# Burkholderia Cepacia Selective Supplement

## PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

G006-1g - Gentamicin Sulfate, Powder, 1g

G006-5g - Gentamicin Sulfate, Powder, 5g

G006-25g - Gentamicin Sulfate, Powder, 25g

G035-10mg - Gentamicin A Sulfate, EvoPure™, 10mg

G031-10mg - Gentamicin C1 Sulfate, EvoPure<sup>™</sup>, 10mg

G032-10mg - Gentamicin C1a Sulfate, EvoPure™, 10mg

G033-10mg - Gentamicin C2 Sulfate, EvoPure<sup>™</sup>, 10mg

G034-10mg - Gentamicin C2a Sulfate, EvoPure™, 10mg

T027-1g - Ticarcillin Disodium, Powder, 1g

## **DESCRIPTION**

Burkholderia Cepacia Agar Base with Burkholderia Cepacia Selective Supplement is a medium for the selective isolation of *Burkholderia cepacia* from the respiratory secretions of patients with cystic fibrosis and for routine testing of non-sterile inorganic salt solutions containing preservative.

### **BACKGROUND**

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa*. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Gentamicin is an aminoglycoside antibiotic which is synthesized by *Micromonospora*, a genus of Grampositive bacteria widely present in the environment (water and soil). Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving,

which makes it particularly useful in the preparation of some microbiological growth media.

Ticarcillin is a carboxypenicillin. It is almost invariably sold and used in combination with clavulanate as Timentin. Because it is a penicillin, it also falls within the larger class of beta-lactam antibiotics.

#### Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis

Ticarcillin's antibiotic properties arise from its ability to prevent cross-linking of peptidoglycan during cell wall synthesis, when the bacteria tries to divide, causing cell death.

## APPLICATION IN BURK-HOLDERIA CEPACIA AGAR BASE

Burkholderia cepacia (formerly known as Pseudomonas cepacia) is a motile aerobic oxidase positive Gramnegative bacillus commonly found in liquid reservoirs and moist environments. The cells are 0.5 to 1.0 mm wide and 5 mm in length. It is an important opportunistic pathogen and causes pulmonary infection among individuals with cystic fibrosis (CF). Isolates from CF patients often display multidrug resistance and as many as 20% of colonised individuals will succumb to Burkholderia cepacia syndrome, a necrotizing pneumonia associated with fever that culminates into a rapid and fatal clinical deterioration. Burkholderia cepacia Agar is based on PC Medium originally devised by Gilligan et al. where it was shown to be superior for growth of Burkholderia cepacia after 48 hours when compared to MacConkey.

Originally isolated from onions, *Burkholderia cepacia* can survive for long periods and multiply in hostile environments such as antiseptic and disinfectant solutions, distilled water, whirlpool baths, nebulizers and commercially packaged urinary catheter kits. An outbreak in Arizona in 1998 due to contaminated alcohol-free mouthwash, was investigated by the Food and Drug

Administration (FDA), who suggested an association with the deionisation procedure of the water used to prepare the product. The organism may be present in low numbers in many non-sterile products used in hospitals. It has been isolated from various water sources and can grow in distilled water with a nitrogen source due to its ability to fix carbon dioxide from air. Suction catheters rinsed in acetic acid solution have reduced incidence of transmission of *Burkholderia cepacia* and other pseudomonads.

The slower growing *Burkholderia cepacia* can be missed on conventional media such as blood or MacConkey Agar due to overgrowth caused by other faster growing organisms found in the respiratory tract of CF patients such as mucoid *Klebsiella* species, *Pseudomonas aeruginosa* and *Staphylococcus* species. This may lead to the infection being missed or wrongly diagnosed.

#### **Content concentrations**

Typical Formula*	mg/litre
Burkholderia Cepacia Agar Base	
Peptone	5
Yeast Extract	4
Sodium pyruvate	7
Potassium dihydrogen phosphate	4.4
Disodium hydrogen phosphate	1.4
Bile salts	1.5
Ammonium sulphate	1
Magnesium sulphate	0.2
Ammonium ferrous sulphate	0.01
Phenol red	0.02
Crystal violet	0.001
Agar	12
Final pH 6.2 ± 0.2 @ 25°C	
Burkholderia Cepacia Selective Supplement	
Polymixin B	150,000 IU
<u>Gentamicin</u>	5.0
<u>Ticarcillin</u>	100.0
* Adjusted as required to meet performance standards	

Table 1 typical formula for Burkholderia Cepacia Agar Base and Burkholderia Cepacia Selective Supplement

#### **METHOD**

#### **Preparation**

Suspend appropriate amount of Burkholderia cepacia Agar Base in distilled water, mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of Burkholderia cepacia Selective Supplement, reconstituted as directed. Mix well and distribute into sterile Petri dishes.

#### **Protocol**

- 1. Take a routine respiratory sample from the patient e.g. sputa, deep pharyngeal swabs or bronchial washings.
- 2. Dilute the sample, if necessary, in Ringer's solution to give a 1:2 dilution. Streak onto Burkholderia cepacia Medium and incubate at 37°C for 48 to 72 hours.
- 3. Examine after 48 hours for sage green colonies and the medium turning from straw-green to bright pink.
- 4. All colonies should be further identified and confirmed. Re-incubate for a further 24 hours if necessary.
- 5. Typical colonies of *Burkholderia cepacia* are circular, and entire. Colour formation is based on natural pigment expression and colonies vary from grey to sage green, with the medium changing from orange to bright pink.

## **Quality control**

Positive control:

*Burkholderia cepacia* ATCC° 25608: Good growth, grey colonies with bright pink medium. Low numbers of colonies may not produce a colour change of the medium.

*Burkholderia cepacia* ATCC\* 25416: Good growth, sage green colonies with bright pink medium. Low numbers of colonies may not produce a colour change of the medium.

Negative control:

Pseudomonas aeruginosa ATCC® 27853: Inhibited

#### REFERENCES

- 1. Whitby, P. W. (1998) Journal of Clinical Microbiology 36: 1642-1645
- 2. Gilligan, P. H. et al. (1985) Journal of Clinical Microbiology 22: No.1 5-8.
- 3. Geftic, S. G., Heymann, H. and Adair, F. W. (1979) Applied and Environmental Microbiology 37: 505-510
- 4. Matrician, L. (1998) Virtual Hospital: Morbidity and Mortality Weekly Report Volume 47: No. 43
- 5. Koneman, E. W. et al. (1997) Color Atlas and Textbook of Diagnostic Microbiology Fifth Ed.: 269-272