Bacto™ M Broth

Intended Use

Bacto™ M Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure.

Summary and Explanation

M Broth, prepared according to the formula of Sperber and Diebel,[1] contains all the nutrients necessary for good growth and flagella development of *Salmonella*.

Fantasia, Sperber and Deibel[2] compared the enrichment serology (ES) procedure with the traditional procedure that was outlined in the *Bacteriological Analytical Manual*[3] (BAM) and reported excellent agreement between the two. They found the ES procedure not only to be faster and less complicated but also as accurate and sensitive as the BAM procedure.

M Broth also conforms to the testing standards recommended by the *Compendium of Methods for the Microbiological Examination of Foods*[4] (APHA) for the isolation and identification of foodborne *Salmonella*.

Monoclonal enzyme immunoassay (EIA) methods have been described in *Official Methods of Analysis of AOAC International*[5] using M Broth. These methods are screening procedures for the presence of *Salmonella* and positive results must be confirmed by culture.

Principles of the Procedure

Yeast extract is a source of B-complex vitamins. Peptone provides organic nitrogen. D-Mannose and sodium citrate are fermentation energy sources. Mannose prevents fimbrial agglutination.[1] Sodium chloride helps maintain osmotic equilibrium, while dipotassium phosphate acts as a buffer. The inorganic salts stimulate bacterial growth. Polysorbate 80 is a surfactant and dispersing agent.

Formula

Bacto™ M Broth

Approximate Formula* Per Liter

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

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Bacto™ M Broth

Dehydrated appearance:	Beige, homogeneous with a tendency to clump.
Solution:	3.62% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 3.62% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Bacto™ M Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Choleraesuis var. Kunzendorf	12011	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good

Uninoculated Tube

Salmonella Choleraesuis ATCC™ 12011

Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at 35 ± 2°C for 18-24 hours.
2. Transfer 1 mL of the above preenrichment culture to 9 mL of Selenite Cystine Broth and 1 mL to 9 mL of Tetrathionate Broth. Incubate both enrichment media at 35 ± 2°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 ± 2°C for 6-8 hours.
4. Prepare a formalin-salt solution by adding 4.2 g of NaCl and 3 mL of formalin to 100 mL of purified 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 z_6 (Salmonella H Antisera Spicer-Edwards Set) 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 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1.5 hours.

Expected Results

Agglutination in the Kahn tube containing antiserum indicates the presence of *Salmonella*. Agglutination in the Kahn tube containing 0.85% 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.

Alternative Testing Procedures

Refer to *Official Methods of Analysis of AOAC International*[5] for screening procedures using enzyme immunoassay or DNA hybridization to detect *Salmonella* antigen in test samples.

References

1. Sperber and Deibel. 1969. Appl. Microbiol. *17*:533.
2. Fantasia, Sperber and Deibel. 1969. Appl. Microbiol. *17*:540.
3. Bacteriological Analytical Manual, 2nd ed. 1969. USDHEW, Washington, D.C.
4. Andrews, Flowers, Silliker and Bailey. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Md.

Availability

Bacto™ M Broth

AOAC COMPF USDA

Cat. No.	294020	Dehydrated – 500 g*
	294010	Dehydrated – 2 kg*

**Store at 2-8°C.*