Technical Information

Tetrathionate Broth Medium

Product Code: DM 1032M

Application:-Tetrathionate Broth Medium is recommended as an selective enrichment broth for isolation of *Salmonella* Typhi and other Salmonellae from clinical specimens and biologicals as specified in Indian Pharmacopoeia, 1996.

Composition**

Ingredients	Gms / Litre
Beef extract	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

Principle & 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 flourishes 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, beef extract 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 (DM1016M), MacConkey Agar (DM1081M), Bismuth Sulphite Agar (DM1027M).

Methodology

Suspend 77.40 grams of powder media in 980 ml purified/ distilled water. Shake well & heat just. Cool below 45°C and add 20 ml iodine solution (iodine - 6 grams and potassium iodide - 5 grams 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.

Quality Control

Physical 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/Characteristics

DM 1032M: Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on Xylose Lysine Deoxycholate Agar (DM1031M) after enrichment in Tetrathionate medium, after an incubation at 35-37°C for 18-72 hours.

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

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: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Mueller, 1923, Compt. Rend. Sco. Biol., 89:434.
2. The Indian Pharmacopoeia (1996), Vol. II.
3. Downes F P and Ito K(Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. 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.
5. Pollock M.R. and Knor R., 1943, Biochem J., 37:476.
6. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria., Vol. 1, Williams and Wilkins, Baltimore.

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