BD BBL™ Prepared Plated Media for Isolation of *Burkholderia (Pseudomonas) cepacia*

OFPBL Agar

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INTENDED USE

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

SUMMARY AND EXPLANATION

Burkholderia cepacia is an opportunistic pathogen generally associated with nosocomial infections.[1] Studies indicate that *B. cepacia* may be an important pulmonary pathogen for patients with cystic fibrosis (CF).[2] The incidence of this organism in the respiratory tract of CF patients is often accompanied by rapid deterioration in pulmonary status and death.[3]

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*.[2] Crystal violet, bile salts and two antimicrobial agents are used as selective agents. 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.[2]

Welch et al. developed a differential but less selective medium for the recovery of *B. cepacia*.[4,5] This medium, OFPBL Agar, is OF (oxidation-fermentation) basal medium supplemented with polymyxin B, bacitracin, lactose and agar. The indicator, bromthymol blue, aids in the detection of *B. cepacia* isolates through a color change in the medium. These investigators reported isolating *B. cepacia* on OFPBL Agar from 58 CF patients, while only isolating this organism from 19 patients on MacConkey Agar.[4]

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, polymyxin B inhibits gram-negative flora, while bacitracin inhibits the gram-positive organisms and Neisseria.[4]

OFPBL Agar contains the pH indicator bromthymol blue to facilitate detection of *B. cepacia*. Acid end products from the metabolism of lactose lower the pH of the medium resulting in a yellow color change. *B. cepacia* colonies will also have a yellow color.

REAGENTS

Formula

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	2.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	0.3	g
Agar	15.0	g
Bromthymol Blue	0.03	g
Lactose	10.0	g
Bacitracin	500	U
Polymyxin B	300,000	U

*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"[6–9] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared plates, specimen containers and other contaminated materials by autoclaving.

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.

SPECIMEN COLLECTION AND HANDLING

Specimens include bronchoalveolar lavage fluid (preferred, but difficult to obtain), sputum, nasolaryngeal aspirates and oropharyngeal swabs.[10]

Refer to appropriate texts for details of specimen collection and handling procedures.[11,12]

PROCEDURE

Material Provided: OFPBL Agar

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

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.[10,13]

User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

TEST STRAIN	EXPECTED RESULT
Burkholderia cepacia ATCC® 25416	Growth; yellow colonies with yellow zones.
Stenotrophomonas maltophilia ATCC 13637	No growth to trace growth.
Staphylococcus aureus ATCC 25923	No growth to trace growth.

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.

RESULTS

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

Typical colonies of *B. cepacia* on OFPBL Agar are yellow with yellow zones in the surrounding medium.

LIMITATIONS OF THE PROCEDURE

Other organisms, e.g., *B. gladioli*, may grow on OFPBL Agar and resemble *B. cepacia* (yellow colonies). Therefore, this medium should not be used as the sole method of identification of *B. cepacia*.[14]

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.[1,15]

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.

PERFORMANCE CHARACTERISTICS

B. cepacia was isolated on OFPBL Agar from 58 (8%) of 725 respiratory specimens collected from 428 cystic fibrosis patients. Most of the isolates were detected by 48 hours of incubation.[4]

AVAILABILITY

Cat. No.	Description
299970	**BD BBL™** OFPBL Agar

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