Technical Information

Fluid Tetrathionate Medium w/o Iodine and BG (Tetrathionate Broth Base w/o Iodine and BG)

Product Code: DM 1032

Application: - Fluid Tetrathionate Medium with added iodine and brilliant green is recommended for the selective enrichment method for isolating Salmonellae from faeces, urine, food and other material of sanitary importance.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	2.500
Peptic digest of animal tissue	2.500
Bile salts	1.000
Calcium carbonate	10.000
Sodium thiosulphate	30.000

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Though *Salmonella* are ubiquitous in nature yet these organisms are usually present in small numbers that requires to examine a relatively large sample to isolate the organisms [1]. *Salmonella* present in food samples may be sublethally damaged during various stages of food processing when they are exposed to low temperatures, heat drying, radiations, and various chemicals [2]. These damaged cells are able to cause spoilage, and if ingested cause diseases under favourable conditions. Therefore it is important to resuscitate these damaged bacteria before enumeration in different food samples. Fluid Tetrathionate Medium (with added iodine and brilliant green) is recommended for the selective enrichment of Salmonella including *Salmonella* Typhi from faeces, urine, food and other material of sanitary importance. The medium, originally formulated by Mueller [3] is also recommended by APHA [4-6] for enrichment of Salmonella.

Due to the addition of iodine and potassium iodide, tetrathionate is formed in the medium. Organisms possessing the enzyme tetrathionate reductase grow in this medium.

Casein enzymic hydrolysate and peptic digest of animal tissue are the sources of carbon, nitrogen, vitamins and minerals. Bile salts inhibit accompanying gram-positive microorganisms. The selectivity depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organism [7, 8]. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Brilliant green also helps to select *Salmonella* by inhibiting the accompanying bacteria. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green Agar (DM1016), MacConkey Agar (DM1081) and Bismuth Sulphite Agar (DM1027).

Aseptically inoculate test specimen into Fluid Tetrathionate medium (with added iodine and brilliant green) and incubate at 35-37°C for 18-24 hours. Following the incubation, isolate onto selective media plates. Refer standard procedures for enrichment and isolation [4-6]

Methodology

Suspend 46 grams of powder media in 1000 ml distilled water and heat to dissolve completely. DO NOT AUTOCLAVE. Cool below 45°C and add 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 in 10 ml quantities. This complete medium should be used on the day of preparation otherwise sterilized broth base may be stored for some time. Do not heat after the addition of iodine solution. Use the medium immediately after addition of iodine.

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

Quality Control

Physical 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.

Cultural Response/ characteristices
DM 1032: Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on MacConkey

Organism	Inoculum (CFU)	Recovery	Colour of colony
Escherichia coli ATCC 25922	50-100	little or no increase in number	pink-red with bile precipitate
Salmonella Choleraesuis ATCC 12011	50-100	good-excellent	colourless
Salmonella Typhi ATCC 6539	50-100	good-excellent	colourless
Salmonella Typhimurium ATCC 14028	50-100	good-excellent	colourless
Escherichia coli NCTC 9002	50-100	little or no increase in number	pink-red with bile precipitate
Escherichia coli ATCC 8739	50-100	little or no increase in number	pink-red with bile precipitate

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Cherry et al, 1972, Appl. Microbiol., 24:334
2. Hartman and Minich, 1981, J. Food and Prot., 44:385
3. Mueller, 1923, Compt. Rend. Sco. Biol., 89:434.
4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
5. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
6. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
7. Pollock M. R. and Knor R., 1943, Biochem J., 37:476.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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