



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Pseudosel™ Agar is a medium for the isolation and identification of *Pseudomonas aeruginosa*.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
  - a. Inoculate the slant surfaces with a 0.01 mL calibrated loop using 10<sup>-1</sup> dilutions of 18- to 24-h Trypticase™ Soy Broth cultures.
  - b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
  - c. Include Trypticase Soy Agar Slants as nonselective controls for all organisms.
2. Examine tubes after 18–24 and 42–48 h for growth, selectivity, pigmentation and fluorescence. Use a Wood's lamp for determination of fluorescent growth.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Pseudomonas aeruginosa</i>	10145	Growth with blue-green pigment due to pyocyanin production and fluorescence
* <i>Stenotrophomonas maltophilia</i>	13637	Complete inhibition

\*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2.
4. Incubate uninoculated representative tubes aerobically at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Pseudosel Agar is used for the selective isolation and identification of *Pseudomonas aeruginosa*.

V SUMMARY AND EXPLANATION

King et al. devised a medium known as Tech Agar for the enhancement of pyocyanin production by *Pseudomonas*.<sup>1</sup> BBL 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*.

In 1951, Lowbury described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*.<sup>2</sup> Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.<sup>3</sup> Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 h of incubation.<sup>4</sup>

Strains of *Pseudomonas aeruginosa* are identified from clinical specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology<sup>5</sup> and the characteristic grapelike odor of aminoacetophenone.<sup>6</sup> *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. 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*.

VI PRINCIPLES OF THE PROCEDURE

The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium.<sup>7</sup> Cetrimide is a quaternary ammonium compound which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*.

VII REAGENTS

Pseudosel™ Agar

Approximate Formula\* Per Liter Purified Water

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

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

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

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>8-11</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

## VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>12,13</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Pseudosel Agar Slants

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

**Test Procedure:** Observe aseptic techniques.

Inoculate tubes of Pseudosel Agar with either pure cultures or with specimen material. Incubate tubes at 35 ± 2°C for 18–24 h in an aerobic atmosphere.

**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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed in the central portion of the agar mass in solid media.

## X RESULTS

Examine tubes for the presence of characteristic blue-green pigmentation surrounding the growth and for fluorescence under short wavelength (254 nm) ultraviolet light. The presence of pyocyanin can be confirmed by extracting it with chloroform.<sup>7</sup> *P. aeruginosa* strains typically produce both pyocyanin and fluorescein.

## XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>12-17</sup>

## XII AVAILABILITY

Cat. No.	Description
221344	BBL™ Pseudosel™ Agar Slants, Pkg. of 10 size K tubes
221345	BBL™ Pseudosel™ Agar Slants, Ctn. of 100 size K tubes

## XIII REFERENCES

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