



## M-HD Endo Broth

M1110

M-HD Endo Broth is recommended for the detection of coliforms in water samples by membrane filter technique.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Yeast extract	3.000
Lactose	20.000
Sodium deoxycholate	0.200
Sodium chloride	5.000
Dipotassium phosphate	6.000
Sodium sulphite	2.100
Basic fuchsin	0.840
Final pH ( at 25°C)	7.5±0.2

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

### Directions

Suspend 57.14 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. DO NOT AUTOCLAVE. Use on the same day of preparation.

### Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. Estimation or enumeration of these bacteria in water can be done by employing the membrane filter procedure. As related to the membrane filter technique, the coliform group is defined as those facultative anaerobic, gram-negative, non-spore forming rod shaped bacteria that develop red colonies with a metallic sheen at 35°C within 24 hours on an Endo- type medium containing lactose (1). M-HD Endo Broth is formulated as per Hajna and Damon (2) and is used for the estimation of coliforms in water samples by membrane filtration technique (3).

M-HD Endo Broth contains casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract as source of essential nutrients including vitamins and B-complex nutrients. Lactose is the fermentable carbohydrate and energy source. Sodium deoxycholate is the selective agent, which helps to inhibit non-coliform bacteria. Sodium chloride maintains the osmotic equilibrium of the medium while dipotassium phosphate buffers the medium. Lactose-fermenting coliforms produce aldehyde and acid. The aldehyde in turn liberates fuchsin from the fuchsin-sulphite complex, giving rise to red coloured colonies. With *Escherichia coli*, this reaction is more pronounced as the fuchsin crystallizes, exhibiting a permanent greenish metallic luster (fuchsin luster) to the colonies.

Sterile cotton absorbent pads are saturated with around 2 ml of M-HD Endo Broth. Membrane filter through which the test water sample has been passed is aseptically placed on these saturated absorbent cotton pads containing the medium. Following an incubation at 35-37°C for 18-24 hours, lactose fermenting coliforms produce pink to rose red colonies with similar colouration to the medium. Non-lactose fermenting coliforms form colourless to faint colonies against the pink background.

### Quality Control

#### Appearance

Light pink to purple homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Light pink coloured clear solution without any precipitate

#### Reaction

Reaction of 5.71% w/v aqueous solution at 25°C. pH : 7.5±0.2

#### pH

7.30-7.70

**Cultural Response**

M1110: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<b>Organism</b>	<b>Inoculum (CFU)</b>	<b>Growth</b>	<b>Colour of colony (on membrane filter)</b>
<b>Cultural Response</b>			
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	red to black with metallic sheen
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	red to black
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	colourless
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	

**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. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of water and Wastewater, 20th Ed. American Public Health Association, Washington, D.C.
2. Hajna A. A. and Damon S. R., 1954, Public Health Rep., 69, 58
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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