IFU

Instructions for Use

BURKHOLDERIA CEPACIA SELECTIVE AGAR (BCSA), USP

Cat. no. G09	Burkholderia cepacia Selective Agar (BCSA), USP, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. GA09	Burkholderia cepacia Selective Agar (BCSA), USP, 15x100mm Plate, 18ml (reduced staking ring)	10 plates/bag

INTENDED USE

Hardy Diagnostics Burkholderia cepacia Selective Agar (BCSA), USP is recommended for the selective isolation and differentiation of *Burkholderia cepacia* from sputum samples from cystic fibrosis patients and other clinical specimens. In addition, the medium complies with the U.S. Pharmacopoeia <60> for the *Microbial Examination of Non-sterile Products–Tests for Burkholderia cepacia complex.*[8]

SUMMARY

Burkholderia cepacia Selective Agar (BCSA) was developed by Henry, Campbell, LiPuma, and Speert for the selective isolation of *Burkholderia* (*Pseudomonas*) *cepacia*.[2] *Burkholderia cepacia* is commonly isolated from cystic fibrosis patients. Correct identification of the organism is critical to patient care.[2] It was found that BCSA had a lower false-positivity rate compared to either Oxidation-Fermentation-Polymyxin-Bacitracin-Lactose (OFBPL) Agar or PC (*Pseudomonas cepacia*) Agar. This finding was confirmed later by Henry, Campbell, McGimpsey, Clarke, Louden, Burns, Roe, Vandamme, and Speert.[1]

B. cepacia is a member of a group of at least 18 closely related species in the *B. cepacia* complex (Bcc) group. Bcc species are known to present a significant health risk to immune compromised patients, patients on mechanical ventilation, and those suffering from underlying disease, such as cystic fibrosis. In addition, members of the Bcc group are highly opportunistic and capable of rapidly establishing themselves in water systems, on equipment and surfaces, and within non-sterile water-based products. The group has a reputation for surviving antimicrobial preservative systems and antiseptics, and has been found in multiple-use preserved oral liquids, topical products, and nasal sprays. Members of the *B. cepacia* complex can also form biofilms, making it more difficult to eliminate this group from pharmaceutical water systems.

Hardy Diagnostics BCSA, USP contains peptones and sugars that supply nutrients for the growth of *Burkholderia cepacia* and other microorganisms. Crystal violet is added to inhibit growth of gram-positive organisms. Antimicrobics are incorporated to inhibit organisms other than *Burkholderia cepacia* that may be in the sample. Hardy Diagnostics BCSA, USP is formulated in accordance with the U.S. Pharmacopoeia standard formula for this medium.[8]

FORMULA

Ingredients per liter of deionized water:*

Casein peptone	10.0g

Lactose	10.0g
Sucrose	10.0g
Sodium chloride	5.0g
Yeast extract	1.5g
Phenol red	0.08g
Gemtamicin	10.0mg
Vancomycin	2.5mg
Crystal violet	2.0mg
Polymyxin B	600,000U
Agar	14.0g

Final pH 6.8 +/- 0.3 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

Formulated in accordance with USP <60>.[8]

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Clinical Method of Use:

Specimen Collection: Consult listed references for information on specimen collection.[1-4] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Allow plates to warm to room temperature prior to use. The agar surface should be dry before inoculating.

1. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop.

2. Incubate plates aerobically at 35-37ºC. for 48-72 hours. Protect from light.

3. Examine plates for colony morphology and the medium for a color change.

USP Method of Use:

Performance Testing and Preparation of Test Strains: Use stable standardized suspensions of test strains per reference method. Use appropriate diluent for making test suspensions, such as Buffered Sodium Chloride Peptone Solution (Cat. no. U255) or Phosphate Buffer pH 7.2 (Cat. no. U438) to make the test suspension, and use suspensions within the specified time period or maintain under appropriate storage practices.[8] Allow plates to warm to room temperature prior to use. The agar surface should be dry before inoculating.

1. Prepare a 1:10 dilution of the product using not less than 1.0g of sample. Add 1.0g or 1.0ml of sample to 10ml of Tryptic Soy Broth, USP (Cat. no. K82) as determined by *Method Suitability*.[8] Alternatively, use a suitable volume of TSB, USP corresponding to the amount of sample tested to achieve a 1:10 dilution.

2. Mix the sample and incubate at 30-35ºC for 48-72 hours.

3. Subculture the broth to BCSA and streak for isolation.

4. Incubate plates at 30-35ºC for 48-72 hours. Protect from light.

5. Examine plates for colony morphology and the medium for a color change.

INTERPRETATION OF RESULTS

Burkholderia cepacia colonies are typically translucent and rough. On BCSA, USP the growth of *B. cepacia* will cause a color change in the medium from red-orange to yellow surrounding the colonies. Bcc colonies may also appear as greenish-brown with yellow halos, or as white colonies surrounded by a pink-red zone in the medium. Consult references for further procedures for identification and confirmation of isolates.[3-6,8]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Organisms other than *Burkholderia cepacia* may grow on BCSA.[1] Colonies that grow may require further biochemical testing for complete identification.

Some strains of *Burkholderia cepacia* isolated from patients who have been colonized for a number of years may show minor changes in biochemical reactions. These strains may show good growth but may not change the color of the medium to yellow. Further biochemical testing should be performed on isolates showing good growth but no color change.

Accurate counting may be difficult with spreading colonies.

Rare, fastidious microorganisms may not grow on general non-selective media formulations.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media (Cat. nos. K82, U255, U438), incinerators, incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Burkholderia cepacia ATCC® 25416	J	48-72hr	30-35°C	Aerobic	Growth; yellow colonies with media color change to yellow
Burkholderia cenocepacia ATCC® BAA-245	J	48-72hr	30-35°C	Aerobic	Growth; light purple colonies with media color change to pink
Burkholderia multivorans ATCC® BAA-247	J	48-72hr	30-35°C	Aerobic	Growth; purple colonies with media color change to pink
Pseudomonas aeruginosa ATCC® 9027	B	72hr	30-35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 6538	B	72hr	30-35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

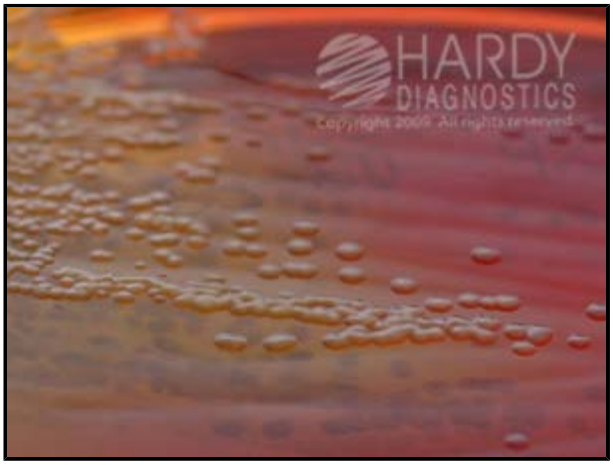
Tested in accordance with USP <60>.[8]

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Burkholderia cepacia Selective Agar (BCSA), USP should appear slightly opalescent, and reddish-orange in color.



Burkholderia (Pseudomonas) cepacia (ATCC® 25416) colonies growing on Burkholderia cepacia Selective Agar, USP (Cat. no. G09). Incubated aerobically for 24 hours at 35ºC.

Pseudomonas aeruginosa (ATCC® 9027) growth inhibited on Burkholderia cepacia Selective Agar, USP (Cat. no. G09). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Henry, D., et. al. 1999. Comparison of Isolation Media for Recovery of *Burkholderia cepacia*Complex from Respiratory Secretions of Patients with Cystic Fibrosis. *J. Clin. Micro.*; 37:1004-1007.

2. Henry, D., et. al. 1997. Identification of *Burkholderia cepacia* Isolates from Patients with Cystic Fibrosis and Use of a Simple New Selective Medium. *J. Clin. Micro.*; 35:614-619.

3. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

4. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I & II. American Society for Microbiology, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22-A2. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

8.*United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

IFU-10049[B]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207H [D]