A-1 Medium

Intended Use

A-1 Medium is used for detecting fecal coliforms in water.

Summary and Explanation

Since the early 1900s enumeration of coliform organisms, specifically *Escherichia coli*, has been used to determine water purity. Elevated-temperature, most-probable-number (MPN) methods are routinely used for the analysis of water and food samples for the presence of fecal coliforms. One limiting factor in using *E. coli* is the length of time required for complete identification.[1] A-1 Medium was formulated to hasten the recovery of *E. coli* and reduce the incidence of false positive cultures.

In 1972 Andrews and Presnell developed A-1 Medium. A-1 Medium recovers *E. coli* from estuarine water in 24 hours instead of 72 hours, and in greater numbers without the preenrichment step.[2] Using a 3-hour preincubation step for the enumeration of coliforms in chlorinated wastewater gave results that were statistically comparable to those obtained in the two-step MPN technique.[3]

A-1 Medium can be used in a single-step procedure for the detection of fecal coliforms in source water, seawater, treated wastewater and foods. Prior enrichment in a presumptive medium is not required.[4] A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.[4,5]

Principles of the Procedure

Peptone provides the nitrogen, vitamins, minerals and amino acids in A-1 Medium. Lactose is the carbon source and, in combination with salicin, provides energy for organism growth. Sodium chloride maintains the osmotic balance of the medium. Triton™* X-100 is a surfactant.

**Triton is a trademark of Rohm and Haas Company.*

Formula

Difco™ A-1 Medium

Approximate Formula* Per Liter		
Tryptone	20.0	g
Lactose	5.0	g
Sodium Chloride	5.0	g
Salicin	0.5	g
Triton X-100	1.0	mL

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

Directions for Preparation from Dehydrated Product

1. Suspend 31.5 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. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: For 10 mL water samples, prepare double-strength medium to ensure ingredient concentrations are not reduced below those of the standard medium.[4]

User Quality Control

Identity Specifications

Difco™ A-1 Medium

Dehydrated Appearance:	Light beige, lumpy.
Solution:	3.15% solution, soluble in purified water upon boiling. Solution is light amber, opalescent immediately after autoclaving. Upon cooling clear, may have a flocculent precipitate.
Prepared Appearance:	Light amber, clear, may have a flocculent precipitate.
Reaction of 3.15% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Difco™ A-1 Medium

Prepare the medium per label directions. Prepare tubes by placing fermentation vials and 10 mL amounts of medium into tubes. Inoculate and incubate at 35 ± 2°C for 3 hours. Transfer tubes to a 44.5°C water bath for 21 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS
Bacillus subtilis	6633	10^2	None	–
Enterobacter aerogenes	13048	10^2	Poor to good*	–
Enterococcus faecalis	19433	10^2	None to poor	–
Escherichia coli	25922	10^2	Good	+
Escherichia coli	13762	10^2	Good	+

**May or may not produce gas.*

Uninoculated Tube

Escherichia coli ATCC™ 25922 with fermentation vial

Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.[4,5]
2. Incubate at 35 ± 0.5°C for 3 hours.
3. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hours.
4. Maintain water level in bath above level of liquid in inoculated tubes.

Expected Results[4,5]

Gas production in the inverted vial, or dissolved gas that forms fine bubbles when slightly agitated, is a positive reaction indicating the presence of fecal coliforms. Calculate fecal coliform densities using MPN tables from standard methods.

Limitations of the Procedure

1. Fecal coliform counts are usually greater than *E. coli* counts.[5]
2. Interpretation of test procedure using A-1 Medium requires understanding of the microflora of the specimen.[5]

References

1. Andrews, Diggs and Wilson. 1975. Appl. Microbiol. 29:130.
2. Andrews and Presnell. 1972. Appl. Microbiol. 23:521.
3. Standridge and Delfino. 1981. Appl. Environ. Microbiol. 42:918.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ A-1 Medium

COMPF EPA SMWW

Cat. No. 218231 Dehydrated – 500 g