Tryptone Soya Yeast Extract Agar

M1214

Tryptone Soya Yeast Extract Agar is recommended for confirmation of *Listeria* in Henry's light.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose	2.500
Yeast extract	6.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Tryptone Soya Yeast Extract Agar is formulated as per APHA (1) for the isolation and cultivation of *L. monocytogenes* from foods. ISO Committee (2) has recommended this medium for confirmation of *Listeria* species and can also be used for the cultivation and maintenance of a wide variety of heterotrophic microorganisms (3).

Casein enzymic hydrolysate and papaic digest of soyabean meal provide amino acids and other complex nitrogenous substances. Dextrose is the energy source. Dipotassium hydrogen phosphate buffers the medium. Yeast extract is the rich source of vitamin B complex.

According to FDAs enrichment procedure (4) for isolation of *L. monocytogenes* from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 24-48 hours. This culture is streaked on Modified McBride Listeria Agar (M891) with cycloheximide or Lithium-Phenylethanol-Moxalactam (LPM) Agar (M1228) and incubated at 35°C for 48 hours. Presumptive *Listeria* colonies are selected under 45° transillumination and colonies are further purified on Tryptone Soya Yeast Extract Agar under the light illumination. *Listeria* colonies are dense white to iridescent white appearing as crushed glass. Other colonies tend to be yellowish or orange.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

Reaction
Reaction of 5.1% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1214: Cultural characteristics observed after an incubation at 30-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery

Cultural Response

Listeria monocytogenes ATCC 19111	50-100	good-luxuriant	>=70%
Listeria monocytogenes ATCC 19118	50-100	good-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. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1993, Draft, ISO/DIS 10560.
3. Atlas R. M. 2004, 3rd Ed., Handbook of Microbiological Media, Parks, L.C. (Ed.), CRC Press, Boca Raton.
4. FDA, Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.

Revision : 02 / 2015

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 (1) for the isolation and cultivation of *Listeria monocytogenes* from foods. ISO Committee (2) has recommended for the cultivation and maintenance of a wide variety of heterotrophic microorganisms (3).

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 (4) 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 (4) 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 (5,6).

Reference

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1993, Draft, ISO/DIS 10560.
3. Atlas R. M. 2004, 3rd Ed., Handbook of Microbiological Media, Parks, L.C. (Ed.), CRC Press, Boca Raton.
4. FDA, Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
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 : 04 / 2022

Fraser Broth Base

M1327

Intended use

Recommended, recommended as a primary as well as secondary enrichment medium, for the isolation and enumeration of *Listeria monocytogenes* from food and animal feeds. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017.

Composition**

ISO 11290 Specification - Half Fraser & Fraser

Ingredients	Gms / Litre
Enzymatic digest of animal tissues	5.000
Enzymatic digest of casein	5.000
Yeast extract	5.000
Meat extract	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Fraser Broth : Half Fraser & Fraser broth

Ingredients	Gms / Litre
Peptone #	5.000
Tryptone $	5.000
Yeast extract	5.000
HM extract ##	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Supplements to be added after autoclaving

	Half fraser Gms / Litre	Fraser Gms / Litre
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
Ammonium Iron citrate	0.5	0.50

	Half fraser Gms / Litre	Fraser Gms / Litre
FD125I	**1 vial**	**2 vials**
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
FD141	**2 vials**	**2 vials**
Ammonium Iron citrate	0.5	0.50

**Formula adjusted, standardized to suit performance parameters # - Equivalent to Enzymatic digest of animal tissues

$ - Equivalent to Enzymatic digest of casein

- Equivalent to Meat extract

Directions

Suspend 54.92 grams (the equivalent weight of dehydrated medium per litre) 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. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Fraser Selective Supplement (FD125I) and 2 vials of Fraser Supplement (FD141) to 1000 ml medium for primary enrichment or 1 vial of each to 500 ml medium for secondary enrichment. Mix well and dispense in tubes or flasks as desired.

Principle And Interpretation

Listeria species are widely distributed in the environment. They have been isolated from soil, decaying vegetable matter, silage, sewage, water, animal feed, fresh and frozen poultry, meats, raw milk, cheese and asymptomatic human and animal carriers (1). *L.monocytogenes* primarily causes meningitis, encephalitis or septicemia in humans (2,3). In pregnant women, *L.*monocytogenes often causes influenza like bacteremic illness that, if untreated, may leaded to ammionitis and infection of the fetus, resulting in abortion, still birth or premature birth. Contaminated foods are the primary vehicles of transmission (4). Fraser Broth Base is based on the formulation of Fraser and Sperber (5) is used for the detection of *Listeria* species in food products (6). Fraser Broth Base is formulated so as to provide optimum conditions for the growth of *Listeria*. This medium is recommended by ISO for primary and secondary enrichment of *Listeria* species (7,8).

Peptone, Tryptone, yeast extract, and HM extract make the media highly nutritive by providing essential nutrients including carbonaceous and nitrogenous substances. Phosphates maintain the buffering capacity of the medium. All Listeria species exhibit beta-glucosidase activity which is evident by the blackening of the media. Listeria species hydrolyze esculin (substituted glucoside) to glucose and esculetin. The latter combines with ferric ions of ferric ammonium citrate (FD141),

resulting in the formation of 6-7 dihydroxycoumarin, a black brown complex. Ferric ammonium citrate also enhances the growth of *L.monocytogenes* (9). The high salt tolerance (of sodium chloride) of *Listeria* is used as means to inhibit the growth of Enterococci. Lithium chloride is also used to inhibit Enterococci, which also possess the ability to hydrolyze esculin. Growth of accompanying bacteria is largely inhibited by the addition of Nalidixic acid and Acriflavin hydrochloride (FD125I).

Type of specimen :

Food samples

Specimen Collection and Handling:

1. **Initial suspension**
   This broth is used as an dilution fluid for the preparation of initial suspension
   25grams/25 ml of sample to 225 ml of the medium (M1327 + 1 vial of FD125I + 2 vials of FD141)
2. **Primary enrichment**
   The dilution prepared in Half Fraser broth is incubated at 30°C ± 1°C for 24-26 hours.
   The preenriched sample after incubation can be stored at 5°C for a maximum of 72 hours before transfer to Fraser Broth (secondary enrichment)
   A black colouration can develop during incubation.
3. **Secondary Enrichment**
   0.1 ml of culture from primary enrichment is added to 10 ml of Fraser Broth (secondary enrichment). It is incubated at 37°C ± 1°C for 24 ± 2 hours.
   Additional incubation of 24 hours for *Listeria* species other than *L.monocytogenes* is recommended to allow recovery of more species.

The sample from primary enrichment and secondary enrichment is then subcultured on L.mono Differential Agar Base (M1540) and on Listeria Oxford Medium Base (M1145) or Listeria Identification Agar Base (PALCAM) (M1064I). Incubate at 37 ± 1 °C for 24 ± 2 hours. Additional incubation at 37 ± 1 °C for 24 ± 2 hours is recommended for *Listeria* spp. other than *L.monocytogenes* for recovery of more species. (7,8)

Warning and Precautions :

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

Limitations :

1. Presence of *L.monocytogenes* is often masked by other *Listeria* species like *L.inocua* and *L.ivanovii.*
2. Further subculture 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 theexpiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal medium : Yellow coloured clear solution with slight precipitate. After addition : Fluorescent yellow coloured clear solution with slight precipitate forms in tubes.

Reaction

Reaction of 5.49% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Half Fraser (Primary Enrichment)

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis	Recovery on M1540*	Colour of colony on M1540*

Productivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour.
Further subculture is carried out on M1540 at 37 ± 1°C for 48± 4 hours.

Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*)+ *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo

Selectivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	-	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	-	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	-	<100 colonies
*Enterococcus faecalis A*TCC 19433 (00009*)	>=10^4	none-poor	-	<100 colonies

Fraser (Secondary

Enrichment) Organism	**Inoculum (CFU)**	**Growth**	**Esculin Hydrolysis**	**Recovery on M1540***	**Colour of colony on M1540***

Productivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hours. Further subculture is carried out on M1540 at 37 ± 1°C for 48± 4 hours.

Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo

Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo

Selectivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	-	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	-	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	-	<100 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	none-poor	-	<100 colonies

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with 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. Nieman R. E., and Lorber B., 1980, Rev. Infect. Dis. 2 : 207-2
3. Schuchat A. B., Swaminathan and C. V. Broome, Clin. Microbiol., Rev. 4 : 169-1
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. Fraser and Sperber, 1988, J. Food Prot., 51:762-76
6. 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.

7.Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1 , Detection method ; ISO 11290-1:2017
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.Cowart R. E. and Foster BG., 1985, J. Infect. Dis.; 151:17

Revision : 06/ 2022

Rappaport Vassiliadis Soya Broth (RVS Broth)

M1491

Intended Use:

Selective enrichment medium for *Salmonellae* species from food and animal feeding stuffs and clinical specimens.

Composition**

Ingredients	Gms / Litre
Soya peptone	4.500
Sodium chloride	8.000
Potassium dihydrogen phosphate	0.600
Dipotassium hydrogen phosphate	0.400
Magnesium chloride hexahydrate	29.000
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.11 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 115°C for 15 mins.

Principle And Interpretation

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

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

Type of specimen

Clinical samples - faeces; Food samples and animal feeding stuffs.

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 (7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. This medium contains inhibitory substances and may not support the growth of certain *Salmonella* species like *S.* Typhi.
2. Less selective enrichment broth must be used in conjunction.
3. After enrichment the organisms must be isolated on less selective media along with selective media.
4. Further biochemical and serological testing 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

Light yellow to light blue homogeneous free flowing powder.

Colour and Clarity of prepared medium

Greenish blue clear to slightly opalescent with a slight precipitate.

Reaction

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

pH

5.00-5.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
***E.coli* +*S.*Typhimurium (mixed culture)**				
E.coli	50 -100	none-poor	<=10 %	yellow
*S.*Typhimurium	50 -100	luxuriant	>=50 %	red with black centers
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Salmonella Abony NCTC 6017 (00029*)	50-100	luxuriant	>=70 %	red with black centers
Salmonella Typhimurium *subsp. aureus* ATCC 14028 (00031*)	50-100	luxuriant	>=70 %	red with black centers
Staphylococcus aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10	yellow
Salmonella Enteritidis ATCC *13076* (00030*)	50-100	luxuriant	>=70 %	red with black centre
Escherichia coli ATCC 8739 (00012*)	50-100	none-poor	<=10 %	yellow
Salmonella Paratyphi B ATCC 8759	50-100	luxuriant	>=70 %	red with black centre

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

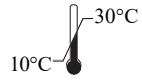
Reference

1. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
4. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C.,Funke., 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. 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 : 04/2023

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

Disclaimer :

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

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 **M1494I**

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. This medium is also recommended by APHA for pre-enrichment of *Salmonella, Cronobacter* and *Listeria* (1). Edel and Kampelmacher (2) 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 (3). 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* (4), *Salmonella* (5), *Cronobacter* (6) and *Listeria* species(7) species from from food stuffs and other materials. It is also recommended as a diluent for enumerations of all microorganisms (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 (8) / ISO 11290-1:2017 (7)

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

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 (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 (4)
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 (6)
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 subculture, isolation and 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

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.

Recovery of

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1.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.

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

3.Sadovski A. Y., 1977, J. Food Technol., 12.85.

4.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.

5.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.

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 — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2 , Detection method ; ISO 11290-2:2017.
8. 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.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. 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 : 07/2022

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

HiCrome™ Listeria Ottaviani-Agosti Agar Base

M1540I

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes*. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017. This medium is also recommended by FDA BAM and APHA Food.

Composition**

ISO 11290 Specification / FDA BAM/ APHA - Agar Listeria according to Ottaviani and Agosti

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

M1540I - HiCrome™ Listeria Ottaviani-Agosti Agar Base

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

**Formula adjusted, standardized to suit performance parameters

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

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

FD212A - 2 vials OA Selective Supplement	mg / vial
Nalidixic acid sodium salt	10.000
Ceftazidime	10.000
Polymyxin B sulfate	38350 IU
Amphotericin B	5.000
(FD214) - 2 vials	
LP Enrichment Supplement 1	1.000g

Directions

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

Principle And Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L. monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). The media is based on the formulation of Ottoviani and Agosti (1,2) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds which is adopted by ISO Committee (3,4). It is also recommended by APHA (5) & FDA-BAM (6).

HM peptone, tryptone and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium pyruvate provide essential growth nutrients. Glucose (Dextrose) is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212A) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate (5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside) which produces blue to green coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Type of specimen

Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling

For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

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 strains of *L.monocytogenes* exposed to stress condition particularly acid stress may show a very weak halo (or even no halo).
2. Further biochemical tests must be carried out to differentiate between *L.monocytogenes* and *L. ivanovii*, sine both shows opaque halo of PIPLC activity.
3. Some organisms other than *Listeria* spp. may also produce blue colonies on this medium, so biochemical characterization is required for differentiation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, opalescent gel forms in Petri plates

Reaction

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

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation for 48 ± 4 hours at 37° ± 1°C.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
Productivity					
Listeria monocytogenes ATCC 13932 (00021*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Listeria monocytogenes ATCC 35152 (00109*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity

Specificity					
Listeria innocua ATCC 33090 (00017*)	50-100	luxuriant		Blue-green	negative
Selectivity					
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%		
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%		
Additional Microbiological Testing					
Listeria ivanovii ATCC 19119	50-100	luxuriant	>=50%	greenish-blue	positive, opaque halo around the colony exhibiting phophatidyl -inositol specific phospholipase acivity

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1.Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.

2.Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p. 6, A.D.R.I.A. Quimper, France, 16-18 June 1997.

3.Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1 , Detection method ; ISO 11290-1:2017.

4.Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2 , Enumeration method ; ISO 11290-2:2017.

5.Salfinger Y. and Tortorello M. L., (Eds.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,APHA, Washington, D.C.

6.BAM Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods, 2022.

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

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

Revision : 02/2023

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

0.1% Peptone Salt Solution

M1748

Intended use

Recommended as diluent for different test method

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Sodium chloride	8.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.50 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle.

Principle And Interpretation

0.1% Peptone Salt solution is recommended as a diluent for dilution of sample by different test methods widely used for examination of foodstuffs. Standard methods for the examination of foodstuffs require sample dilution to be carried out accurately for enumerating the microorganisms. This medium is also recommended by ISO Committee (1) for use as an isotonic diluent.

It contains peptone at low concentration which provides nutrients for survival of microorganisms and hence protecting the organisms (2). Sodium chloride at 0.85% concentration maintains osmotic balance of medium thereby maintaining cell morphology and integrity (3). The pH of this diluent medium is near neutral range optimum for viability of microorganisms. Therefore it can be successfully used as a diluent for carrying out dilutions of different samples. It is recommended to use 10 gm of test sample along with 90 ml of 0.1% Peptone salt solution for enumeration. The prepared dilution may be blended at 15,000 to 20,000 revolutions per minute. Further a ten fold dilution may be prepared using 1 ml of it in 9ml of sterile diluent within 15 minutes and mixed well. This is considered as 10-1 dilution. Sequential dilutions can be prepared using same diluent and counts obtained by spread plate or pour plate technique. Tests may be performed in duplicates as described in technique and checked for equivalent yields of organisms between the diluent batches.

Incubate the tubes with test organisms. At time of zero minutes and after 30 minutes and 2 hours, subculture an inoculum (approximately 0.01ml) or a loop full onto Soyabean Casein Digest Agar (M290) using streak plate technique. If desired SCDA may be also enriched with 5% v/v sheep blood depending on intended organisms to be isolated. Incubate plates at 35 ±2ºC for 18-24 hours.

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,6). For water samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Some strains may show poor growth due to nutritional variations.
3. Further serological and biochemical 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
Off white to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Cream to pale yellow clear solution in tubes
Reaction
Reaction of 0.95% w/v aqueous solution at 25°C. pH : 7.0±0.2
pH
6.80-7.20
Cultural Response
Cultural characteristics observed on Soyabean Casein Digest Agar (M290), after an incubation at 35-37°C for 18-48 hours of cultures suspended in 0.1% Peptone Salt solution for 30 minutes.

Organism	Inoculum (CFU)	Recovery (after 30 minutes)
Escherichia coli ATCC 25922 (00013*)	50-100	no change in numbers
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	no change in numbers

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 (8,9).

Reference

1. International Organization for Standardization (ISO),ISO/DIS 6649.
2. Straker R.P.and Stokes J.L., 1957,Appl.Microbiol.,5:21.
3. Patterson J.W. and Cassells J.A., 1963,J.Appl.Bacteriol., 26:493.
4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. 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.
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.

Revision : 04 / 2022

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).

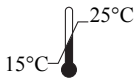
Reference

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

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

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.