



## Tetrathionate Brilliant Green Bile Broth

M1255

Tetrathionate Brilliant Green Bile Broth is used for the isolation and identification of Salmonellae.

### Composition\*\*

| Ingredients                    | Gms / Litre |
|--------------------------------|-------------|
| Peptic digest of animal tissue | 8.600       |
| Ox 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 distilled water. Heat just to boiling. DO NOT AUTOCLAVE OR REHEAT. Dispense 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 (1) and later modified by Kauffman (2, 3). 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. (5) for isolation and identification of *Salmonella* species from foods, water and other materials of sanitary importance.

Peptic digest of animal tissue in the medium provides nitrogenous nutrients for growth of Salmonellae. Brilliant green and ox-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).

### 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.

#### Cultural Response

| Organism                                 | Inoculum (CFU)    | Growth    | Recovery | Colour of colony                  |
|--|-------------------|-----------|----------|-----------------------------------|
| <b>Cultural Response</b>                 |                   |           |          |                                   |
| <i>Escherichia coli</i> ATCC 25922       | 50-100            | fair      | 20-30%   | pink to red with bile precipitate |
| <i>Salmonella Typhi</i> ATCC 6539        | 50-100            | luxuriant | >=50%    | colourless                        |
| <i>Salmonella Typhimurium</i> ATCC 14028 | 50-100            | luxuriant | >=50%    | colourless                        |
| <i>Salmonella Enteritidis</i> ATCC 13076 | 50-100            | luxuriant | >=50%    | colourless                        |
| <i>Staphylococcus aureus</i> ATCC 25923  | >=10 <sup>3</sup> | inhibited | 0%       |                                   |
| <i>Staphylococcus aureus</i> ATCC 6538   | >=10 <sup>3</sup> | inhibited | 0%       |                                   |
| <i>Escherichia coli</i> ATCC 8739        | 50-100            | fair      | 20-30%   | pink to red with bile precipitate |
| <i>Escherichia coli</i> NCTC 9002        | 50-100            | fair      | 20-30%   | Pink to red with bile precipitate |
| <i>Staphylococcus aureus</i> NCIMB 9518  | >=10 <sup>3</sup> | inhibited | 0%       |                                   |

### Storage and Shelf Life

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

### Reference

1. Mueller L., 1923, C. R. Soc. Biol., (Paris), 89, 434.
2. Kauffman F., 1930, Hyg. Abt. I. Orig., 113, 148.
3. Kauffman F., 1935, Z. Hyg. Infektionskr., 117, 26.
4. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.). 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
5. Indian Pharmacopoeia, 1996, Ministry of Health and Family Welfare, Govt. of India,
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

Revision : 1 / 2011



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