



BBL™ Pseudosel™ Agar (Cetrimide Agar)

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Pseudosel™ Agar (Cetrimide Agar) is used for the selective isolation and presumptive identification of *Pseudomonas aeruginosa* from clinical and nonclinical specimens.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopeia (EP)* and *Japanese Pharmacopeia (JP)* performance specifications, where applicable.¹⁻³

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.

- For non-USP organisms, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield $10^6 - 10^7$ CFU/mL. Incubate at $36 \pm 1^\circ\text{C}$ for 18 – 48 h (72 h for *S. maltophilia*) under appropriate atmospheric conditions.
- For *E. coli* ATCC 8739, spread inoculate 100 – 1000 CFU from a 4 – 5 h culture of **Trypticase** Soy Broth. Incubate at $30 - 35^\circ\text{C}$ for 72 h under appropriate atmospheric conditions.
- For *P. aeruginosa* ATCC 9027, spread inoculate 10 – 100 CFU from a 4 – 5 h culture of **Trypticase** Soy Broth using Phosphate Buffer as a diluent. Incubate at $30 - 35^\circ\text{C}$ for 18 h under appropriate atmospheric conditions.
- Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for all organisms.

2. Examine plates at time intervals specified above for growth, colony color, agar pigmentation and fluorescence.

3. Expected Results

For clinical and other non-USP/EP/JP applications:

Organisms	ATCC™	Recovery	Colony Color	Fluorescence
<i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A	N/A
* <i>Pseudomonas aeruginosa</i>	10145	Moderate to heavy growth	Blue-green pigmentation	Yes†
<i>Pseudomonas aeruginosa</i>	27853	Moderate to heavy growth	Blue-green pigmentation	Yes†
* <i>Stenotrophomonas maltophilia</i>	13637	Complete inhibition	N/A	N/A

*Recommended organism strain for User Quality Control.

†Examine colonies with a long-wave (365 nm) UV lamp for fluorescence.

For USP/EP/JP applications:*

Organisms	ATCC™	Recovery	Colony Color	Fluorescence
<i>Escherichia coli</i>	8739	Inhibited	N/A	N/A
<i>Pseudomonas aeruginosa</i>	9027	Growth	Blue-green pigmentation	Yes†

*For USP/EP/JP applications, inoculum level and incubation conditions are prescribed.

†Examine colonies with a long-wave (365 nm) UV lamp for fluorescence.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Pseudosel Agar (Cetrimide Agar) is used for the selective isolation and presumptive identification of *Pseudomonas aeruginosa* from clinical and nonclinical specimens.

Meets USP/EP/JP performance specifications, where applicable.¹⁻³

V SUMMARY AND EXPLANATION

King et al. developed Medium A (Tech Agar) for the enhancement of pyocyanin production by *Pseudomonas*.⁴ Cetrimide (**Pseudosel**) Agar has the formula for Tech Agar but is modified by the addition of cetrimide (cetyl trimethyl ammonium bromide) for the selective inhibition of organisms other than *P. aeruginosa*.⁵

In 1951, Lowbury described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*.⁵ Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.⁶ Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.⁷

Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology⁸ and the characteristic grape-like odor of aminoacetophenone.⁹ *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide (**Pseudosel**) Agar, therefore, is a valuable culture medium in the identification of this organism.

In addition to the promotion of pyocyanin production, **Pseudosel** Agar also enables the detection of fluorescent products produced by *P. aeruginosa*.

Cetrimide (**Pseudosel**) Agar is widely recommended for use in the examination of cosmetics,¹⁰ clinical specimens^{8,11} for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.¹² It is also used in the microbiological examination of nonsterile pharmaceutical products for *Pseudomonas aeruginosa*.¹

VI PRINCIPLES OF THE PROCEDURE

Gelatin peptone supplies the nutrients necessary to support growth. The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium.¹³ Cetrimide is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*. Agar is a solidifying agent. Glycerol is a source of carbon.

VII REAGENTS

Pseudosel™ Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	20.0 g	Agar	13.6 g
Potassium Sulfate	10.0 g	Cetrimide	0.3 g
Magnesium Chloride	1.4 g	Glycerol	10.0 mL

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

For clinical specimens, refer to appropriate texts for details of specimen collection and handling procedures.^{11,14}

For industrial samples, follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹⁵⁻¹⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

IX PROCEDURE

Material Provided: Pseudosel Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface. Streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35 ± 2°C for 18 – 48 h.

Refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and tests for isolating *P. aeruginosa* using Pseudosel (Cetrimide) Agar.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After 18 – 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Colonies that are surrounded by a blue-green to green pigment and fluoresce under short wavelength (254 nm) ultraviolet light may be presumptively identified as *Pseudomonas aeruginosa*. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light. Most non-*Pseudomonas* species are inhibited, and some species of *Pseudomonas* may also be inhibited. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.^{9,11,19,20}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Cultures of specimens grown on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII PERFORMANCE CHARACTERISTICS

Lambe and Stewart performed a study comparing BBL Pseudosel Agar with Technicolor Agar for detection of pyocyanin (blue-green) and pyorubin (red-brown) pigments by strains of *P. aeruginosa*.²¹

Three hundred and four (304) strains of *P. aeruginosa* and 512 strains of other gram-negative bacilli (including 262 strains of *Pseudomonas* sp.) isolated from clinical specimens were chosen at random and inoculated to the test media. Sixty-three percent (190/304) of *P. aeruginosa* produced pyocyanin, pyorubin, or both pigments on Technicolor Agar, but 81% (246/304) produced

one or both pigments on Pseudosel Agar. Therefore, Pseudosel Agar was a more satisfactory medium to detect the production of pyocyanin, pyorubin or both, than Technicolor Agar.²¹ Strains (512) of gram-negative bacilli other than *P. aeruginosa*, failed to produce pyocyanin or pyorubin.²¹

XIII AVAILABILITY

Cat. No.	Description
297882	BBL™ Pseudosel™ Agar, Pkg. of 10 plates

XIV REFERENCES

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