Listeria Oxford Medium Base

M1145

Intended use

Recommended for isolation of *Listeria* species from pathological specimens.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Lithium chloride	15.000
Sodium chloride	5.000
Corn starch	1.000
Esculin	1.000
Ammonium ferric citrate	0.500
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.75 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Oxford Listeria Supplement (FD071) or 1 vial of Listeria Moxalactam Supplement (FD126). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Listeria monocytogenes is the only species of the genus *Listeria* that is important as a human pathogen. *Listeria seeligeri, Listeria welshimeri* and *Listeria ivanovii* have been related with animal diseases. In any case, all the species are pathogenic between the ovine and bovine cattle. Positive diagnosis of listeriosis can be obtained only by the isolation and cultivation of the responsible bacteria from blood or CSF samples of the affected organisms. Listeria Oxford Medium Base is based on the formulation described by Curtis et al (2) for isolation of *L. monocytogenes* from clinical and food specimens. Peptone special serves as the source of essential nutrients to the organisms. Corn starch serves to neutralize the toxic metabolites formed. Lithium chloride and the antibiotics inhibit gram-negative bacteria and most gram-positive organisms but certain strains of Staphylococci may grow as esculin negative colonies. Cycloheximide is used to reduce fungal contamination; cefotetan and phosphomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively moxalactam (FD126) can be added which inhibits both gram-positive and gram-negative bacteria. *L. monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ions and produces black zones around the colonies. Although the selectivity of the medium is enough to allow the isolation and differentiation by direct surface inoculation, a previous dilution of the inoculum is advisable or even more when the sample is highly polluted. The techniques for isolation vary with the material under examination (8). For all specimens selective and cold enrichment is recommended (3,4). For faecal and biological specimens, the sample is homogenized in 0.1% Peptone Water (M028) and 0.1 ml amount is either directly plated on Listeria Selective Medium or inoculated into the Selective Enrichment Broth and incubated at 30°C for 7 days and then further inoculated on Listeria Selective Medium. For food and environmental samples selective enrichment is generally used.

For isolation of Listeria from food (milk and milk products), add 25 ml or 25 grams of sample to 225 ml of Listeria Enrichment Broth, UVM (M890A). Homogenize and mix carefully. Incubate for 48 hours at 30°C. Streak the enriched cultures onto Listeria Oxford medium Base and incubate aerobically for 48 hours at 37°C. Take 5 typical colonies (esculin positive) and inoculate onto Soyabean Casein Digest Medium (M290). Incubate for 24 hours and then use these colonies for biochemical confirmation.

Type of specimen

Clinical samples - Body tissues or body fluids, Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Further biochemical tests are needed for a final identification of the isolated organisms.

Performance and Evaluation

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

Quality Control

Appearance
Light yellow to dark yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Dark amber coloured clear to slightly opalescent gel with a blue cast forms in Petri plates

Reaction
Reaction of 5.55% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Oxford Listeria Supplement (FD071) or Listeria Moxalactam supplement(FD126), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Bacillus subtilis ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Enterococcus hirae ATCC 10541	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Listeria monocytogenes serovar 1 ATCC 19111 (00020*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony

Listeria monocytogenes ATCC 19117	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	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 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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).

References

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Curtis G. D. W., Mitchell R. G, King A. F., Griffin E. J., 1989,Lett. Appl. Microbiol.
3. Fernandez G. J. F., Dominguez R. L., Vazzuez B. J. A., Rodriguez F.E. F., Briones D. V., Blanco L. J. L., Suarez F. G., 1986, Can. J. Microbiol., 32:149.
4. Hayes P. S, Feeley J. L, Groves L. M, Ajello G. W. and Fleming D. W, 1986, Appl. Environ. Microbiol., 51:43
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. 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.
8. Van Netten P., Peroles I., Van de Mosdik A., Curtis G. D. W., Mossel D. A. A, 1988, Int. J. Food Microbiol., 6:18
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03/ 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
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Tryptone Soya Yeast Extract Broth

M1263

Intended Use:

Recommended for confirmation of *Listeria* in Henry's light.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose (Glucose)	2.500
Yeast extract	6.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Tryptone Soya Yeast Extract Broth is formulated as per APHA (6) for the isolation and cultivation of *Listeria monocytogenes* from foods. ISO Committee (3) has recommended for the cultivation and maintenance of a wide variety of heterotrophic microorganisms (1).

Tryptone and soya peptone provide nitrogeneous and carbonaceous compounds, long chain amino acids and other essential nutrients. Dextrose is the energy source. Dipotassium hydrogen phosphate acts as buffering system to control pH. Yeast extract is the rich source of vitamin B complex.

According to FDAs enrichment procedure (2) for isolation of *Listeria monocytogenes* from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 24-48 hour

Type of specimen

Food and dairy samples

Specimen Collection and Handling:

According to FDAs enrichment procedure (2) for isolation of *Listeria monocytogenes* from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 24-48 hours.

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 confirmation of organisms on selective media is required.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured clear solution in tubes.

Reaction
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 30-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Listeria monocytogenes ATCC 19117	50-100	good-luxuriant
Listeria monocytogenes ATCC 19111 (00020*)	50-100	good-luxuriant
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Atlas R. M. 2004, 3rd Ed., Handbook of Microbiological Media, Parks, L.C. (Ed.), CRC Press, Boca Raton.

2. FDA, Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.

3. International Organization for Standardization (ISO), 1993, Draft, ISO/DIS 10560.

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

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

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Revision : 03 / 2019

Tryptophan Medium

M1339

Tryptophan Medium is recommended for detection of indole production.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Sodium chloride	5.000
DL-Tryptophan	1.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Enterohemorrhagic *Escherichia coli* (EHEC) is a defined subset of Shiga-like (vero) toxin-producing *E. coli* . EHEC infections are waterborne or food borne. EHEC is ingested most commonly with undercooked ground beef (1, 2, 3). There are more than 50 serotypes of EHEC. However, *E. coli* O157:H7 is the prototype EHEC.

E. coli O157:H7 can cause an asymptomatic infection, mild diarrhea, or a diarrheal illness that is characterized by non-bloody (progressing to bloody) diarrhea and abdominal cramps (together known as hemorrhagic colitis), few leukocytes in stools and lack of significant fever (1, 2, 4).Tryptophan Medium is prepared as per the formula approved by ISO Committee (5), that is a modification of original formula of APHA where the medium is devoid of tryptophan (6). This medium is useful for the detection of indole production by *Escherichia coli* O157: H7, which is a key feature in differentiation of coliforms.

Casein enzymic hydrolysate provides carbonaceous and nitrogenous sources required for the growth of microorganisms. Tryptophan is an amino acid, which serves as a substrate to study indole reaction. Certain microorganisms breakdown tryptophan with the help of the enzyme tryptophanase that mediate the production of indole by hydrolytic activity (7). The indole produced can be detected by Kovacs or Ehrlichs reagent (8). Indole combines with the aldehyde present in the above reagent to give red colour in the alcohol layer. The alcohol layer extracts and concentrates the red colour complex.

The test sample is enriched in Modified Soyabean Bile Broth Base (M1286I) by incubating at 42°C for 18-24 hours. *E. coli* O157:H7 is then isolated on MacConkey Sorbitol Agar Base (M298I). Pale coloured colonies obtained on incubation at 35-37°C for 18-24 hours are reported as presumptive *E. coli* O157:H7. Presumptive colonies are subjected to indole test that makes the use of Tryptophan Medium (M1339).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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

Reaction
Reaction of 1.6% aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
M1339: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum	Growth	Indole production

Cultural Response

Enterobacter aerogenes ATCC 13048	50-100	luxuriant	negative reaction, no colour development / cloudy ring
Escherichia coli 0157:H7 NCTC 12900	50-100	luxuriant	positive reaction, red ring at the interface of the medium
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction, red ring at the interface of the medium

Storage and Shelf Life

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

Reference

1. Centers for Disease Control and Prevention, 1993, Morbid. Mortal. Weekly Rep. 42: 257:253.
2. Griffin P. M. and Tauxe R. V., 1991, Epidemiol. Rev. 13: 60-91
3. Kay B. A., Griffin P. M., Strockbine N. A. and Wells J. G., 1994, Clin. Microbiol., Newsletter, 16:17-19.
4. 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.
5. International Organization for Standardisation (ISO) Draft: ISO/DIS 16654:1999.
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7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

Revision : 02 / 2015

Rappaport Vassiliadis Soyabean Meal Broth (RVSM Broth) M1448

Rappaport Vassiliadis Soyabean Meal Broth is recommended as selective enrichment medium for the isolation of *Salmonella* species.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	4.500
Sodium chloride	7.200
Potassium dihydrogen phosphate	1.260
Dipotassium hydrogen phosphate	0.180
Magnesium chloride	13.580
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.75 grams in 1000 ml distilled water. Heat gently if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Rappaport Vassiliadis Soyabean Meal Broth (RVSM) is modification of the Rappaport Vassiliadis Enrichment Broth, revised by van Schothorst (1-3). This medium is recommended as the selective enrichment medium for isolation of *Salmonella* . van Schothorst modified the original formula by addition of dipotassium hydrogen phosphate to buffer the medium and addition of anhydrous magnesium chloride to enhance the reliability of enrichment broth. Peterz (4) et al have also emphasized the importance of the concentration of magnesium chloride in the final medium.

The test specimen is added to Buffered Peptone Water (M614) and incubated at 35°C for 16 - 20 hours. This pre-enriched peptone water culture is inoculated into RVSM Broth and incubated at 42 ± 1°C for 24 - 48 hours and further subcultured on Brilliant Green Agar (M016). For faecal specimens, no pre-enrichment is needed. Add 1 or 2 loopfuls of liquid faeces (or an emulsion of faeces in saline) to 10 ml of RVSM Broth pre-warmed to 42°C. Incubate at 42 ±1°C for 24 hours and streak on to a selective agar.

The medium contains papaic digest of soyabean meal which provides essential growth nutrients. Magnesium chloride raises the osmotic pressure in the medium. Malachite green is inhibitory to organisms other than Salmonellae. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* species. Phosphates buffer the medium to maintain the constant pH. Sodium chloride maintains the osmotic balance.

Quality Control

Appearance
Light yellow to light blue homogeneous free flowing powder

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

Reaction
Reaction of 2.67% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH
5.00-5.40

Cultural Response
M1448: Cultural characteristics observed after an incubation for 18-24 hours for following temperature.

Organism	Inoculum (CFU)	Growth at 42±1°C	Recovery	Growth at 35-37°C

Cultural Response

Escherichia coli ATCC 25922	50-100	fair	10-20%	poor
Salmonella Paratyphi B ATCC 8759	50-100	good	40-50%	good
Salmonella Typhi ATCC 6539	50-100	fair-good	30-40%	fair
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	>=50%	good-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. Rappaport F., Konforti N. and Navon B., 1956, J. Clin. Pathol., 9, 261-266
2. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11-18.
3. Van Schothorst M. and Renauld A., 1983, J. Appl. Bacteriol., 54:209-215.
4. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bacteriol., 66,523-528.

Revision : 1 / 2011

Buffered Peptone Water

M1494I

Intended use

Recommended as a pre-enrichment medium of *Enterobacteriaceae* organisms such as *Salmonella* and *Cronobacterium* species from food and animal feeding stuffs, water, milk, milk products and other products. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017, ISO 6887-1:2017, ISO 21528-1:2017, ISO 22964:2017.

Composition**

ISO 6579-1:2017, ISO 6887-1:2017 (E), ISO 21528-1:2017, ISO 22964:2017, Specification - Buffered peptone water (BPW)

Ingredients	Gms / Litre
Enzymatic digest of casein	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate, dodecahydrate,($Na_2HPO_4.12H_2O$)	9.000
Potassium dihydrogen phosphate (KH_2PO_4)	1.500
Final pH (at 25°C)	7.0±0.2

Buffered Peptone Water

Ingredients	Gms / Litre
Peptone#	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate, dodecahydrate,($Na_2HPO_4.12H_2O$)	9.000
Potassium dihydrogen phosphate (KH_2PO_4)	1.500
FinalpH (at 25°C)	7.0±0.2

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

Directions

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

Principle And Interpretation

Microorganisms that are subjected to environmental stresses may become structurally or metabolically damaged or injured. These microorganisms are unable to replicate in selective environments. Therefore these injured organisms must be resuscitated or permitted to repair the damage by incubation in an appropriate, non-selective environment (10). Edel and Kampelmacher (1) noted that sub-lethal injury to Salmonellae may occur in many food preservation processes. Pre-enrichment in Buffered Peptone Water (M1494I) at 35°C for 18-24 hours results in repair of injured cells (9). The buffering system prevents bacterial damage due to change in the pH of the medium. ISO committee has also recommended this pre-enrichment medium for the detection of *Enterobacteriaceae* (3), *Salmonella* (4) *a*nd *Cronobacter* (6) species from from food stuffs and other materials. It is also recommended as a diluent for enumerations of all microorganisms (7) and *Listeria* species(8).

Type of specimen

ISO 6579-1:2017/ ISO 6887-1:2017/ ISO 11290-1:2017/ ISO 21528-1:2017/ ISO 22964:2017

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 6887-1:2017 (7) / ISO 11290-1:2017 (8)

Dilution samples : Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

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 (M1428) 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.

Processesing : ISO 21528-1:2017 (6)
Pre-enrichment : Samples (10 grams in 90 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 37 ± 1°C for 18 h ± 2 hours.
Isolation : The culture thus obtained is then plated on Violet red bile glucose (VRBG) agar (M1684) and incubated at 37 ± 1 °C for 24±2hours.
Confirmation : Biochemical and serological tests are performed for confirmation.

Processesing : ISO 22964:2017 (5)
Pre-enrichment : Samples (10 grams in 90 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 Cronobacter Selective Broth (M1786I) and incubated at 41.5 ± 1°C for 24±2hours.
Isolation : The culture thus obtained is then plated on HiCrome™ Cronobacter Isolation Agar(CCI Agar)(M2062I) and incubated at 41.5 ± 1°C for 24±2hours.
Confirmation : Biochemical and serological tests are performed for confirmation.

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. Individual organisms differ in their growth requirement and may show variable growth patterns in 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.
3. Further biochemical tests must be carried outfor confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate
Reaction
Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.0±0.2
pH
6.80-7.20
Cultural Response

Organism	Inoculum CFU)	Recovery
ISO 6887-1:2017		
Dilution : Recovery of ± 30% of the original count (recovered on Tryptone Soya Agar, M290), when the inoculated sample holding time is 45 minutes to 1 hour at 20-25°C. The plates are incubated at at 37°±2°C for 18 h ± 2 hours.		
Escherichia coli ATCC 8739 (00012*)*	50-100	±30% of the original count
Escherichia coli ATCC 25922 (00013*)	50-100	±30% of the original count

Staphylococcus aureus ATCC 6538 (00032*)*	50-100	±30% of the original count
Staphylococcus aureus ATCC 25923 (00034*)*	50-100	±30% of the original count

ISO 6887-1:2017

Dilution : Recovery of ± 30% of the original count (recovered on Tryptone Soya Agar, M290), when the inoculated sample holding time is 1 hour ± 5 minutes at 20 ± 2°C. The plates are incubated at at 37°±2°C for 18 h ± 2 hours.

Listeria monocytogenes ATCC 13932 (00021*)	50-100	±30% of the original count
Listeria monocytogenes ATCC 35152 (00109*)	50-100	±30% of the original count

ISO 6579-1:2017 & ISO 21528-1:2017

Productivity

Cultural characteristics observed after an incubation at at 34°C to 38°C for 18 h ± 2 hours.

Organism	**Inoculum CFU)**	**Growth**
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant
Escherichia coli ATCC 25922 (00013*)*	50-100	good-luxuriant

ISO 22964:2017

Productivity

Cultural characteristics observed after an incubation at at 34°C to 38°C for 18 h ± 2 hours.

Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good-luxuriant

Key :* Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 (2,3).

Reference

1. Edel W. and Kampelmacher E. H., 1973, Bull. Wld. Hlth. Org., 48: 167.

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. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of Salmonella . International Organization for Standardization (ISO), ISO/DIS 6579-1:2017.
5. Microbiology of the food chain —Horizontal method for the detection and enumeration of *Enterobacteriaceae* —Part 1: Detection of Enterobacteriaceae. International Organization for Standardization (ISO), ISO 21528-1:2017.
6. Microbiology of the food chain- Horizontal method for the detection of Cronobacter spp. International Organization for Standardization. Draft ISO/ TS 22964, 2017 (E).
7. Microbiology of the food chain- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1 General rules for the preparation of the initial suspension and decimal dilutions. International Organization for Standardization (ISO), 6887-1:2017.
8. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2 , Detection method ; ISO 11290-2:2017.
9. Sadovski A. Y., 1977, J. Food Technol., 12.85.
10. 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 : 05/ /2020

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 (6) recommended Tetrathionate Broth as a selective medium for the isolation of Salmonella. Kauffman (4) modified the formula to include ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus s*pecies. The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat, meat

products, and from poultry and poultry products (7). ISO committee has also recommended this pre-enrichment medium for the detection of *Salmonella* species from from food stuffs and other materials (5). 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 (4).

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 (5)

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 meidum 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 period when stored at 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)
Oqnc t bshulsx			
Salmonella Enteritidis ATCC 13076 (00030*)+	50-100	>10 colonies	red colonies w/ black centre
Esccherichia coli ATCC 8739 (00012*) +	>=10^4		
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4		
Salmonella Typhimurium ATCC 14028 (00031*)+	50-100	>10 colonies	red colonies w/ black centre
Esccherichia coli ATCC 25922 (00013*) +	>=10^4		
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4		

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. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

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

Reference

1. Harvey R. W. S. and Price T. S., 1976, J. Hyg. Camb., 77:333.
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. Kauffman F., 1935, Ztschr. F. Hyg., 117:26.

5. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Detection of Salmonella spp. ISO 6579-1:2017

6. Mueller L., 1923, C. R. Soc. Biol., (Paris) 89:434.

7. Public Health Laboratory Service, 1974, Monograph Series No. 8, Public Health Laboratory Service, London, England.

Revision : 03 / 2020

Tryptone Bile Glucuronic Agar (TBX Agar)

M1591

Intended use

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

Composition**

Ingredients	Gms / Litre
Bile salt mixture	1.500
Tryptone	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 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 in sterile Petri plates.

Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (3). Tryptone Bile Glucuronic Agar contains the enzyme ß -D- glucuronidase which differentiates most *E.coli* species from other coliforms.
E.coli absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (2).The enzyme ß-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the ß-D-glucuronide. *E.coli* colonies are blue green coloured (5,6). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

Type of specimen

Clinical samples - urine, blood, Food samples ; Water 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 (7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
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.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii ATCC 8090	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	>=50%	blue-green
Enterococcus faecalis ATCC 29212 (00087)*	>=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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. 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.

3.International Standard ISO 16649-2: 2018. 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.

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

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

6. Killian M. and Bolow P 1976 Rapid diagnosis of Enetrobacteriacea I. Detection of bacterial glycosidases. Acta Rattol. Microbiol Scand Sct B 84245:251.

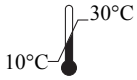
7. Ley A N, Bowers R J, Wolfe S 1988 Indocyl –B-D-glcuaoride, a novel chromogenic coli reagent for the detection and enumeration of *Escherichia coli* in environmental samples. Canadian Journal of Microbiology 34:690-693.

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

Revision : 03/ 2019

IVD In vitro diagnostic medical device

CE Marking



Storage temperature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Skim Milk Plate Count Agar

M1623

Skim Milk Plate Count Agar is recommended for determining the microbial count in milk and dairy products.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	2.500
Skim milk powder	1.000
Glucose	1.000
Agar	10.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20 grams in 1000 ml distilled water. Allow it to stand for about 15 minutes, place in a cold water bath and heat gently with frequent shaking to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Skim Milk Plate Count Agar complies with the recommendation of the International Dairy Federation (1, 2) and the DIN Norm 10192 (3) for the examination of milk and dairy products.

Casein enzymic hydrolysate provides amino acids and other complex nitrogenous substances. Yeast extract supplies vitamin B complex. Addition of skim milk in the medium makes the conditions optimal for microorganisms which grow in milk. A wide range of microorganisms can be cultured and enumerated on this medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.05% Agar gel.

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

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

pH
6.80-7.20

Cultural Response
M1623: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Lactococcus lactis spp. LactisATCC 19435	50-100	luxuriant	>=70%
Listeria monocytogenes ATCC 19118	50-100	Luxuriant	>=70%
Bacillus cereus ATCC 11778	50-100	luxuriant	>=70%

Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%
Candida albicans ATCC 10231	50-100	luxuriant	>=70%

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. DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung; Bestimmung der Keimzahl (Referenzverfahren) - DIN 10192.
2. Internationaler Milchwirtschaftsverband: Milch u. Milchprodukte, Zählung von Mikroorganismen (Koloniezählung bei 30 °C) - Internationaler Standard 100 (1991).
3. Internationaler Milchwirtschaftsverband: Flüssige Milch. Zählung von psychotrophen Mikroorganismen (Koloniezählung bei 6,5°C). - Internationaler Standard 101 (1991).

Revision : 02 / 2015

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).

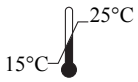
Reference

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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Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) M1881

Intended Use:

Recommended for selective isolation of fungi-yeasts and moulds of significance in food spoilage. The composition and performance criteria are in accordance with ISO 21527-1:2008.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Rose Bengal	0.025
Chloramphenicol	0.100
Dichloran	0.002
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.6 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

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) is formulated by as described by King et.al (4) and is recommended for selective isolation of yeasts and moulds especially in food samples. It is recommended by ISO (5) This medium is a modification of Rose Bengal Chloramphenicol Agar which additionally contains dichloran.

Peptone provides nitrogeneous compounds, carbon, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Rose Bengal exhibits an improved inhibitory activity at pH 5.6 and hence the final pH of the medium is maintained at 5.6 for the inhibition of spreading fungi (4) The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and colonies of the more rapidly growing moulds. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. Additionally Rose Bengal is taken by yeast and moulds colonies, which allows these colonies to be easily recognized and enumerated.

This medium should not be exposed to direct light as rose bengal undergoes photo-degradation leading to formation of toxic chemicals for fungi (6,7).

Type of specimen

Food sample : Eggs, Meat, Dairy products (except milk powder), Fruits, Vegetables,Fresh pastes, etc.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,5,6,8,9).

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.16% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for upto 6 days.

Organism	Inoculum (CFU)	Growth	Recovery
*Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)**	>=10^4	inhibited	0%
*Candida albicans ATCC 10231 (00054)**	50-100	good-luxuriant	>=50%
*Escherichia coli ATCC 25922 (00013)**	>=10^4	inhibited	0%
*Escherichia coli ATCC 8739 (00012)**	>=10^4	inhibited	0%
*Mucor racemosus ATCC 42647 (00181)**		good-luxuriant	
*Saccharomyces cerevisiae ATCC 9763 (00058)**	50-100	good-luxuriant	>=50%
*Aspergillus brasiliensis ATCC 16404 (00053)**		good-luxuriant	

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store the dehydrated powder and the prepared medium between 15-25°C in a tightly closed container. 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. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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. King D.A. Jr., Hocking A.D. and Pitt J.I., 1979, J. Appl. Environ. Microbiol., 37:959.
5. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0,95, ISO 21527-1:2008
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
7. Sharp A.N. and Jackson A.K., 1972, J. Appl. Bact., 24:175.
8. U.S. Food and Drug Administration, 1995, Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, Md.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 04/ 2019

Violet Red Bile Glucose Agar

MH581

Intended use

Recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP .

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Gelatin peptone #	7.000
Bile salts	1.500
Sodium chloride	5.000
Glucose monohydrate	10.000
Agar	15.000
Neutral red	0.030
Crystal violet	0.002
pH after heating (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Pancreatic digest of gelatin

Directions

Suspend 40.62 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. DO NOT HEAT IN AN AUTOCLAVE. Cool to 45 - 50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Glucose Agar is a selective medium recommended for detection and enumeration of *Enterobacteriaceae* especially the bile tolerant gram negative bacteria in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (7,1,2,3,4) from non-sterile products and pharmaceutical preparations.

Gelatin peptone and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts positive organisms especially Staphylococci. Neutral red indicator helps to detect glucose fermentation. Glucose fermenting and crystal violet. Crystal violet inhibits gram-strains produce red colonies with pink-red halos in the presence of neutral red. Sodium chloride maintains the osmotic equilibrium in the medium. The red colour is due to absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.

Type of specimen

Pharmaceutical samples, Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (7,1,2,3,4).

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. 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. Though the medium is for selective isolation of *Enterobacteriaceae*, further biochemical and serological testing must be carried out for further confirmation.
2. Over incubation may result in reverting of reaction.

Performance and Evaluation

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

Quality Control

Appearance
Light yellow to pinkish beige homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel.

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

pH
7.20-7.60

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. Cultural response was observed after an incubation at 30-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean 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).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <= 100 cfu (at 30-35°C for 18-24 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	25 -100	>=50 %	pink to red	18 -24 hrs
Additional Microbiological Testing						
Escherichia coli NCTC 9002	50 -100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Salmonella Enteritidis ATCC 13076 (00030)*	50 -100	good-luxuriant	25 -100	>=50 %	light pink	18 -24 hrs
# Klebsiella aerogenes ATCC 13048 (00175)*	50 -100	good-luxuriant	25 -100	>=50 %	pink-red	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited	0	0%		>=24 hrs
Staphylococcus aureus subsp. aureus ATCC ATCC 6538 (00032)*	>=10^3	inhibited	0	0%		>=24 hrs

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

Storage and Shelf Life

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

Disposal

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

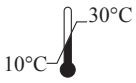
Reference

1. British Pharmacop eia, 2017, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2016, European Dept. for the quality of Medicines.
3. Japanese Pharmacopoeia, 2016.
4. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. The United States Pharmacopoeia, 2019 The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg,Mumbai-86,MS,India

EC REP

CE Partner 4U ,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu

Technical Data

Fluid Thioglycollate Medium

MU009

Intended Use:

Recommended for sterility testing of biologicals and for cultivation of aerobes, anaerobes and microaerophiles in accordance with USP.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	5.000
Dextrose monohydrate	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750
pH after sterilization	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.25 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 25°C and store in a cool dark place preferably below 25°C.
Note: If more than the upper one-third of the medium has acquired a pink-purple colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink-purple colour disappears.

Principle And Interpretation

Brewer (1) formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes by adding a reducing agent and small amount of agar. The USP (10), BP (2), EP (3)and AOAC (11) have recommended the media for sterility testing of antibiotics, biologicals and food products and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials. Dextrose monohydrate, tryptone, yeast extract, L-cystine provide the growth factors necessary for bacterial multiplication. Sodium thioglycollate and L-cystine act as a reducing agent lowering the oxidation-reduction potential by removal of oxygen This condition helps to prevent the accumulation of peroxides which is toxic in nature. The SH group also neutralizes the antibacterial effect of mercurial preservatives and other heavy metal compounds which exert a bacteriostatic effect in the materials under examination. Any increase in the oxygen content is indicated by a colour change of redox indicator-resazurin; to red (7,8,9). The small amount of agar helps in maintaining low redox potential and stabilizes the medium (6).
In sterility checking, it is recommended to dilute the sample containing preservatives, with this broth to reduce the toxicity and enhance the growth of contaminants, if any.

Type of specimen

Pharmaceutical samples for sterility testing.

Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (10)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

1. It is intended for the examination of clear liquid or water-soluble materials.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light straw coloured clear to slightly opalescent solution with upper 10% or less medium pink- purple on standing.

Reaction

After sterilization,reaction of 2.92% w/v aqueous solution. pH : 7.1±0.2

pH

6.90-7.30

Growth Promotion Test

As per United States Pharmacopoeia

Stability test

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

Cultural response

Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days.

Organism	Inoculum (CFU)	Growth
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	luxuriant
Clostridium sporogenes ATCC 11437	50 -100	luxuriant
Clostridium perfringens ATCC 13124 (00007*)	50 -100	luxuriant
Bacteroides fragilis ATCC 23745	50 -100	luxuriant
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant
Micrococcus luteus ATCC 9341	50 -100	luxuriant
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant
Escherichia coli NCTC 9002	50 -100	luxuriant
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant
Salmonella Abony NCTC 6017	50 -100	luxuriant
Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant

Key : * Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle 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 (4,5).

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2. British Pharmacopoeia, 2020, The Stationery office British Pharmacopoeia.
3. European Pharmacopoeia, 2020 European Department, for the Quality of Medicines.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of, Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
7. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.
8. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287.
9. Portwood, 1944, J. Bact., 48:255.
10. U.S. Pharmacopoeia, 2019,Vol.4 United States Pharmacopoeia Convention, Inc., Rockville, MD.
11. Williams. (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., AOAC,Washington, D.C.

Revision :03/2021

*Buffer solution, pH 7.0 ± 0.02

R063

Intended Use:

Buffer solution, pH 7.0 ± 0.02 is used to establish and maintain an ion activity within narrow range. It is most commonly used to establish hydrogen-ion activity for the calibration of pH meters, in analytical procedures. It is also used to maintain stability of various dosage forms.

Composition**

Ingredients

Sodium dihydrogen phosphate	1.20gm
Disodium hydrogen phosphate	0.885gm
Distilled water	1000.00ml

**Formula adjusted, standardized to suit performance parameters

Principle And Interpretation

Buffer is defined as a solution which resists changes in the activity of an ion on addition of substances that are expected to change the activity of that ion. Buffer capacity refers to the amount of material that may be added to solution without causing a significant change in ion activity. Buffered solutions are systems in which the ion is in equilibrium with substances capable of removing or releasing the ion. For successful completion of many pharmacopeial tests and assay requires adjustment or maintenance of a specified pH by addition of buffer solutions .In pH measurements standard buffer solutions are required for reference purposes.

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.

Performance and Evaluation

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

Quality Control

Appearance

Colourless liquid

Clarity

Clear with no insoluble particles.

Results

The buffer solution gives a pH value of 7.0 ± 0.02 at 25°C

Storage and Shelf Life

On receipt store between 2-8°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 (3,4).

Reference

1)U.S. Pharmacopeia USP 42,NF37 vol 4.

2)Delloyd's Lab Tech resources reagent and solution: Preparation of pH buffer solutions.

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

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

Revision : 1 / 2019

*Buffer solution, pH 4.0 ± 0.02

R064

Intended Use:

Buffer solution, pH 4.0 ± 0.02 is used to establish and maintain an ion activity within narrow range. It is most commonly used to establish hydrogen-ion activity for the calibration of pH meters, in analytical procedures. It is also used to maintain stability of various dosage forms.

Composition**

Ingredients

Disodium hydrogen phosphate,12H2O	8.954gm
Potassium dihydrogen phosphate	3.4023gm
Distilled water	1000.00ml

**Formula adjusted, standardized to suit performance parameters

Principle And Interpretation

Buffer is defined as a solution which resists changes in the activity of an ion on addition of substances that are expected to change the activity of that ion. Buffer capacity refers to the amount of material that may be added to solution without causing a significant change in ion activity. Buffered solutions are systems in which the ion is in equilibrium with substances capable of removing or releasing the ion. For successful completion of many pharmacopeial tests and assay requires adjustment or maintenance of a specified pH by addition of buffer solutions .In pH measurements standard buffer solutions are required for reference purposes.

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.

Performance and Evaluation

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

Quality Control

Appearance
Colourless liquid

Clarity
Clear with no insoluble particles.

Result
The buffer solution gives a pH value of 4.0 ± 0.02 at 25°C.

Storage and Shelf Life

On receipt store between 2-8°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 (3,4).

Reference

1)U.S. Pharmacopeia USP 42,NF37 vol 4.

2)Delloyd's Lab Tech resources reagent and solution: Preparation of pH buffer solutions.

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

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

Revision : 1 / 2019

McFarland Standard set **R092**

McFarland standards are used to perform spectrophotometric comparisions of bacterial densities in water, saline or liquid growth medium. It provides laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedure requiring a standardization of the inoculum like growth promotion test (GPT).

Set Contains:

R092A (Standard 0.5)- 1 tube

R092B (Standard 1)-1 tube

R092C (Standard 2)- 1 tube

R092D (Standard 3)- 1 tube

R092E (Standard 4)- 1 tube

Directions

Prepare the inoculum of culture required for testing by using sterile saline. Match the density of the resultant suspension with the density of the desired standard. The standards must be thoroughly mixed on a vortex mixture at the time of use to obtain a uniform suspension. Adjust the density of cell suspension by adding saline if it is more turbid as compared to the desired standard or by adding culture if it is dilute. Check the density of the turbidity by determining the absorbance of 0.5 McFarland standard using a spectrophotometer with a 1 cm light path. The absorbance at 625 nm should be 0.08 to 0.10. The standards should be checked regularly to ensure the density accuracy.

Interpretation

McFarland standards are a set of tubes with increasing concentration of Barium Sulphate suspension. The turbidity of Barium Sulphate's white precipitation is used as a point of comparision of bacterial suspensions to known bacterial turbidity.

McFarland Standard	0.5	1	2	3	4
Approximate Corresponding suspension x 10^8 CFU/ml	1.5	3	6	9	12

Limitation of procedure

1. Coloured media may interfere with result interpretation and give incorrect results.

2. Bacterial suspensions of older cultures may not be comparable with expected bacterial counts.

Storage

Store the standards at 2-8°C, away from light after each use.

Reference

1. McFarland, J.1907. Nephelometer: JAMA 14:1176-1178

2. Murry,PR; Baron,EJ; Jorgensen,JH;Landry,ML;Pfaller,MA; Manual of Clinical Microbiology 9th edition ASM press, Washington DC.