

**H Broth****M243**

H Broth is recommended for the preparation of H antigen used in the identification and differentiation of *Salmonella* species.

**Composition\*\*\***

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Peptic digest of animal tissue	5.000
Beef extract	3.000
Dextrose	1.000
Dipotassium phosphate	2.500
Sodium chloride	5.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 21.5 grams in 1000 ml distilled water. Heat if necessary to ensure complete solution. Dispense in 4 ml amounts in 13 x 100 mm test tubes and sterilize by autoclaving at 115°C for 15 minutes.

**Principle And Interpretation**

The genus *Salmonella* is a member of the family *Enterobacteriaceae*. *Salmonella* has three kinds of major antigen with diagnostic or identifying applications: Somatic (O) or Cell Wall Antigens, Surface (Envelope) Antigens and Flagellar (H) Antigens (1). Identification and differentiation of *Salmonellae* based on H antigen were found to be less labor-intensive than the standard methods while requiring no more technical skill. H Broth, used for the preparation of H antigen in the identification and differentiation of *Salmonellae*, was originally formulated by Hajna and Damon (2). The medium is also used for differentiating *Enterobacteriaceae* (3). The combination of casein enzymic hydrolysate and peptic digest of animal tissue makes the medium highly nutritive for the growth of gram-negative enteric bacteria (4). Peptic digest of animal tissue, casein enzymic hydrolysate and beef extract in the medium provide nitrogen, vitamins and minerals necessary to support bacterial growth. Dextrose is the carbon and energy source. Dipotassium phosphate provides buffering to the medium. Sodium chloride provides essential ions and maintains the osmotic equilibrium. Presumptive typical colonies should be recovered from SS Agar (M108) or Bismuth Sulphite Agar (M027) and used to inoculate tubes of TSI Agar (M021), Motility Test Medium (M260) and H Broth (M243). *Salmonella Typhi* or *Salmonella*-like organisms identified from these tests are subjected to serological testing including indole test.

**Quality Control****Appearance**

Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Light amber coloured, clear solution without any precipitate

**Reaction**

Reaction of 2.15% w/v aqueous solution at 25°C. pH : 7.2±0.2

**Cultural Response**

M243: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth				
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant				
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant				
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant				
<i>Salmonella Paratyphi A</i> ATCC 9150	50-100	luxuriant				
<i>Salmonella Paratyphi B</i> ATCC 8759	50-100	luxuriant				
<i>Salmonella Arizonae</i>	50-100	luxuriant				

**Reference**

- 1.Gruenewald R., Dixon D. P., Brun M., Yappow S., Henderson R., Douglas J. E., and Backer M. H., Appl. Environ. Microbiol., 1990, 56 (1),24-30
- 2.Hajna A. A. and Damon S. R., 1950, Pub. Health Rep., 65:116,
- 3.Hajna A. A., 1951, Pub. Health Lab., 9:23,
- 4.Hajna A. A., 1951, Personal communication.

**Storage and Shelf Life**

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.