



Pseudomonas Asparagine Broth

M1096

Pseudomonas Asparagine Broth is used for presumptive determination of *Pseudomonas aeruginosa* from recreational or natural water as per APHA.

Composition**

Ingredients	Gms / Litre
DL-Asparagine	3.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 4.5 grams in 1000 ml distilled water. Gently boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

Principle And Interpretation

Recreational water like from swimming pool is a body of water in a holding structure. Microorganisms of concern are those causing infections of ear, skin and upper respiratory tract etc. *Pseudomonas aeruginosa* is one of those organisms which account for a large percentage of swimming pool associated illness. Asparagine Medium is recommended for the microbiological analysis of water. Pseudomonas Asparagine Broth is an excellent enrichment medium for *P. aeruginosa*, since it is composed of a mineral base and the only carbon source is asparagine. It is also used in the multiple-tube technique for microbiological analysis of recreational waters. Pseudomonas Asparagine Broth is formulated as recommended by APHA (1) for presumptive detection of *P. aeruginosa* from recreational or natural waters. Pseudomonas Asparagine Broth medium is a relatively simple medium containing an amino acid DL-asparagine and two salts dipotassium phosphate and magnesium sulphate. Asparagine is the amino acid and carbon source while phosphate and sulphate provide the ions for the growth of *P. aeruginosa*. Dipotassium phosphate also helps in maintaining the buffering conditions of the medium. This medium is only a presumptive medium for *P. aeruginosa*, and further confirmatory tests are necessary for the identification. For five tubes multiple tube test, use 10 ml of single strength Asparagine Broth for inocula of 1 ml or less and 10 ml double strength Asparagine Broth for 10 ml inocula. For swimming pools, higher dilutions may be necessary. Incubate inoculated tubes at 35-37°C. After 24 hours and again after 48 hours of incubation examine tubes under long wave ultraviolet light in a darkened room. Production of a green fluorescent pigment constitutes a positive presumptive test. Confirmation is performed by subculturing a loop from each tube in Acetamide Medium (M148). Development of purple colour within 24-36 hours of incubation at 35-37°C is a positive confirmed test for *P. aeruginosa*.

Quality Control

Appearance

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless clear solution with slight precipitate.

Reaction

Reaction of 0.45% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M1096: Cultural characteristics observed after an incubation at 35-37°C for 20 - 24 hours.

Organism	Inoculum (CFU)	Growth
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	luxuriant

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. 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.

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