Tetrathionate Brilliant Green Bile Broth

M1255

Intended Use:

Recommended for isolation and identification of Salmonellae.

Composition**

Ingredients	g/ L
Peptone	8.600
Bile	8.000
Sodium chloride	6.400
Calcium carbonate	20.000
Potassium tetrathionate	20.000
Brilliant green	0.070
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 63.07 grams in 1000 ml purified / distilled water. Heat just to boiling. DO NOT AUTOCLAVE OR REHEAT.

Dispense into sterile tubes or flasks as desired.

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

Principle And Interpretation

Salmonella are gram-negative, facultatively anaerobic, non-sporulating, non-motile rods in the family *Enterobacteriaceae.* They are widely distributed in animals affecting mainly the stomach and the intestines. These organisms are difficult to differentiate biochemically from *Escherichia coli.* Tetrathionate Broth was originally described by Mueller (7) and later modified by Kauffman (4,5). Tetrathionate Brilliant Green Bile Broth is used as an enrichment medium for *Salmonella.* Enrichment broth is usually recommended to facilitate the recovery of small numbers of *Salmonella* species (4). Tetrathionate Brilliant Green Bile Broth is also mentioned in I.P. (1) for isolation and identification of *Salmonella* species from foods, water and other materials of sanitary importance.

Peptone in the medium provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and nutrients for growth of Salmonellae. Brilliant green and bile inhibit both gram-positive as well as some selected gram-negative organisms. Potassium tetrathionate inhibits normal flora of faecal specimens. Sodium chloride helps in maintaining osmotic equilibrium.

After incubation, streak the culture from Tetrathionate Brilliant Green Bile Broth (M1255) onto differential medium for isolation and identification.

Tetrathionate Brilliant Green Bile Broth is not suitable for growth of *Salmonella* Typhi and *Salmonella* Paratyphi (6).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green Agar (M016), MacConkey Agar (M081) and Bismuth Sulphite Agar (M027).

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 pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Bluish green coloured opalescent solution with white precipitate.

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed when subcultured on MacConkey Agar (M082) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	50-100	fair	20-30%	pink to red with bile precipitate
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	>=50%	colourless
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739	50-100	fair	20-30%	pink to red with bile precipitate

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

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., 1930, Hyg. Abt. I. Orig., 113, 148.
5. Kauffman F., 1935, Z. Hyg. Infektionskr., 117, 26.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
7. Mueller L., 1923, C. R. Soc. Biol., (Paris), 89, 434.
8. 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.
9. 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.

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