IFU

Instructions for Use

CRITERION™ BURKHOLDERIA CEPACIA SELECTIVE AGAR (BCSA) BASE

Cat. no. C8880	CRITERION™ Burkholderia cepacia Selective Agar Base	121.2gm
Cat. no. C8881	CRITERION™ Burkholderia cepacia Selective Agar Base	500gm
Cat. no. C8882	CRITERION™ Burkholderia cepacia Selective Agar Base	2kg
Cat. no. C8883	CRITERION™ Burkholderia cepacia Selective Agar Base	10kg
Cat. no. C8884	CRITERION™ Burkholderia cepacia Selective Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Burkholderia cepacia Selective Agar (BCSA) Base is used for the selective isolation of *Burkholderia* (*Pseudomonas*) *cepacia*. It is recommended for the isolation and cultivation of *Burkholderia* (*Pseudomonas*) *cepacia*.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

Burkholderia cepacia Selective Agar (BCSA) was developed by Henry, Campbell, LiPuma, and Speert for the selective isolation of *Burkholderia* (*Pseudomonas*) *cepacia*.[7] *Burkholderia cepacia* is commonly isolated from cystic fibrosis patients. Correct identification of the organism is critical to patient care.[7] 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.[8]

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.

CRITERION™ BCSA Base contains peptones and sugars that supply nutrients for the growth of *Burkholderia cepacia* and other microorganisms. Crystal violet is added to inhibit the growth of gram-positive organisms. Antimicrobics must be added when making the prepared formulation to inhibit organisms other than *Burkholderia cepacia.*

FORMULA*

Gram weight per liter:	60.6gm/L
Casein Peptone	10.0gm
Lactose	10.0gm
Sucrose	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	1.5gm
Phenol Red	0.08gm
Crystal Violet	2.0mg
Agar	14.0gm

Final pH 6.8 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light tan to beige.

Store the prepared culture media at 2-8ºC. away from light.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 60.6gm of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45ºC.

5. Aseptically add 10.0mg gentamicin, 600,000U polymyxin B, and 2.5mg vancomycin.

6. Stir to mix thoroughly and dispense as desired.

General Method of Use:

Specimen Collection: Consult listed references for information on specimen collection.[2,3,7,8] 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 medium 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 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, 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.[9] 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 (e.g. Cat. no. K82) as determined by Method Suitability.[9] 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 30-35◦C for 48-72 hours.

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

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

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

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. no. G09.

Burkholderia cepacia colonies are typically translucent and rough. On BCSA, 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.[2-5,9]

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Organisms other than *Burkholderia cepacia* may grow on BCSA.[(2)] Colonies that grow may require further biochemical testing for complete identification.

Accurate counting may be difficult with spreading colonies.

Rare, fastidious microorganisms may not grow on some 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 antibiotics (gentamicin, polymyxin B, and vancomycin), autoclaves, incinerators, and incubators, other culture media (Cat. nos. K82, U255, U438, G09), etc., 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**	A	24-72hr	35°C	Aerobic	Growth; color change in media from red-orange to yellow
Pseudomonas aeruginosa ATCC® 27853**	B	24hr	35°C	Aerobic	Partial to complete inhibition
Staphylococcus aureus ATCC® 25923**	B	24hr	35°C	Aerobic	Partial to complete inhibition

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

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Burkholderia cepacia Selective Agar (BCSA) Base powder should appear homogeneous, free-flowing, and light tan to beige in color. The prepared media should appear slightly opalescent, and reddish-orange in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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

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

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

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

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Henry, D.A., Campbell, M,E., LiPuma, J.J., Spreert, D.P. 1997. Identification of *Burkholderia cepacia* Isolates from Patients with Cystic Fibrosis and Use of a Simple Selective Medium. *J. Clin. Microbiol.*; 35(3), 614-619. American Society for Microbiology.

8. Henry, D.A., 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.

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

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

HDQA 2207B [D]