

M Broth (NCM0125)

Intended Use

M Broth is used for the cultivation of *Salmonella* spp in a laboratory setting. M-Broth is not intended for use in the diagnosis of disease or other conditions in humans.

Description

M-Broth is prepared according to the formula of Sperber and Diebel and contains the nutrients necessary for good growth and flagella development of *Salmonella*. This medium is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure. M-Broth conforms to standard procedures recommended in food testing and monoclonal and polyclonal enzyme immunoassays (EIA).

Typical Formulation

Enzymatic Digest of Casein	12.5 g/L
Yeast Extract	5.0 g/L
D-Mannose	2.0 g/L
Sodium Citrate	5.0 g/L
Sodium Chloride	5.0 g/L
Potassium Phosphate	5.0 g/L
Manganese Chloride	0.14 g/L
Magnesium Sulfate	0.8 g/L
Ferrous Sulfate	0.04 g/L
Polysorbate 80	0.75 g/L

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Dissolve 36.2 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous with small lumps and light beige.

Prepared Appearance: Prepared medium is yellow to amber and clear to trace hazy with no to light precipitate.

Expected Cultural Response: Cultural response in M-Broth at 35 ± 2°C and examined for growth after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Salmonella arizonae</i> ATCC® 13314	10 - 300	Good growth
<i>Salmonella choleraesuis</i> ATCC® 13076	10 - 300	Good growth
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Good growth
<i>Salmonella typhi</i> ATCC® 19430	10 - 300	Good growth

The organisms listed are the minimum that should be used for quality control testing.

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Test Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at $35 \pm 2^\circ\text{C}$ for 18 - 24 hours.
2. Transfer 1 mL of the above pre-enrichment culture to 9 mL of Selenite Cystine Broth, and 1 mL to 9 mL of Tetrathionate Broth. Incubate both enrichment media at $35 \pm 2^\circ\text{C}$ for 24 hours.
3. Inoculate one 10 mL tube of M-Broth, tempered to 35°C , with one drop from each of the above cultures. Incubate at $35 \pm 2^\circ\text{C}$ for 6 - 8 hours.
4. Prepare a formalin-salt solution by adding 4.2 grams of NaCl and 3 mL of formalin to 100 mL of distilled water. Place one drop in each of two Kahn tubes.
5. Carefully insert a pipette about 1 inch below the surface of the M-Broth culture and transfer 0.85 mL of culture to each of the above Kahn tubes containing formalin-salt solution.
6. Prepare a pooled antiserum by combining together 0.5 mL each of rehydrated Salmonella H Antiserum Poly D and Salmonella H Antiserum z₆ in 11.5 mL of 0.85% NaCl.
7. Add 0.1 mL pooled Salmonella H Antiserum to one of the Kahn tubes (above). Add 0.1 mL of 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1 ½ hours.

Note: An alternative testing procedure can be found in AOAC International³ for screening procedures using enzyme immunoassay or DNA hybridization to detect Salmonella antigens in test samples.

Results

Agglutination in the Kahn tube containing salmonella antiserum indicates the presence of *Salmonella*. agglutination in the Kahn tube containing 0.8% NaCl solution (control tube) indicates a rough culture which should be streaked for isolation, passed through Motility GI Medium to enhance flagella, and then retested with pooled antiserum.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2-8°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Sperber, W. H., and R. H. Deibel. 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. *Appl Microbiol.* 17:533-539.
2. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Association of Official Analytical Chemists. 2016. Official methods of analysis of AOAC International, 20th ed. AOAC International, Arlington, VA.

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