PSEUDOMONAS SELECTIVE BROTH

Dehydrated culture medium

1 - INTENDED USE

For the enumeration of *Pseudomonas aeruginosa* and other *Pseudomonas* spp. in liquid samples by the membrane filtration method and for the confirmation test of *P. aeruginosa*.

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

Pancreatic digest of gelatin	16.0 g
Acid digest of casein	10.0 g
Potassium sulphate	10.0 g
Magnesium chloride	1.4 g
Cetrimide	0.2 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

King, Ward, and Raney[1] in 1954 described two media, one of which (medium A) enhances the production of pyocyanin by *P. aeruginosa*, while the other (medium B) enhances the production of fluorescein. Pseudomonas Selective Broth is prepared according to the formulation of Tech Agar/Medium A without agar and with the addition of cetrimide for the inhibition of microorganisms other than *Pseudomonas*, originally proposed at the concentration 0.1% by Lowbury[2] and later decreased by Lowbury and Collins in 1955[3].
Pseudomonas Selective Broth can be used for the enumeration of *Pseudomonas* spp. by membrane filtration technique and for the confirmation test of *P. aeruginosa* colonies cultivated on solid selective media.[4]
Pancreatic digest of gelatin and acid digest of casein provide nitrogen, carbon and amino acids for bacterial growth and contributes to the pyocyanin and fluorescein production. Cetrimide has bactericidal activity against a broad range of Gram-positive organisms and some Gram-negative organisms other than *P. aeruginosa*. Magnesium chloride and potassium sulphate provide necessary cations for the activation and stimulation of fluorescein and pyocyanin production.[5] Glycerol is present in the medium as a carbon source for microbial growth and as a stimulant for the production of pyocyanin.[5]

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37.6 g in 1000 mL of cold purified water and add 10 mL of glycerol. Heat to boiling with frequent agitation, distribute 5 mL in tubes and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pseudomonas Selective Broth	Dehydrated medium	4019642	500 g (13.3 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Water and other liquid samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Membrane filtration method

1. Filter an appropriate volume of sample onto the membrane depending on the expected *Pseudomonas* number. When the bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate.
2. Using aseptic technique, place a sterile absorbent pad in each culture dish and pipet at least 2 mL of broth. Carefully remove any excess of liquid from culture dish by decanting plate.
3. Roll the membrane filter used to collect the sample onto the surface of the pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Incubate the inverted Petri dish for 24 - 72 hours at 25-35° C. Incubation at 35° C during 24 hours is favourable to *P. aeruginosa*, 25° C for *P. fluorescens*.

Confirmation technique[4]

Select five typical colonies cultivated onto primary selective medium and subculture into tubes of Pseudomonas Selective Broth and perform cytochrome oxidase test. Incubate the tubes of Pseudomonas Selective Broth at 42 ± 0.5°C for 24 hours. The oxidase positive colonies that grow on Pseudomonas Selective Broth tubes are identified and enumerated as *P. aeruginosa*

10 - READING AND INTERPRETATION

Enumerate the number of colonies per plate and calculate the microbial count.

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 are listed some test strains useful for the quality control.

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CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
P. aeruginosa ATCC 9027	37°or 42°C / 24-48 H-A	good growth
E. coli ATCC 25922	37° or 42°C/ 24-48 H-A	inhibited

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 Pseudomonas Selective Broth is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 42°C for 24 hours and recording the highest dilution showing growth 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 9027.
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, *E. faecalis* ATCC 19433, *E. coli* ATCC 25922, *C. albicans* ATCC 18804. After incubation at 42°C for 24 hours, the growth of other non-target strains is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- Inhibition of some strains of *P. aeruginosa* has been reported using a selective agar containing cetrimide.[6]
- Occasionally some enteric organisms (e.g., *Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia*), *Alkaligenes* and *Aeromonas* will exhibit growth with a slight yellowing of the medium; however, this coloration is easily distinguished from slight fluorescein production since this yellowing does not fluoresce.[5]
- There are non-pigmented strains of *P.aeruginosa* that grow on the medium but do not produce the typical green-blue or yellow-green colour.
- Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. Some *Serratia* strains may exhibit a pink pigmentation.[5]
- Studies of Lowbury and Collins[3] showed that *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. However, fluorescence reappears when tubes are re-incubated.
- Some mucoid *P.aeruginosa* strains have a delayed oxidase positive reaction and therefore may require further confirmation tests.
- 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.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- 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 plates 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 the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
2. Lowbury EJ. Improved culture methods for the detection of Pseudomonas pyocyanea. J Clin Pathol 1951; 4:66-72
3. Lowbury EJ, Collins AG. The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J Clin Pathol 1955; 8:47-8.
4. Manuel Suisse des Denrées Alimentaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore : Williams & Wilkins ; 1985.
6. Hoibi N, Ciofu O, Bjarnsholt T. Pseudomonas. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015

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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