OFPBL Agar Base (Oxidation Fermentation Polymyxin Bacitracin Lactose Agar Base)

M1811

OFPBL Agar Base (Oxidation Fermentation Polymyxin Bacitracin Lactose Agar Base) when supplemented with Polymyxin and Bacitracin is recommended for the selective isolation of *Burkholderia cepacia* from clinical specimens as well as non-clinical samples.

Composition**

Ingredients	Gms / Litre
Casein Enzyme Hydrolysate	2.000
Dipotassium hydrogen phosphate	0.300
Sodium chloride	5.000
Lactose	10.000
Bromothymol blue	0.030
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.33 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C Aseptically add rehydrated contents of one vial of OFPBL Selective Supplement (FD269). Mix well and pour into sterile Petri plates or as desired.

Principle And Interpretation

Burkholderia cepacia is an opportunistic pathogen generally associated with nosocomial infections. Due to its ability to survive for extended period of time in hostile environments, it is found in such widely varied and inhibitory items such as equipment, medications, mouthwash and disinfectants. Nosocomial infections caused by this organism include bacteremia, urinary infections, and respiratory infections. However, the most serious implication is when identified in patients with Cystic Fibrosis (CF) Patients with cystic fibrosis (CF) have a predisposition for infection and infected patients, if untreated, show a rapid decline in lung function, frequent bacteremia, and death due to lung failure. It is also reported to be a primary cause of bacteremia, pneumonia, and death in the Chronic granulomatous disease CGD patient population (1,2). Therefore, it is critical that isolation and proper identification be fast and accurate. Burkholderia Cepacia Agar (M1640) as well as OFPBL Agar (M1811) is recommended for isolation of *Burkholderia cepacia* from clinical specimens (1,3,4).

OFPBL medium is a modified version of OF (Oxidation Fermentation) basal medium developed by a group of researchers, Welch et al. in 1987 (5). They demonstrated that OFPBL agar resulted in improved recovery and isolation of *Burkholderia cepacia* when compared to other selective mediums such as MacConkey agar, XLD agar and various blood blood agars. OFPBL Agar contains the casein enzyme hydrolysate which provides necessary nitrogeneous compounds and lactose serves as carbohydrate source. Lactose is readily utilized by *Burkholderia cepacia* . The fermentation of lactose results in the release of acid end-products which is detected by the pH indicator, bromothymol blue, present in the medium. When sufficient acid is produced the medium changes from green to yellow providing the colonies their yellow coloration. Dipotassium hydrogen phosphate in the medium buffers the medium well. Sodium chloride helps to maintain osmotic balance. The selectivity of the medium owes itself to the presence of the antibiotics polymixin B and bacitracin together these antibiotics provide good suppression of the bacterial flora present in respiratory secretions and sputum (for the inhibition of gram-positive organisms and *Neisseria*) (5).

Appropriate patient samples for testing include sputum, bronchial washings, and pharyngeal swabs. Using a direct or diluted inoculum from the sample, a four-quadrant streak may be performed to obtain well-isolated colonies. If the sample is collected

on a swab, the swab may be rolled over a small area near the edge of the plate and further streaked for isolation using a sterile loop.

It is recommended to incubate at 30-35°C and examine the plates daily upto five days. before discarding. Generally colonies develop within 48-72 hours.

Typically, *Burkholderia cepacia* colonies appear as yellow colonies with yellow halos. Most strains will grow in 48 hours but some strains may require up to 5 days for the color development. Most other microorganisms are inhibited on this medium. Other similar bacteria such as *Pseudomonas aeruginosa* may also grow as yellow colonies and further testing must be performed to differentiate these bacteria from *Burkholderia cepacia. Burkholderia gladioli* which has been shown to occur in respiratory tract specimens of CF patients will grow on OFPBL Agar and may resemble *B.cepacia* (6)

Any growth is considered a positive result and additional biochemical and/or serological tests should be performed on isolated colonies from pure culture (1). The yellowing of the medium signifies carbohydrate fermentation and some rare, atypical strains of *Burkholderia cepacia* may not produce this reaction

Quality Control

Appearance
Yellow to yellowish green coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Green coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.23% w/v aqueous solution at 25°C. pH : 6.8±0.2

Cultural Response
M1811: Cultural characteristics observed with added OFPBL Selective Supplement (FD269), after an incubation at 30-35°C for 48 - 72 hours. Examine upto five days.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Burkholderia cepacia ATCC 25416	50-100	good-luxuriant	>=50%	yellow w/ yellow halo
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2- 8°C. Use before expiry date on the label.

Reference

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5.Welch DF, Muszynski M. J, Pai CH, Marcon MJ, Hribar MM, Gilligan PH, Matsen JM, Ahlin PA, Hilman BC, Chartrand SA. 1987. J. Clin.Microbiol; 25:1730-4.
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