

Burkholderia Cepacia HiCynth™ Agar Base

MCD1640

Intended use

Recommended as a selective medium used for isolation of *Burkholderia cepacia* from the respiratory secretions of patients with cystic fibrosis and other non-clinical specimens

Composition**

Ingredients	g / L
HiCynth™ peptone No.1#	5.000
HiCynth™ peptone No.5#	4.000
Sodium pyruvate	7.000
Potassium dihydrogen phosphate	4.400
Disodium hydrogen phosphate	1.400
Synthetic detergent 1	1.500
Ammonium sulphate	1.000
Magnesium sulphate	0.200
Ammonium ferrous sulphate	0.010
Phenol red	0.020
Crystal violet	0.001
Agar	12.000
Final pH (at 25°C)	6.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Chemically defined peptones

Directions

Suspend 18.26 gram in 500 ml purified / 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 and aseptically add the rehydrated contents of 1 vial of PGT Selective Supplement (FD232). Mix well and pour in sterile Petri plates.

Principle And Interpretation

Burkholderia cepacia is an important opportunistic pathogen and causes pulmonary infection among individuals with cystic fibrosis (CF). The organism may lead to *Burkholderia cepacia* syndrome, a neutralizing pneumonia associated with fever that culminates in to a rapid and fatal clinical deterioration (1). *B.cepacia* is difficult to isolate on routinely used laboratory media like MacConkey Agar, since *B.cepacia* is a slow grower and therefore it is usually outgrown by the faster growing *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Burkholderia Cepacia Agar is based on PC medium, which was originally devised by Gilligan (2). This medium was found to be superior to MacConkey Agar for growth of *B.cepacia*. Burkholderia Cepacia HiCynth™ Agar Base is similar to Burkholderia Cepacia Agar Base wherein the animal based peptones are completely replaced with chemically defined peptones to avoid BSE / TSE risks associated with animal peptones. The medium is made selective for *B.cepacia* by the incorporation of synthetic detergent No.1, crystal violet and antibiotics in supplement (FD232) provides additional selectivity.

HiCynth™ peptone No. 1 and HiCynth™ peptone No.5 in the medium provides the nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B source and other essential nutrients. Crystal violet, synthetic detergent No. 1 and antimicrobial agents are used as selective agents. Crystal violet and ynthetic detergent No. 1 inhibits gram-positive cocci including Enterococci and Staphylococci. The antibiotics (FD) namely ticarcillin, polymyxin B and gentamycin inhibit gram-negative bacteria. *B.cepacia* metabolizes pyruvate forming alkaline end products. These end products elevate the pH of the medium. The phenol red indicator changes colour from pink orange to pink red in alkaline pH. Inoculate the plate with the specimen so as to obtain isolated colonies. The plates should be incubated for a period of 4 days to allow *B.cepacia* to grow and form colonies and subsequent colour change (3,4). The medium is not selective only for *B.cepacia*. Other organisms forming similar colonies may also grow on this medium. Therefore results obtained on this media should not be the sole criteria for identification of *B.cepacia* (5).

Type of specimen

Clinical samples - Respiratory secretions

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to varying nutritional requirements, some strains may show poor growth.
2. Further serological and biochemical testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period when stored at the recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Orange coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.65% w/v aqueous solution at 25°C. pH : 6.2±0.2

pH

6.00-6.40

Cultural Response

Cultural characteristics observed, with addition of PGT Selective Supplement (FD232), after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Burkholderia cepacia</i> ATCC 25608	50-100	good-luxuriant	≥50%	sage green colonies with bright pink medium
^ <i>Pseudomonas paraeruginosa</i> ATCC 9027 (00026*)	≥10 ⁴	inhibited	0%	

Key : (*) Corresponding WDCM numbers, ^ Formerly known as *Pseudomonas aeruginosa*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Whitby P. W., 1998, J. Clin. Microbiol., 36:1642 1645.
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4. MacDonald Gilligan, Welch, Reller and Menegus, 1994, Vol. 5:1, Cystic Fibrosis Foundation, Washington, D.C.
5. Christensen et al, 1980, J. Clin. Microbiol., 27:270.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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Disclaimer :

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