Fluid Tetrathionate Medium w/o Iodine and BG, Modified

M032F

Fluid Tetrathionate Medium on addition of Iodine and Brilliant Green is recommended for the selective enrichment method for isolating Salmonellae from food and other materials of sanitary importance in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
BioPeptone	5.000
Bile salts	1.000
Calcium carbonate	10.000
Sodium thiosulphate, pentahydrate	30.000
Final pH (at 25°C)	8.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.12 grams of dehydrated medium (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water and heat to boiling to dissolve completely. DO NOT AUTOCLAVE. Cool below 45°C and add freshly prepared solutions : 20 ml iodine solution (iodine-6 grams and potassium iodide-5 grams in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution. Mix well and dispense 10 ml portions into 20 x 150 or 16 x 150 mm sterile test tubes. This complete solutions should be used on the day of preparation otherwise sterilized broth base may be stored for sometime. Do not heat after addition of Iodine and dye solutions. Use the medium immediately after addition of iodine.

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

Principle And Interpretation

Salmonellosis is a disease caused by *Salmonella* present in raw or undercooked food (1) . *Salmonella* is the most common bacterial form responsible for food poisoning causing diarrhoea, vomiting, abdominal cramps and fever lasting for 4-7 days. It can cause serious illness due to consumption of such foods in older adults, infants and persons with chronic diseases. These are present in small numbers compared to coliforms that necessitates the examination of a relatively large sample for the isolation of organism (2). Due to processing of foods by physical and mechanical methods (3), sublethally injured *Salmonella* species are often present in foods and needs to be enumerated by resuscitating in suitable enrichment medium (1,4). Enrichment medias include Rappaport Vassilidias medium (M880F)and Fluid Tetrathionate Broth w/o Iodine and BG, Modified (M032F) . It is a second selective enrichment medium recommended by FDA, BAM for enriching such *Salmonella* species from food samples under study(4).

Fluid Tetrathionate Medium was originally devised by Mueller (5) for enrichment of *Salmonella* . Organisms possessing the enzyme tetrathionate reductase are known to grow in this media. Biopeptone act as source of carbon , nitrogen, vitamins and minerals. Bile salts selectively inhibits gram positive organisms. Sodium thiosulphate in combination with tetrathionate supresses commensal coliform organisms(6,7). Calcium carbonate neutralizes the acidic products of tetrathionate decomposition. Brilliant green also helps to select salmonella by inhibiting the accompanying bacteria.

As per protocol (4) depending on type of food sample, 25 g of sample (guar gum, milk, egg, tomatoes, mammy pulp etc.) are pretreated in suitable diluents such as Lactose Broth (M1003) or Buffered Peptone Broth (M614) or Universal Preenrichment Broth (M1372F). The ratio of sample : broth being 1:9. In case of food samples with high microbial load, 0.1 ml of sample mixture is mixed with 10 ml of Tetrathionate broth (M032F)and incubated at 43°C ± 0.2 . In case of low microbial load, same is incubated at 35°C ± 0.2 for 24 ± 2 h. On incubation 10 µl is isolated on solid selective media as Bismuth Sulphite Agar (M027) , Xylose Lysine Deoxycholate Agar (M031F) or Hektoen agar (M467F) and checked for typical *Salmonella* colonies which are further identified and confirmed biochemically.

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 heavy white precipitate, which on standing the precipitate settles down.

pH

8.20-8.60

Cultural Response

Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on XLD Agar (M031F) after enrichment in Tetrathionate medium, after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Recovery	Colour of colony
Cultural Response			
Escherichia coli ATCC 25922	50-100	little or no increase in number	yellow
Salmonella Choleraesuis ATCC 12011	50-100	good-excellent	Red with black centres
Salmonella Typhi ATCC 6539	50-100	good-excellent	red with black centres
Salmonella Typhimurium ATCC 14028	50-100	good-excellent	red with black centres
Escherichia coli NCTC 9002	50-100	little or no increase in numbers	yellow
Escherichia coli ATCC 8739	50-100	little or no increase in numbers	yellow
Salmonella Paratyphi A ATCC 9150	50-100	good-excellent	red
Salmonella Paratyphi B ATCC 8759	50-100	good-excellent	red with black centres

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.Downes F.P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
2.Cherry et al., 1972, Appl. Microbiol., 24: 334.
3.Hartman and Minich, 1981, J. Food and Prot., 44:385.
4.FDA, US. Bacteriological Analytical Manual 8 Ed. Gaithersburg, MD: AOAC international ; 1998.
5.Mueller, 1923, Compt. Rend. Sco. Biol., 89:434.
6.MacFaddin JF. Media for Isolation-Cultivation- Identification-Maintenance of Medical Bacteria. Baltimore: Williams and Wilkins; 1985.
7.Eaton A. D., Clesceri L.S. and Greenberg A. E., (Eds) 2005, Standard Methods for the Examination of Water and Waste Water, 20th Ed., , APHA. Washington, D.C.

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