



## Tetrathionate Broth Medium

MM032

### Intended Use:

Recommended an enrichment broth for isolation of Salmonellae from specimens suspected to be contaminated with *Salmonellae* in accordance with IP.

### Composition\*\*

Ingredients	Gms / Litre
HM peptone B #	0.900
Peptone	4.500
Yeast extract	1.800
Sodium chloride	4.500
Calcium carbonate	25.000
Sodium thiosulphate	40.700

\*\*Formula adjusted, standardized to suit performance parameters

# Equivalent to Beef extract

### Directions

Suspend 77.40 grams in 980 ml purified/ distilled water. Heat just to boiling. Cool below 45-50°C and aseptically add 20 ml iodine solution (6 grams of iodine and 5 grams of potassium iodide in 20 ml distilled water). Mix well and dispense in 10 ml quantities in sterile tubes. This complete medium should be used on the day of preparation. Do not heat after the addition of iodine solution. Use the medium immediately after addition of iodine.

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

Note : Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. User must ensure its compatibility with the latest edition.

### Principle And Interpretation

Tetrathionate Broth Medium was originally described by Mueller (1) and found that the medium selectively inhibit coliforms and permit unrestricted growth of enteric pathogens. The medium is now formulated according to Indian Pharmacopoeia (2). Compendium of Microbiological Examination of Foods(3) and Standard Methods for the Examination of Water and Wastewater (4) specify this medium as enrichment medium for *Salmonella* species. *Salmonella* is the common causative agent of mild gastroenteritis to typhoid. It is common contaminant in food and other biological products. This medium supports the rejuvenation of *Salmonella* cells injured by food processing which are incapable of forming colonies on plate, but on injection can cause infection.

The selectivity depends on the ability of thiosulphate and tetrathionate (formed by addition of Iodine and Potassium iodide) in combination to suppress commensal coliform organisms (5,6). The microorganism harboring tetrathionate reductase flourish in this broth. Sodium thiosulphates are inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Yeast extract, HM peptone B and peptone provides essential nutrients, growth factors and vitamins in this medium. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Sodium chloride maintains osmotic balance For further confirmation, streak the enriched cultures after incubation, on the plates of Brilliant Green Agar (MM016), MacConkey Agar (MM081), Bismuth Sulphite Agar (MM027).

### Type of specimen

Pharmaceutical samples

### Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (2). 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 the plates of Brilliant Green Agar (MM016), MacConkey Agar (MM081), Bismuth Sulphite Agar (MM027).

## 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

Complete medium with added brilliant green and iodine solution - Light green opalescent with white precipitate, on standing the precipitate settles down.

### Growth Promotion Test

As per Indian Pharmacopoeia.

### Cultural Response

Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on Xylose Lysine Deoxycholate Agar (MM031) after enrichment in Tetrathionate medium, after an incubation at 35-37°C for 18-72 hours.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs

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 (7,8).

## Reference

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- 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.
- 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.
- MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria., Vol. 1, Williams and Wilkins, Baltimore.
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