PRODUCT SPECIFICATION SHEET

A-1 Broth (DM001)

Intended Use
A-1 Broth (DM001) is recommended for detection of faecal coliforms in water samples and foods by MPN technique.

Product Summary and Explanation
Escherichia coli is used as the indicator organism to detect the faecal contamination of water. 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 identifcation.[1] A-1 Broth was formulated to accelerate the recovery of *E. coli* and reduce the incidence of false positive cultures. In 1972 Andrews and Presnell[2] devised A-1 Broth, 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*[1] by the elevated temperature and most probable number (MPN) methods, routinely used for water analysis. A-1 Broth 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 Broth) 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 Broth also conforms to the standard methods identified for the isolation of faecal coliforms in food, water and wastewater.[2,7]

Principles of the Procedure
A-1 Broth contains casein enzymic hydrolysate which provides the nitrogen, vitamins, minerals and amino acids required for bacterial metabolism. Lactose is the carbon source and, in combination with salicin, provides energy for organism growth. Sodium chloride maintains osmotic equilibrium. Polyethylene glycol p-isooctylphenyl ether acts as a surfactant.

Formula / Liter

Ingredients	Gms / Liter
Part A	--
Casein enzymic hydrolysate	20.00
Lactose	5.00
Sodium chloride	5.00
Salicin	0.50
Part B	--
Polyethylene glycol p-isooctylphenyl ether (Triton 100)	1.00
Final pH: 6.9 ± 0.1 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 31.5 grams of medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Distribute 10 ml amounts into tubes containing inverted Durham's tubes.
4. Autoclave at 121°C, 15 psi pressure, for 10 minutes.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear solution after cooling to room temperature
Reaction of 3.15% Solution	pH : 6.9 ± 0.1 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth at 35°C	Growth at 44.5°C
1.	*Bacillus subtilis ATCC 6633*	50-100	none	none
2.	*Enterobacter aerogenes ATCC 13048*	50-100	luxuriant (may or may not produce gas)	poor-fair
3.	*Escherichia coli ATCC 25922*	50-100	luxuriant with gas	luxuriant with gas
4.	*Salmonella Typhimurium ATCC 14028*	50-100	luxuriant without gas	good without gas
5.	*Enterococcus faecalis ATCC 19433*	50-100	poor	none - poor

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

Test Procedure

1. Inoculate tubes of A-1 broth as directed in standard methods.[3,7]
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.
5. Refer to appropriate references for standard test procedures.

Results

1. 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.
2. Calculate fecal coliform densities using MPN tables from standard methods.[3,7]
3. Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Fecal coliform counts are usually greater than *E. coli* counts.
2. Interpretation of test procedure using A-1 Broth requires understanding of the microflora of the specimen.
3. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : A-1 Broth
Product Code : DM001
Available Pack sizes : 100gm/500gm

References

1. Andrews, Diggs and Wilson. 1975. Appl. Microbiol. *29*:130.
2. Andrews and Presnell, 1972, Appl. Microbiol., 23:521.
3. 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.
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.

Further Information

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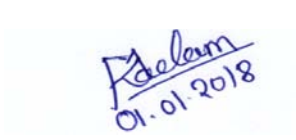		
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