of the food chain- Horizontal method for the detection of *Cronobacter* spp. International Organization for Standardization.Draft ISO/ TS 22964, 2017 (E).
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 02/ 2022

Buffered Sodium Chloride-Peptone Solution pH 7.0

MH1275

Intended use

Recommended as a diluent for carrying out microbial limit testing by harmonized methodology of pharmaceutical products in accordance with USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Potassium dihydrogen phosphate	3.600
Disodium hydrogen phosphate dihydrate	7.200
Sodium chloride	4.300
HMC Peptone #	1.000
Final pH (at 25°C)	7.00

**Formula adjusted, standardized to suit performance parameters

Peptone (meat or casein)

Directions

Suspend 14.64 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified /distilled water. Heat if necessary to dissolve the medium completely. For preparation of non-fatty products insoluble in water, add 0.1% w/v Polysorbate 80 to assist the suspension of poorly wettable substances. Dispense in tubes or flasks or as desired and sterilize by autoclaving at 15 lbs pressure 121°C for 15 minutes or as per validated cycle.

Principle And Interpretation

The composition of this medium is in accordance with the harmonized methodology of USP/EP/BP/JP/IP (1-5). This medium is recommended for preparation of stable test strain suspension employed for validating the microbiological testing procedures of non-sterile products. The standardized stable suspensions are used so that the suitability of this test to detect microorganism in presence of product can be established. Non-fatty products insoluble in water and water-soluble products are diluted/dissolved using this solution.

HMC Peptone serves as nutrient source and maintains the cell viability. Phosphates in the medium act as good buffering agents. Sodium chloride maintains the osmotic balance and cell integrity. Polysorbates reduce surface tension and also inactivate phenolic compound, if present in the test sample.

Preparation of test strain is recommended in Buffered Sodium chloride-Peptone solution pH 7.0 (MH1275) at 30-35°C wherein there is no multiplication of organisms or there is no decrease in count for upto 4 hours.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling:

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (1-5).

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

Warning and Precautions

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

Limitations :

1. This medium contains less nutrients and is not recommended for the growth of microorganisms.
2. 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

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless to pale yellow clear solution w/o any precipitate

pH

7.00

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of ICH(USP/EP/BP/JP/IP).

Cultural response

Cultural characteristics observed after recovery on Soybean Casein Digest Agar after an incubation at 30-35°C for 18-24 hours for bacteria and Sabouraud Dextrose Agar at 30-35°C for 24-48 hours .

Organism	Inoculum (CFU)	Recovery within 2 hours of incubation	Recovery within 4 hours of incubation	Recovery within 24 hours of incubation
Preparation of test strain				
Escherichia coli ATCC 8739 (00012*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli ATCC 25922 (00013*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli NCTC 9002	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Abony *NCTC* 6017 (00029*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Bacillus subtilis subsp. *spizizennii* ATCC 6633 (00003*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at

				2-8°C)
Micrococcus luteus ATCC 9341	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Candida albicans ATCC 10231 (00054)*	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Candida albicans ATCC 2091 (00055)*	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)

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 inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

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

Reference

1.The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
2. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
3. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
4. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022
5.The Japanese Pharmacopoeia, 17th edition, 2016, The Ministry of Health, Labour and welfare.
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 : 06 / 2022

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

Barritt Reagent A (for VP test)

R029

Intended use

Barritt Reagent A (for VP test) is used in Voges-Proskauer test for detection of acetoin production by bacterial culture.

Composition**

Ingredients

a-Naphthol (1-Naphthol)	5.0 gm
Absolute ethanol	100.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Grow test culture in MR-VP Medium (M070).
2. Add 0.6 ml of Reagent A and 0.2 ml (2 drops) of Reagent B (R030) for 10 ml medium.
3. Shake tubes gently for 30 seconds to 1 minute to expose the medium to atmospheric oxygen in order to oxidize the acetoin (acetylmethylcarbinol) so as to obtain a colour reaction.
4. Allow tube to stand at least 10 to 15 minutes.

Principle And Interpretation

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

Type of specimen

The specimen is any isolated colony on primary or subculture plates.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. 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. Increased exposure of organisms to atmospheric oxygen in the microtechnique decrease the incubation period.
2. False-positive VP results may occur if VP tests are read beyond one hour following the addition of reagents. A copper like color may develop, resulting in a potential false positive interpretation.
4. Shaking the tubes enhance VP reaction.
3. With prolonged incubation, some VP positive organisms can produce acid condition in the medium, yielding weak positive reaction or false negative VP reaction.
5. Do not add more than 2 drops of KOH per 2ml of medium. Excess amount of KOH can give a week positive reaction, which may be masked by the formation of copper like color because of the reaction of KOH with alphanaphthol alone.
6. Reagents must be added in specified order. A reversal of order may result in the weak positive or false negative VP results.

Performance and Evaluation

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

Quality Control

- **Appearance :** Colourless to reddish brown coloured solution.
- **Clarity :** Clear without any precipitate.

Cultural Response

Organism	Growth	VP Test
Cultural Responce	24-48 hours old cultures grown in MR-VP Medium (M070). reagent (Part A) (R029) and 0.2 ml of Barritt Reagent (Part B) (R030) in Biochemical identification was carried out by adding 0.6 ml of Barritt R	
**Klebsiella aerogenes* ATCC 13048 (WDCM 00175)	Luxuriant	Positive (Cherry red colour formation)
Escherichia coli ATCC 25922 (WDCM 00013)	Luxuriant	Negative (No red colour formation)
Klebsiella pneumoniae ATCC13383 (WDCM 00097)	Luxuriant	Positive (Cherry red colour formation)

(*) formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

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.

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition. Vol. 2.
2. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A,Academic Press, London.
7. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
8. Color Atlas and Textbook of Diagnostic Microbiology,4th edition ,Elmer W. Koneman. Stephen D.Allen., William M. Janda., Paul C. Schreckenberger.,Washington C.Winn.

Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

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Revision : 03/2022

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