M-Broth

M846

Intended use

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

Ingredients	Gms / Litre
Tryptone	12.500
Yeast extract	5.000
D-Mannose	2.000
Sodium chloride	5.000
Sodium citrate	5.000
Dipotassium hydrogen phosphate	5.000
Manganese chloride	0.140
Magnesium sulphate	0.800
Ferrous sulphate	0.040
Polysorbate 80 (Tween 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 purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. 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, H_2S 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 (6) 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 (1) by maintaining the accuracy and sensitivity of the method. M-Broth also conforms to testing standards recommended by APHA (5) for isolation and identification of foodborne *Salmonella*. M-Broth contains all the nutrients necessary for good growth and flagella development of *Salmonella*.

Tryptone 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* (6). Sodium chloride helps to maintain osmotic equilibrium. Dipotassium hydrogen 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.

Type of specimen

Food and feed samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Further biochemical testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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 (00030*)*	50-100	luxuriant
Salmonella Typhi *ATCC 6539*	50-100	luxuriant
Salmonella Typhimurium *ATCC 14028 (00031*)*	50-100	luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. FDA Bacteriological Analytical Manual, 2005, 18th ed., AOAC, Washington, DC.
2. Fantasia L. D., Sperber W. H. and Deibel R. H., 1969, Appl. Microbiol., 17:540.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Sperber W. H. and Deibel R. H., 1969, Appl. Microbiol., 17:533.

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