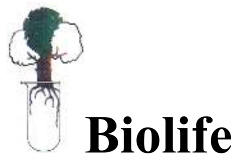


INSTRUCTIONS FOR USE

PSEUDOMONAS AGAR P

Dehydrated culture medium

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

1 - INTENDED USE

In vitro diagnostic. Pseudomonas Agar P (King Medium A) is intended for the differentiation of *Pseudomonas aeruginosa* isolated from clinical and non-clinical specimens, by ability to produce pyocyanin.

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

Peptone	20.0 g
Potassium sulphate	10.0 g
Magnesium chloride	1.4 g
Agar	15.0 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-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 wounds infections, corneal ulcers and keratitis, septicaemia, gastroenteritis in newborns, 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 be also 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 P, also known as King's Medium A or Tech Agar, is a modification of the formula described by King, Ward and Raney[4], it conforms to the formulation recommended by FDA-BAM[5] and is used for the pyocyanin production test. Pyocyanin producing strains develop a blue to blue green colour on the growth and on the medium around the growth.
Potassium sulphate and magnesium chloride enhance the production of pyocyanin and inhibit the production of fluorescein.[6] Peptone provides nitrogen and carbon compounds for bacterial growth and furthermore, thanks to a low phosphorus content, it has a reduced inhibitory effect on the production of pyocyanin; glycerol, added to the medium, is a carbon source for the growth and for the production of pyocyanin.[6]
Pseudomonas Agar P, combined with Pseudomonas Agar F, allows to perform the conventional phenotypic tests for the differentiation of *P.aeruginosa* from other species of the genus *Pseudomonas*, isolated from clinical specimens.[7]
FDA-BAM[5] 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 46.4 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 P can also be distributed in tubes before sterilization and let solidify in slanted position with a short slant.

5 - PHYSICAL CHARACTERISTICS

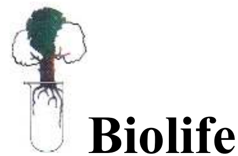
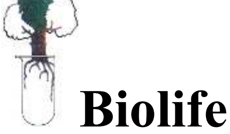
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	whitish, lightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pseudomonas Agar P	Dehydrated medium	4019622	500 g (10,8 L) CND: W0104010101; EDMA:14.01.01.01; RDM: 1874652/R

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).

8 - SPECIMENS

The sample consists of pure cultures of bacteria isolated from clinical samples or other material. 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 caps loosened, 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.[5]
FDA-BAM[5] recommends the incubation temperature at 25°C for at least 3 days.

10 - READING AND INTERPRETATION

Examine daily and note the presence of growth colour: record as positive the presence of blue to blue-green colour on the growth diffusing into the medium.
FDA-BAM[5] recommends the following result reading: break up Pseudomonas Agar P with a glass rod in approximately equal amount of distilled water and shake vigorously until water has removed as much pigment as possible. Decant into a separator. In a chemical hood, add 5-10 ml chloroform to water in separator and shake (venting occasionally to prevent internal pressure). The blue pyocyanin will migrate to chloroform. Draw off chloroform layer into a test tube. Add about 3 ml distilled water. Add 1 drop 1N H_2SO_4. Pyocyanin becomes red and migrates to water.

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 14207	35 ± 2°C / 18-24H / A	blue to blue-green growth
B.cepacia	ATCC 25415	35 ± 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, a representative sample of all lots of dehydrated Pseudomonas Agar P is tested for the specific performance characteristic (production of pyocyanin) comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of 5 *Pseudomonas* strains are inoculated by smearing the plated medium surface: *P.aeruginosa* ATCC 9027, *P.aeruginosa* ATCC 14207, *P.aeruginosa* ATCC 27853, *P.aeruginosa* ATCC 10299, *P.aeruginosa* clinical isolate, *B.cepacea* ATCC 25415. After incubation at 35 ± 2°C for 18-24 hours aerobically, pyocyanin production is observed and recorded. All *P.aeruginosa* strains show a blue to blue-green colour on the growth diffusing into the medium, while *B.cepacea* grows with white colonies.

13 - LIMITATIONS OF THE METHOD

- The presence of colourless colonies does not completely exclude the presence of *P.aeruginosa*.[6]
- Mucoid isolates of *P.aeruginosa* from cystic fibrosis patients may undergo several phenotypic changes including the loss of pigment production.[7]
- Occasionally a *Pseudomonas* strains will produce small quantities of fluorescein which, normally, should be inhibited on resulting in a blue-green colour on the medium.[6]
- Pseudomonas Agar P 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 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 diseases; 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
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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 Med1954;44:301-7.
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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.