

Instructions for Use

REF - PP2230

Cetrimide Agar (USP)

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use. PP2230 Cetrimide Agar (USP) is a selective medium for the isolation and detection of *Pseudomonas aeruginosa* from, clinical, pharmaceutical and cosmetic samples ⁽¹⁾.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Pancreatic digest of gelatine	20.0
Cetrimide	0.3
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar	13.6
Glycerol	10.0ml

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Developed by Lowbury in 1951 ⁽¹⁾ as a selective medium for *P. aeruginosa*, the formulation is compliant with the requirements of the Harmonised EP/USP/JP ^(2 3 4).

4. Principle

Detection is achieved using the unique ability of *P. aeruginosa* to synthesise the iron chelating pigments pyoverdine and pyocyanin which combine to produce the characteristic green colonies of *Pseudomonas aeruginosa* ⁽⁵⁾. Production of these pigments is stimulated by the presence of magnesium and potassium ions in the medium. Glycerol (10ml/l) serves as an energy source and pancreatic digest of gelatine supplies the required nitrogen and other essential nutrients. Cetrimide, a quaternary ammonium bromide compound, is present to suppress the growth of other *Pseudomonas* species as well as Gram-negative and Gram-positive organisms ⁽⁶⁾.

5. Physical Characteristics

	Medium
Appearance and Colour	Straw firm gel
pH	7.2 ± 0.2

6. Materials Provided

PP2230 is supplied in packs of 10 x 90mm P090 Petri dishes with an 18-22ml fill (product code: PP2230-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP2230 Cetrimide Agar (USP) is suitable for the testing of the following specimens:

- Cosmetics: liquid, solid, or gel cosmetics
- Clinical: swabs from infected areas, samples of blood or urine

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation of the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Clinical Specimens:

Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 35-37°C for 16-48 hours ⁽⁷⁾.

Non-sterile Pharmaceutical samples:

Prepare a homogenised sample suspension using a ratio of 1:10 of sample (not less than 1g) to Tryptone Soya Broth (E&O BM0432). Incubate at 30-35°C for 18-24 hours.

Subculture pre-enriched sample directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 30-35°C for 18-72 hours.

Cosmetic Specimens:

For solid or viscous samples, prepare a homogenised suspension using a ratio of 1:10 of sample to Lethen Broth Modified (E&O BM4284). With homogenised and liquid specimens, media should be inoculated with 1ml of the specimen. Incubate at 30-35°C for 18-72 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>P. aeruginosa</i> (NCTC 12903)	30-35°C for 72 hours	Green pigmented colonies. Fluorescence = +ve (Greenish white colour)
<i>P. aeruginosa</i> (ATCC 9027)	30-35°C for 72 hours	Green pigmented colonies. Fluorescence = +ve (Greenish white colour)
<i>E. coli</i> (NCTC 12923)	30-35°C for 72 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify PP2230 Cetrimide Agar (USP) performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4 - 10^5 cfu for the non-target organisms then incubated aerobically at 30-35°C for 72 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Pseudomonas aeruginosa* (NCTC 12903) and *Pseudomonas aeruginosa* (ATCC 9027) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12923). Therefore, it can be concluded that PP2230 Cetrimide Agar (USP), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Rarely, some enteric bacterial species may produce a slight yellowing of the medium. This colouration is easily distinguished due to the pale colour and lack of fluorescence by enteric colonies.
- Some non-fermenters and some aerobic spore formers may exhibit a tan to brown pigmentation on this medium.
- *Serratia* strains may exhibit a pink pigmentation.
- *Pseudomonas aeruginosa* colonies may lose fluorescence when cultures are kept at room temperature for a brief time. Fluorescence reappears when plates are re-warmed.
- Additional biochemical, molecular, or mass spectroscopy tests should be performed to confirm identity.
- Due to natural variation, some strains may grow poorly on this medium.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to the E&O Non-hazardous Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP2230 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date, cracked or a precipitate is present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Lowbury E. (1951). Improved Culture Methods for the Detection of *Ps. pyocyanea*. J Clin Pathol., 4(1), pp.66-72.
2. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
3. United States Pharmacopeial Convention (2019) The United States Pharmacopoeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
4. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.
5. King, E., Ward, M. and Raney, D. (1954) Two simple media for the demonstration of pyocyanin and fluorescein. The Journal of

laboratory and clinical medicine, 44(2), pp.301-307.

6. Lowbury, E. and Collins, A. (1955). The use of a new cetrimide product in a selective medium for *Pseudomonas pyocyanea*. J Clin Pathol., 8(1), pp.47-48.

7. Public Health England (2015) UK Standards for Microbiology Investigations: Identification of *Pseudomonas* species and other Non-Glucose Fermenters. SMI ID 17 (Issue 3).

Version History*

001 14/08/23 - New Document Created

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



E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com



Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D15PY00

TABLE OF APPLICABLE SYMBOLS

IFU/PP2230 REV. 001

 REF o REF Catalogue number	 LOT Batch code	 IVD <i>In vitro</i> 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