M-Broth

M846

M-Broth is used for detecting Salmonellae in foods and feeds by the accelerated enrichment serology procedures.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	12.500
Yeast extract	5.000
D-Mannose	2.000
Sodium chloride	5.000
Sodium citrate	5.000
Dipotassium phosphate	5.000
Manganese chloride	0.140
Magnesium sulphate	0.800
Ferrous sulphate	0.040
Polysorbate 80	0.750
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.23 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Salmonella are facultatively anaerobic gram-negative bacilli that are typically oxidase negative, lactose negative, H2S positive and produce gas. *Salmonella* are found in nature and occur in the intestinal tract of many animals, both wild and domestic. *Salmonella* species cause variety of human diseases called Salmonellosis, which includes diarrheal disease and bacteremic conditions leading to enteric fever. The severity of the diarrheal diseases depends on the virulence of the strain and the condition of the human host. Serological procedures that confirm the identification of an organism are usually agglutination reactions.

M-Broth was developed by Sperber and Diebel (1) to accelerate the detection of Salmonellae. The accelerated 50 hour detection procedure consists of an 18 hours pre-enrichment, a 24 hours selective enrichment, a 6 to 8 hours selective enrichment and 2 hours serological testing. In the selective enrichment step, to avoid nonspecific agglutination, Sperber and Diebel modified APT Broth by removing dextrose from it and by adding mannose. Fantasia et al (2) found that enrichment serology method is rapid and less complicated to perform than the method described in the Bacteriological Analytical Manual (3) by maintaining the accuracy and sensitivity of the method. M-Broth also conforms to testing standards recommended by APHA (4) for isolation and identification of foodborne Salmonella. M-Broth contains all the nutrients necessary for good growth and flagella development of *Salmonella* .

Casein enzymic hydrolysate and yeast extract in the medium provide organic nitrogen, carbon, sulphur, vitamins and trace elements essential for the growth of *Salmonella* species. Mannose is the fermentable sugar and energy source and it prevents fimbrial agglutination of *Salmonella* (1). Sodium chloride helps to maintain osmotic equilibrium. Dipotassium phosphate acts as a buffer. The inorganic salts stimulate bacterial growth while polysorbate 80 supplies fatty acids.

10% suspension of sample is prepared in sterile Lactose Broth (M026) and incubated at 35±2°C for 18 to 24 hours. 1 ml of this pre-enriched culture is added to 9 ml of Selenite Cystine Broth (M025) and Tetrathionate Broth (M032) and incubated at 35±2°C for 24 hours. This enriched culture is subsequently inoculated in M-Broth and incubated at 35±2°C for 6-8 hours following, which H-agglutination test is performed as per standard procedures.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent solution with a slight precipitate.

Reaction
Reaction of 3.62% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M846: Cultural characteristics observed after an incubation at 35-37°C for 6-8 hours.

Organism	Inoculum (CFU)	Growth
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant
Salmonella Paratyphi B ATCC 8759	50-100	luxuriant
Salmonella Choleraesuis ATCC 12011	50-100	luxuriant
Salmonella Enteritidis ATCC 13076	50-100	luxuriant
Salmonella Typhi ATCC 6539	50-100	luxuriant
Salmonella Typhimurium ATCC 14028	50-100	luxuriant

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. Sperber W. H. and Deibel R. H., 1969, Appl. Microbiol., 17:533.
2. Fantasia L. D., Sperber W. H. and Deibel R. H., 1969, Appl. Microbiol., 17:540.
3. FDA Bacteriological Analytical Manual, 2005, 18th ed., AOAC, Washington, DC.
4. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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