PSEUDOMONAS AGAR F

Dehydrated and ready-to-use culture medium

From left: *P. aeruginosa* and *B. cepacea* on Pseudomonas Agar F

1-INTENDED USE

Pseudomonas Agar F (King Medium B) is intended for the differentiation of *Pseudomonas aeruginosa* by the ability to produce fluorescein.

2-COMPOSITION *

PSEUDOMONAS AGAR F, DEHYDRATED MEDIUM

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.00 g
Peptone	10.00 g
Dipotassium hydrogen phosphate	1.50 g
Magnesium sulphate	1.50 g
Agar	15.00 g

PSEUDOMONAS AGAR F, READY-TO-USE PLATES

TYPICAL FORMULA

Pseudomonas Agar F	38 g
Glycerol	10 mL
Purified water	1000 mL

*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-spore-forming, 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 may be 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 superficial water, waste and marine waters, on the soil, on vegetation and in all moist 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 wound infections, corneal ulcers and keratitis, septicaemia, gastroenteritis in new-borns, abscesses, and meningitis.[2]

Other characteristics that may be associated with *P. aeruginosa* species (with some exceptions) include secretion of pyoverdine (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions.[2] Additional types of siderophores, such as pyocyanin (blue) pyorubine (red) or pyomelanin (brown) may also be produced by *P. aeruginosa* and thioquinolobactin by *P. fluorescens*.[3]

King, Ward, and Raney[4] in 1954 described two media for pigment detection in *P. aeruginosa*: medium A enhancing the production of pyocyanin and medium B enhancing the production of fluorescein.

Pseudomonas Agar F, also known as King's Medium B or Flo Agar, is a modification of the formula described by King, Ward and Raney[4], it conforms to the formulation recommended by ISO16266[5] and by FDA-BAM[6] and is used for the fluorescein production test for the differentiation of *P. aeruginosa*.

Potassium phosphate has a stimulatory effect on fluorescein production and an inhibitory effect on pyocyanin; meat and casein peptones in equal quantities contribute to the optimal production of fluorescein, activated by the presence of Mg cations of magnesium sulphate; glycerol, added to the base medium, is a source of carbon for the growth and production of the pigment.[7]

A fluorescein-producing *Pseudomonas* will grow with yellow-green colonies, fluorescent under Wood's lamp.

Pseudomonas Agar F, combined with Pseudomonas Agar P, allows to perform the conventional phenotypic test for the differentiation of *P. aeruginosa* from other species of the genus *Pseudomonas*, isolated from clinical specimens.[8]

ISO 16266[5] Standard recommends the fluorescein production on Pseudomonas Agar F (+) as a confirmation test of *P. aeruginosa* colonies isolated from water, together with the oxidase test (+) and the ability to produce ammonia in Acetamide Broth (+).

FDA-BAM[6] recommends the pyocyanin and fluorescein production tests on Pseudomonas Agar P (+) and Pseudomonas Agar F (+) for the confirmation of *P. aeruginosa* colonies isolated from cosmetics, together with arginine dehydrolase (+), citrate and malonate utilisation (+), nitrate reduction (+), motility (+) and growth at 42°C (+).

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38 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. Pseudomonas Agar F can also be distributed in tubes and let solidify in slanted position with a short slant.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution appearance	whitish, lightly opalescent
Ready-to-use plate appearance	whitish, lightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pseudomonas Agar F	Dehydrated medium	4019612	500 g (13.2 L)
Pseudomonas Agar F	Ready-to-use plates	541961	2 x 10 plates ø 90 mm

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

The sample consists of pure cultures of bacteria. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for *Pseudomonas*.

9 - TEST PROCEDURE

Inoculate the medium in a plate or in a test tube with a single colony taken from the primary isolation medium, smear onto the medium surface with a single line streak; do not stab the butt when slanted tubes are used.
Incubate plates or tubes, with loosened caps, at 35 ± 2°C for 18-24 hours. If the isolate fails to grow or grows slowly, re-incubate at 25-30°C for 1-2 days and observe for growth and pigment production.
ISO 16266: incubate plates aerobically at 36 ± 2°C for up to 5 days, examining the cultures daily.[5]
FDA-BAM recommends an incubation temperature at 25°C for at least 3 days. [6]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Examine the growth under Wood's lamp daily: record as positive any appearing fluorescence.

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 of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa	ATCC 14207	36 ± 2°C / 18-24H / A	greenish-yellow growth, fluorescent under Wood's lamp
B. cepacia	ATCC 25416	36 ± 2°C / 18-24H / A	colourless not fluorescent growth

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative sample of all lots of dehydrated and ready-to-use Pseudomonas Agar F are tested for performance characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of 4 *Pseudomonas* strains and an *E. coli*, are inoculated by smearing the plated medium surface: *P. aeruginosa* ATCC 10145, *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 9027, *B. cepacea* ATCC 25416, E coli ATCC 8739. After incubation at 36±1°C for 18-24 hours aerobically, fluorescence production under Wood's lamp is observed and recorded. All *P. aeruginosa* strains show a production of fluorescence while *B. cepacea* and *E. coli* grows with white and non-fluorescent colonies.

13 - LIMITATIONS OF THE METHOD

- The presence of colourless colonies does not completely exclude the presence of *P. aeruginosa*.[7]
- Mucoid isolates of *P. aeruginosa* may undergo several phenotypic changes including the loss of pigment production.[8]
- Occasionally a *Pseudomonas* strain will produce small quantities of pyocyanin which, normally, should be inhibited on resulting in a yellow-green colour on the medium.[7]
- Pseudomonas Agar F should not be used as an isolation medium, but only as a differential medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, 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 culture medium 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.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 Sheets of the products 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

Ready to use plates

Upon receipt, store plates in their original pack at +2°C/+8°Caway from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2°C/+8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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). According to ISO 16266 the tubed medium prepared by the user can be stored at +2°C/+8°C in the dark for up to 3 months.[3]

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
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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 Med 1954;44:301-7.
5. ISO 16266:2006 - Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Microbiological Methods for cosmetics. Updated 07/2017
7. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
8. 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	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/06
Revision 2	Update of “intended use”, “precautions and warnings” and “storage conditions and shelf life”	2022/05
Revision 3	Update of chapters 2, 6, 12, 14, 15	2023/03

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