



A-1 Broth

M874

A-1 Broth is used for detecting faecal coliforms in water samples waste water, seawater and foods by MPN Method.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Lactose	5.000
Sodium chloride	5.000
Salicin	0.500
Polyethylene glycol p-isooctylphenyl ether (Triton 100)	1.000
Final pH (at 25°C)	6.9±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.5 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute 10 ml amounts into tubes containing inverted Durham's tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.

Principle And Interpretation

Escherichia coli is used as the indicator organism to detect the faecal contamination of water. Andrews and Presnell (1) devised A-1 Medium, which was capable of recovering *Escherichia coli* from estuarine waters in 24 hours instead of 72 hours by avoiding the pre-enrichment step as recommended by APHA (2). This greatly reduced the time required for the complete identification of *E. coli* (3) by the elevated temperature and most probable number (MPN) methods, routinely used for water analysis. A-1 Medium substantially reduces the incidence of false positive cultures. Also, Stanbridge and Delfino found that the results obtained by using 3-hours pre-incubation step (using A-1 Medium) were statistically comparable with the two-step MPN technique for the enumeration of *E. coli* in chlorinated waste-water (4). Fast recovery of faecal coliforms from shell fish (5) and sea water (6) was also reported. A-1 Medium also conforms to the standard methods identified for the isolation of faecal coliforms in food, water and wastewater (2,7).

Casein enzymic hydrolysate provides carbonaceous and nitrogenous substances required for bacterial metabolism. Lactose and salicin act as energy sources and sodium chloride maintains osmotic equilibrium. Polyethylene glycol p-isooctylphenyl ether acts as a surfactant. Presence of gas bubbles in the inverted Durhams tubes is a positive indication of presence of faecal coliforms. The density of faecal coliform can be calculated by the standard methods using the MPN table.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution after cooling to room temperature.

Reaction

Reaction of 3.15% w/v aqueous solution at 25°C. pH : 6.9±0.1

pH

6.80-7.00

Cultural Response

Cultural characteristics observed after an incubation at different temperatures for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth at 35°C	Growth at 44.5°C
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Cultural Response

<i>Bacillus subtilis</i> ATCC 6633	50-100	none	none
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<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant (may or may not produce gas)	poor-fair
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant with gas	luxuriant with gas
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant without gas	good without gas
<i>Enterococcus faecalis</i> ATCC 19433	50-100	poor	none - poor

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. Andrews and Presnell, 1972, Appl. Microbiol., 23:521.
2. Eaton A. D., Clesceri L. S., and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
3. Andrews, Diggs and Wilson, 1975, Appl. Microbiol., 29:130.
4. Standridge and Delfino, 1981, Appl. Environ. Microbiol., 42:918.
5. Hunt and Springer, 1978, J. Assoc. Off. Anal. Chem., 61:1317
6. Miescier et al, 1978, J. Assoc. Off. Anal. Chem., 61:772.
7. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.

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