I INTRODUCTION
PC Agar is a selective medium for the isolation and detection of *Burkholderia* (formerly *Pseudomonas*) *cepacia* from clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation using 5-h BD *Trypticase™* Soy Broth cultures diluted to yield $10^3$-$10^4$ CFUs/plate for *B. cepacia* and $10^4$-$10^5$ CFUs/plate for the remaining organisms.
   b. Incubate plates at 30–35 °C in an aerobic atmosphere.
   c. Include BD *Trypticase* Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 48-72 h for amount of growth, reaction and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>25416</td>
<td>Growth with medium surrounding colonies becoming pink to pink-red</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>27853</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>Inhibition (partial to complete)</td>
</tr>
</tbody>
</table>

Additional Organism

| Pseudomonas aeruginosa | 10145 | Inhibition (partial to complete) |

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3. However, monitoring of exempt media used for recovery of *B. cepacia* is strongly recommended.

III ADDITIONAL QUALITY CONTROL
1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates 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 7.0 ± 0.2.
4. Note the firmness of the plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE
PC Agar is used in the selective isolation and detection of *Burkholderia* (formerly *Pseudomonas*) *cepacia* from clinical and nonclinical specimens.

V SUMMARY AND EXPLANATION
*Burkholderia cepacia* is an opportunistic pathogen generally associated with nosocomial infections. Studies indicate that *B. cepacia* may be an important pulmonary pathogen for patients with cystic fibrosis (CF). The incidence of this organism in the respiratory tract of CF patients is often accompanied by rapid deterioration in pulmonary status and death. Recovery of this organism on commonly-used media, such as blood agar or MacConkey Agar, is difficult because common isolates, such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, overgrow the slower-growing colonies of *B. cepacia* and mask its presence. Gilligan et al. developed PC Agar for improved recovery of *B. cepacia*. Crystal violet, bile salts and two antimicrobial agents are used as selective agents. Phenol red facilitates detection of *B. cepacia* by a color change in the medium. They reported isolating *B. cepacia* on PC Agar from respiratory secretions of 35 CF patients, but isolated the organism from only 21 patients on MacConkey Agar.

VI PRINCIPLES OF THE PROCEDURE
This medium provides a variety of enzymatic digests of proteinaceous substrates, inorganic salts and other nutrients to satisfy the nutritional requirements of these organisms. In addition, selective agents are incorporated to improve the recovery of *B. cepacia* by inhibiting common contaminants. For example, crystal violet inhibits gram-positive cocci, especially enterococci and staphylococci; bile salts inhibit most gram-positive cocci other than enterococci; and ticarcillin and polymyxin B inhibit gram-negative bacilli. PC Agar contains the pH indicator phenol red to facilitate detection of *B. cepacia*. Alkaline end products from the metabolism of pyruvate raise the pH of the medium, causing the color of the indicator to change from light orange to pink or pink-red in the area of growth. In areas of heavy growth of *B. cepacia*, the pink color intensifies.
VIII REAGENTS

PC Agar
Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Material Provided</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic Digest of Animal Tissue</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Ammonium Sulfate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Ferrous Ammonium Sulfate</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Bile Salts</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>4.3 g</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>2.1 g</td>
</tr>
<tr>
<td>Sodium Pyruvate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>20.0 mg</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>100.0 mg</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>300,000.0 U</td>
</tr>
</tbody>
</table>

*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.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions” and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

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
Specimens include bronchoalveolar lavage fluid (preferred, but difficult to obtain), sputum, nasolaryngeal aspirates and oropharyngeal swabs. Refer to appropriate texts for details of specimen collection and handling procedures. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material 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. Inoculate by streaking the specimen over the medium with a sterile inoculating loop.

Incubate the plates in an inverted position (agar-side up) at 30–35 °C for a minimum of 4 days to allow sufficient time for colony development and for the color of the indicator to change.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

X RESULTS

The plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Typical colonies of B. cepacia on PC Agar are grayish-white with a pink to pink-red zone in the surrounding medium. As the colonies age, they may become purplish.

XI LIMITATIONS OF THE PROCEDURE

Organisms other than B. cepacia may also grow on PC Agar and produce alkaline end products that cause the medium to become pink. Therefore, this medium should not be used as the sole method of identification of B. cepacia.

For identification, the organism 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.

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

XII AVAILABILITY

Cat. No. Description
297755 BD BBL™ PC Agar, Pkg. of 20 plates

XIII REFERENCES


Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.