



Fluid Tetrathionate Medium

MU032

Intended Use:

Recommended as an enrichment broth for isolation of *Salmonellae* from specimens suspected to be contaminated with *Salmonellae* in accordance with U.S.P.

Composition**

Ingredients	g / L
Tryptone	2.500
Peptone	2.500
Bile salts	1.000
Calcium carbonate	10.000
Sodium thiosulphate	30.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.0 grams in 970 ml purified/distilled water. Heat just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C. On the day of use add 20 ml iodine solution (5 grams of potassium iodide and 6 grams of iodine in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution. Mix well and dispense in 10 ml quantities. Do not heat after 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 white precipitate.

Principle And Interpretation

Fluid Tetrathionate 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 FDA (2), United States Pharmacopoeia, 2019 (Vol. 5) (3), Compendium of Microbiological Examination of Foods (4) and Standard Methods for the Examination of Water and Wastewater (5) which 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. This medium is recommended by USP for microbial limit tests for pharmaceutical preparations. Bile salts inhibit gram-positive microorganisms. 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 (6,7). The microorganism harbouring tetrathionate reductase flourish in this broth. Sodium thiosulphate forms the substrate for enzyme thiosulphate reductase. Sodium thiosulphates are also inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Tryptone and peptone supplies essential nutrients and vitamins in this medium. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Sodium chloride maintains osmotic balance.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (3) 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

Please refer disclaimer Overleaf.

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

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

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

Growth Promotion Test

As per United States Pharmacopoeia.

Cultural Response

Cultural characteristics observed with added brilliant green and iodine solution, after an incubation at 35-37°C for 18-24 hours, when sub cultured on MacConkey Agar (MU081) after enrichment in Tetrathionate medium.

Organism	Inoculum (CFU)	Recovery	Colour of colony
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50-100	good-luxuriant	colourless
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	colourless
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-luxuriant	colourless
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	little or no increase in numbers	white to pink with bile precipitate

Key : *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 (8,9).

Reference

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

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