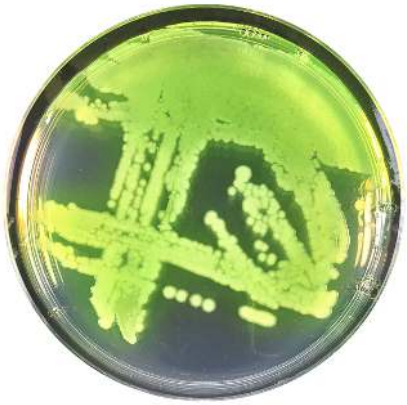
INSTRUCTIONS FOR USE

PSEUDOMONAS SELECTIVE AGAR

Dehydrated culture medium



P.aeruginosa on Pseudomonas Selective Agar

1 - INTENDED USE

In vitro diagnostic. For selective isolation and presumptive identification of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

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

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	14.0 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

Pseudomonas spp. are aerobic, non-sporeforming Gram-negative rods that are straight or slightly curved, 0.5 to 1.0 by 1.5 to 5.0 µm; they have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate has been used as an alternative that allows anaerobic growth.[1] They are usually motile with one or several polar flagella. *Pseudomonas* spp. are catalase positive and most species of clinical interest are oxidase positive (except *P.luteola* and *P.oryzihabitans*).[1]

P.aeruginosa is widely distributed in surface, waste and marine water, on soil, vegetation and in all humid environments; moreover, it is able to grow in distilled water, to survive in disinfectants, in cosmetics and to contaminate food. *P.aeruginosa* is considered an opportunistic pathogen especially in immunocompromised patients and is characterized by multi-resistance to antibiotics, thus representing a health risk in hospital environments. *P.aeruginosa* can cause ventilator-associated pneumonia, urinary tract infections, burns and wounds infections, corneal ulcers and keratitis, septicemia, gastroenteritis in newborns, abscesses, and meningitis.[2]

Other characteristics that tend to be associated with *Pseudomonas* species (with some exceptions) include secretion of pyoverdin (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions.[2] Certain *Pseudomonas* spp. may also produce additional types of siderophore, such as pyocyanin (blue) pyorubin (red) or pyomelanin (brown) by *P.aeruginosa* and thioquinolobactin by *P.fluorescens*.[3]

King, Ward, and Raney[4] in 1954 have 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 Agar is prepared according to the formulation of Tech Agar/Medium A with the addition of cetrimide for the inhibition of microorganisms other than *Pseudomonas*, originally proposed at the concentration 0.1% by Lowbury[5] and later decreased to 0.03% by Lowbury and Collins in 1955[6].

Pseudomonas Selective Agar meets cetrimide agar formulation and performance specifications described by EP, USP, JP harmonized method for the determination of absence of *P.aeruginosa* in non-sterile pharmaceutical products.[7] It is recommended by ISO Standard 22717[8] and by FDA-BAM[9] for the detection of *P.aeruginosa* in cosmetics.

Pancreatic digest of gelatin provides nitrogen and carbon for bacterial growth and, as reported by King et al.[4] and by Goto and Enomoto,[10] contributes to the pyocyanin and fluorescein production. Cetyltrimethylammonium bromide, acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane protein, causes the release of nitrogen and phosphorous from the cell and 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.[11] Glycerol is present in the medium as a carbon source for microbial growth and as a stimulant for the production of pyocyanin.[11] The presumptive identification of *P.aeruginosa* is obtained on the basis of the pigments production and the chromatic characteristics of the colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 45.7 g in 1000 mL of cold purified water and add 10 mL of glycerol (REF 421015). Heat to boiling stirring constantly and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pseudomonas Selective Agar CND: W0104010101; EDMA:14.01.01.01	Dehydrated medium	4019632	500 g (10,9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies; Glycerol (REF 421015).

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8 - SPECIMENS

Pseudomonas Selective Agar may be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as respiratory secretions, damaged tissues, ear, eye, urine etc.[12] Collect specimens before antimicrobial therapy where possible and apply good laboratory practices for collection, transport and storage of the clinical specimens.
Non-clinical samples analysed with Pseudomonas Selective Agar include non-sterile pharmaceutical products and cosmetics. Consult the references for sample collection and preparation.[7-9]

9 - TEST PROCEDURE

Allow plates to come to room temperature; the agar surface should be smooth and moist, but without excessive moisture.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate aerobically at 35-37°C and record the results after 18-24 hours. If typical colonies are not observed re-incubate an additional 24-36 hours (72 h in total). For cultures from cystic fibrosis patients it is recommended that solid media plates be held at 35 to 37°C for 5 days since some strains from chronic infections grow very slowly.[12]
For the detection of *P.aeruginosa* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia[7] and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Subculture on a plate of Pseudomonas Selective Agar and incubate at 30-35 °C for 18-72 h.

The growth of colonies indicates the possible presence of *P.aeruginosa*. This is confirmed by identification tests.
For the detection of *P.aeruginosa* in cosmetics consult the references.[8,9]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Any growth indicates a possible *Pseudomonas* species. Examine colonies under UV light (254 nm) for fluorescence.
Positive: bright yellow green colour diffuses into the agar producing a fluorescent zone in the agar surrounding the growth (*P.aeruginosa, P.fluorescens, P.putida).*
Examine colonies for pigmentation under normal light
The production of pyocyanin is indicated by the blue or green / blue colour of the colonies.
The production of fluorescein is indicated by the yellow-green colour of the colonies
The formation of pyorubin is indicated by the colour from light pink to red or dark brown of the colonies. Pyorubin is frequently formed simultaneously with pyocyanin and or fluorescein.
Fluorescein combines with pyocyanin, to give *P.aeruginosa* its characteristic bright green colour. These chromatic characteristics are typical of *P.aeruginosa*, together with the morphology of the colonies and the typical grape smell caused by the production of aminoacetophenone.

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, it is responsibility of the end-user to perform Quality Control testing 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 of un-supplemented medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P.aeruginosa ATCC 9027	35-37°C / 18-24H / A	good growth, green colonies
E. coli ATCC 25922	35-37°C / 18-24H / A	inhibited
S.aureus ATCC 25923	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection. User quality control of Pseudomonas Selective Agar used for *P.aeruginosa* detection in non-sterile pharmaceutical products and cosmetics should meet the requirements of EP[7] and ISO Standard.[8]

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Pseudomonas Selective Agar (Test Batch: TB), is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative test with the target strain *P.aeruginosa* ATCC 9027. Pseudomonas Selective Agar plates are inoculated with decimal dilutions in saline of the colonies suspension and incubated at 30-35°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr = CFU_{TB}/CFU_{RB}$) is calculated. If *Pr* is ≥ 0,7 and if the colonies morphology is typical (green colonies), the results are considered acceptable and conform to the specifications.
Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the target strains *P.aeruginosa* ATCC 14207 and *P.aeruginosa* ATCC 10299. After incubation at 35-37°C for 18-24 hours the colour of the colonies and the amount of growth is evaluated and recorded. All strains show a good growth with typical green colour. Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922*, S.aureus* ATCC 25923 and *P.mirabilis* ATCC 10005*, A.calcoaceticus* ATCC 19606. The growth of *A.calcoaceticus*, *E.coli* and *S.aureus* is completely inhibited while the growth of *P.mirabilis* is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- Inhibition of some strains of *P.aeruginosa* has been reported using a selective agar containing cetrimide.[12]
- A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. For an optimized recovery of *P.aeruginosa*, mainly in cystic fibrosis patients, it is recommended to use in addition to Pseudomonas Selective Agar, other selective and non-selective media such as Mac Conkey Agar, blood agar and chocolate agar.[12]
- No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.[4]
- 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.[11]

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- 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.[11]
- Studies of Lowbury and Collins[6] 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 plates are re-incubated.
- Some mucoid *P.aeruginosa* strains have a delayed oxidase positive reaction and therefore may require further confirmation tests.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, 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.
- 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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - REFERENCES

1. Public Health England- Identification of Pseudomonas species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. ID 17 Issue 3, 2015
2. Istituto Superiore di Sanità. Metodi analitici per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. A cura di Lucia Bonadonna e Massimo Ottaviani 2007, iv, 204 p. Rapporti ISTISAN 07/5
3. Meyer JM, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P, et al. Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. Appl Environ Microbiol 2002;68:2745-53
4. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med1954;44:301-7.
5. Lowbury EJ. Improved culture methods for the detection of Pseudomonas pyocyanea J Clin Pathol 1951; 4:66-72
6. 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.
7. European Pharmacopoeia, current edition
8. ISO 21717:2015. Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa.
9. U.S. Food and Drug Administration. Bacteriological Analytical Manual BAM Chapter 23: Methods for Cosmetics. Content current as of:10/31/2017
10. Goto S. Enomoto S. Nalidixic Acid Cetrimide Agar. A New Selective Plating Medium for the Selective Isolation of Pseudomonas aeruginosa Jpn J Microbiol 1970 14 (1): 65.
11. 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	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/06

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

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