M-Tetrathionate Broth Base

M1115

M-Tetrathionate Broth Base with added iodine solution is used for selective enrichment of Salmonellae using membrane filter technique.

Composition**

Ingredients	Gms / Litre
Proteose peptone	5.000
Bile salts	1.000
Sodium thiosulphate	30.000
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 3.6 grams in 100 ml distilled water. Heat if necessary to dissolve the medium completely. Cool below 45°C and add 2 ml Iodine solution containing 0.5 grams potassium iodide and 0.6 grams iodine crystals. Complete medium should be used on the day of preparation. Soak the absorbent pads placed in 5-6 cm Petri dishes with 2 ml broth and place membrane filter inoculums on them. Incubate at 35-37°C for 3 hours and then transfer the inoculum membrane filter onto absorbent pads soaked with 2 ml M-Brilliant Green Broth incubate at 35-37°C for 15-24 hours.

Principle And Interpretation

Enrichment media favour the multiplication of a particular species as a step towards their isolation in pure culture (1). M-Tetrathionate Broth is prepared as per the formulation of Kabler and Clark (2) for selective enrichment of *Salmonella* using membrane filter technique. The formulation is similar to Tetrathionate Broth except calcium carbonate. Tetrathionate Broth Base was originally described by Mueller (3) and found that the medium selectively inhibits coliforms and permits the unrestricted growth of enteric pathogens.

Proteose peptone provides nitrogenous nutrients for the bacterial metabolism. Tetrathionate is formed by the addition of iodine solution. The selectivity of the medium depends upon the ability of thiosulphate and tetrathionate in combination, to suppress commensal organisms (4, 5). Only those organisms possessing the tetrathionate reductase enzyme can grow on this medium. Bile salts inhibit many gram-positive microorganisms. Soak sterile cotton absorbent pads placed in 5-6 cm Petri plates with 2 ml of M-Tetrathionate Broth Base and place the membrane filter inoculum on them. Incubate at 35-37°C for 3 hours and then transfer inoculum membrane filter onto absorbent pads soaked with 2 ml M-Brilliant Green Broth (M1102). Incubate at 35-37°C for 15-21 hours. After M-BGB incubation, add urease test reagent (urea- 20 gram, bromothymol blue 0.16 gm and phenol red 0.2 grams in 1000 ml distilled water) to absorbent pads and allow to set for 15-20 minutes to permit reagent to diffuse throughout the medium for development of colour.

Quality Control

Appearance
White to light yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Amber coloured clear solution without any precipitate

Reaction
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH
7.80-8.20

Cultural Response
M1115: Cultural characteristics observed with added Iodine solution (containing Potassium Iodide and Iodine crystals), after an incubation at 35-37°C for 18-24 hours

Organism	Recovery (by Mile Misra test)	Colour of colony (on membrane filter)	Colour (after addition of urease test reagent
Cultural Response			
Escherichia coli ATCC 25922	fair-good	yellow-green	yellow
Salmonella Enteritidis ATCC 13076	good-excellent	pink-red	red
Salmonella Typhimurium ATCC 14028	good-excellent	pink-red	red

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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
2. Kabler P. W. and Clark H. F., 1952, Am. J. Public Health, 42:390.
3. Mueller G. M., 1923, Compt. Rend. Seo. Biol., 89:434
4. Pollock M. R. and Knor R., 1943, Biochem J., 37: 476
5. MacFaddin J. F., 1985, Vol. I, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Williams and Wilkins, Baltimore.

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