

Specification

Solid culture medium for selective isolation of *Pseudomonas aeruginosa* according to the Pharmacopeial Harmonised Method and the ISO standard.

Presentation

20 Prepared Plates
 90 mm
 with: 21 ± 2 ml

Packaging Details

1 box with 2 packs of 10 plates/pack. Single cellophane.

Shelf Life

3 months

Storage

2-14 °C

Composition

Composition (g/l):

Gelatin Peptone.....	20.00
Magnesium chloride.....	1.40
Potassium sulfate.....	10.00
Cetrimide.....	0.30
Agar.....	13.60
Glycerol.....	10.00 ml

Description /Technique

Description

The Cetrimide Agar is based on the resistance of *P. aeruginosa* strains to Quaternary Ammonium Compounds (QAC's). With Cetyltrimethyl-Ammonium Bromide a growth at concentrations of 1g/L has been achieved, but has been very poor and slow.

An inhibitor concentration of 0,3-0,5 g/L does not seem to affect the viability of pyogenic species. But it does inhibit the accompanying bacteria, both Gram positive and Gram negative organisms. Other species of *Pseudomonas* which may develop at lower inhibitory concentrations are also inhibited.

Although *P. aeruginosa* prevails over any other fastidious bacteria after a 48 hour incubation at 30-35°C, an initial incubation at 42°C for 48 hours followed by an incubation at 35°C for 48 hours is recommended. Using this method almost complete inhibition of other microorganisms is obtained.

Technique

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Once solidified on a flat surface, spread the plate streaking methodology or by spiral method.

Incubate the plates right side up aerobically at 30-35 °C for 18-72 h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications,...)

After incubation, enumerate all the colonies that have appeared onto the surface of the agar with a blue-greenish colour (due to pigment production by *Pseudomonas* sp)

Each laboratory must evaluate the results according to their specifications.

Presumptive isolation of *Pseudomonas* sp must be confirmed by further microbiological or biochemical tests.

Quality control

Physical/Chemical control

Color : Off-white / opalescent

pH: 7.2 ± 0.2 at 25°C

Microbiological control

50-100 CFU (productivity)/ 10³-10⁴ CFU (selectivity), according to harmonized pharmacopeial monographs and test methods & ISO 11133:2014/A1:2018

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020

Aerobiosis. Incubation at 30-35°C. Reading at 18h (Productivity) and 72h (Selectivity)

Microorganism

Escherichia coli ATCC® 8739, WDCM 00012

Ps. aeruginosa ATCC® 9027, WDCM 00026

Ps. aeruginosa ATCC® 27853, WDCM 00025

Ps. aeruginosa ATCC® 10145

Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Growth

Inhibited

Good (≥ 50%) Green-yellowish to dark green colonies

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Bibliography

- ATLAS, R.M. and L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton, Fla.
- BROWN, V.I. & J.L. LOWBURY (1965) Use of an improved Cetrimide Agar Medium and of culture methods for *Pseudomonas aeruginosa*. J. Clin. Path. 18.752.
- COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
- EUROPEAN PHARMACOPOEIA 8.0 (2014) 8th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual. 8th ed. Rev. A. AOAC International. Gaithersburg. VA.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 22717 Standard (2015) Cosmetics - Microbiology - Detection of *Pseudomonas aeruginosa*.
- LOWBURY, E.J.L. & A.G. COLLINS (1955) The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa* J. Clin. Path. 8.47.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.