

## A-1 Medium (NCM0124)

### Intended Use

A-1 Medium is used for the detection of coliform organisms in water and food in a laboratory setting. A-1 Medium is not intended for use in the diagnosis of disease or other conditions in humans.

### Description

Since the early 1900's enumeration of coliform organisms, specifically *E. coli*, have been used to determine water purity. Elevated temperature and most-probable-number (MPN) methods are routinely used for analysis of water and food samples for presence of fecal coliforms. A-1 Medium was formulated to hasten *E. coli* recovery and reduce the incidence of false positive cultures.

In 1972 Andrews and Presnell developed A-1 Medium. This medium recovers *E. coli* from estuarine water in 24 hours instead of 72 hours, and in greater numbers without the pre-enrichment step. 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. A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.

### Typical Formulation

Enzymatic Digest of Casein	20.0 g/L
Lactose	5.0 g/L
Sodium Chloride	5.0 g/L
Salicin	0.5 g/L
Triton X-100	1.0 g/L

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Precaution

Refer to SDS

### Preparation

1. Dissolve 31.5 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Distribute into test tubes containing Durham tubes.
4. Autoclave at 121°C for 10 minutes.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free-flowing, forms soft lumps, and light beige to beige.

**Prepared Appearance:** Prepared medium is clear, may have a light precipitate, and light yellow to amber.

**Expected Cultural Response:** Cultural response in A-1 Medium were incubated aerobically at  $35 \pm 2^\circ\text{C}$  for 3 hours and then examined for growth following transfer to a  $44.5^\circ\text{C}$  waterbath for 19 – 22 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Gas
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Growth	+
<i>Escherichia coli</i> ATCC® 35218	10 - 300	Growth	+
<i>Proteus mirabilis</i> ATCC® 12453	10 - 300	Growth	-
<i>Pseudomonas aeruginosa</i> ATCC® 27853	1000	Partial to complete inhibition	-

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.
2. Incubate at  $35 \pm 0.5^\circ\text{C}$  for 3 hours.
3. Transfer tubes to a water bath at  $44.5 \pm 0.2^\circ\text{C}$  and incubate for an additional  $21 \pm 2$  hours.
4. Maintain water level in bath above level of liquid in inoculated tubes.

### Results

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. For MPN, calculate fecal coliform densities using tables from standard methods.

### Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if appearance has changed from the original color and texture. Expiry applies to medium in its intact container.

### Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Fecal coliform counts are usually greater than *E. coli* counts.
3. Interpretation of tests using A-1 Medium requires understanding of the microflora of the specimen.

### Storage

Store dehydrated culture media at  $2-30^\circ\text{C}$  away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

### References

1. Andrews, W. H., and M. W. Presnell. 1972. Rapid recovery of *Escherichia coli* from estuarine water. Appl. Microbiol. 23:521-523.
2. Andrews, W. H., C. D. Diggs, and C. R. Wilson. 1975. Evaluation of a medium for the rapid recovery of *Escherichia coli* from shellfish. Appl. Microbiol. 29:130-131.
3. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 2017. Standard methods for the examination of water and wastewater, 23<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
4. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of food, 4<sup>th</sup> ed. American Public Health Association, Washington, D.C.
5. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm).