ASPARAGINE ENRICHMENT BROTH

Dehydrated culture medium

1 - INTENDED USE

Liquid medium for the presumptive detection and enumeration of *Pseudomonas aeruginosa* in water samples.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

DL-asparagine	3.0 g
Dipotassium hydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

P. aeruginosa is an opportunistic environmental pathogen characterized by a high degree of adaptability, capable of growing in waters with very low nutrients concentrations and surviving in disinfected water.[1]
Asparagine Enrichment Broth is recommended by APHA[2] for the enumeration of *P. aeruginosa* in recreational waters by multiple tube technique.
The medium is a strictly mineral base with asparagine as the sole source of carbon and nitrogen which is converted to aspartic acid by *P. aeruginosa*. Magnesium sulphates provide necessary cations for the activation and stimulation of fluorescein and pyocianin production. Dipotassium hydrogen phosphate acts as a buffer system.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 4.5 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly to completely dissolve the powder if necessary. Distribute 10 mL in tubes and sterilise by autoclaving at 121ºC for 15 minutes. For 10 mL inocula, use tubes with 10 mL of double strength broth.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid with a light precipitate
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Asparagine Enrichment Broth	Dehydrated medium	40109512	500 g (111 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, test tubes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

Recreational waters. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

9 - TEST PROCEDURE

Perform a five tubes MPN test.
Use 10 mL single-strength broth for inocula of 1 mL or less. Use 10 mL double-strength broth for 10 mL inocula. Higher dilutions may be necessary for swimming pools.
Incubate at 35-37°C for 24-48 hours.

10 - READING AND INTERPRETATION

After 24 hours and again after 48 hours of incubation, examine tubes under long-wave UV lamp (black light) in a darkened room.
Production of a green fluorescent pigment constitutes a positive presumptive test.
Confirm positive tubes by inoculating 0.1 mL of culture into Acetamide Broth and incubate at 36 ± 2 °C for 22 ± 2 h. After incubation, add 1 to 2 drops of Nessler reagent and examine the tubes for the production of ammonia. Development of a colour varying from yellow to brick red is a positive confirmed test for *P.aeruginosa*.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below is listed a test strain useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa ATCC 14207	35-37°C / 24 H/ A	growth with green fluorescent ring

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Asparagine Enrichment Broth is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

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Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 24 hours and recording the highest dilution showing growth and green fluorescent ring in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 14207, *P. aeruginosa* ATCC 10299, *P. aeruginosa* CB607. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is tested with the following non-target strains: *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. After incubation at 37°C for 48 hours, the growth of *S. aureus* is totally inhibited, while the growth of *E.coli* is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- Asparagine Enrichment Broth is a presumptive medium for *P. aeruginosa*, and further confirmatory tests are necessary for the identification.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized tubes inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Briancesco R, Paduano S, Semproni M, Vitanza L, Bonadonna L. Behavior of Pseudomonas aeruginosa and Enterobacter aerogenes in Water from Filter Jugs. Int. J. Environ. Res. Public Health 2020; 17: 8263.
2. APHA Standards Methods for the Microbiological of Water and Wastewater. American Public Health Association, Washington D.C. 23rd, 2017.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/12

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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