



A-1 Broth

M874

Intended use

Recommended for detecting faecal coliforms in water samples waste water, seawater and foods by MPN Method.

Composition**

| Ingredients | Gms / Litre |
|---|-------------|
| Tryptone | 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 purified/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 (3). This greatly reduced the time required for the complete identification of *E. coli* (2) 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 (9). Fast recovery of faecal coliforms from shell fish (4) and sea water (7) was also reported. A-1 Medium also conforms to the standard methods identified for the isolation of faecal coliforms in food, water and wastewater (3,9).

Tryptone provides carbonaceous and nitrogenous compounds, long chain amino acids, vitamins and other essential nutrients 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.

Type of specimen

Food samples; Water samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Due to nutritional variations certain strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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.

| Organism | Inoculum (CFU) | Growth at 35°C | Growth at 44.5°C |
|---|----------------|--|--------------------|
| <i>Bacillus spizizenii subsp. subtilis</i> ATCC 6633 (00003*) | 50-100 | none | none |
| # <i>Klebsiella aerogenes</i> ATCC 13048 (00175*) | 50-100 | luxuriant (may or may not produce gas) | poor-fair |
| <i>Escherichia coli</i> ATCC 25922 (00013*) | 50-100 | luxuriant with gas | luxuriant with gas |
| <i>Salmonella</i> Typhimurium ATCC 14028 (00031*) | 50-100 | luxuriant without gas | good without gas |
| <i>Enterococcus faecalis</i> ATCC 19433 | 50-100 | poor | none - poor |

Key : *Corresponding WDCM numbers. #- Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

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 6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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