IFU

Instructions for Use

HARDYCHROM™ BCC

Cat. no. G335	HardyCHROM™ Bcc, 15x100mm Plate, 22ml	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ Bcc is recommended for the selective isolation and differentiation of *Burkholderia cepacia* and other members of the Bcc group based on colony color. The medium is useful for testing non-sterile, water-based pharmaceuticals. Some ingredients are very light sensitive. Store and incubate media in the dark.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Burkholderia cepacia is an aerobic, Gram-negative, oxidase-positive, rod-shaped bacterium. The organism has been identified by the CDC and other public health agencies as a significant concern relative to antibiotic resistance. In addition, the organism 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.

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. Members of the *B. cepacia* complex can also form biofilms making it more difficult to eliminate this group from pharmaceutical water systems.

Conventional phenotypic identification methods are challenging for this group, because of overlapping biochemical characteristics.[4] Species level identification using a single molecular target does not distinguish Bcc from other related members of this genus. However, recent advancements in genome sequencing using single nucleotide polymorphism (SNP) phylogeny may prove useful.[4]

Due to a surge in recalls for products implicated in Bcc infections, the Food and Drug Administration(FDA) advised drug manufacturers of the risks associated with Bcc contamination in non-sterile, water-based drugs. More recently, the United States Pharmacopeia (USP) established the USP-NF Chapter <60> for the *Microbial Examination of Non-sterile products - Tests for Burkholderia cepacia Complex*, which describes tests to aid in detecting members of the Bcc group in non-sterile products.[1]

HardyCHROM™ Bcc contains peptones, yeast extract, and carbohydrates, which provide nitrogenous compounds, vitamins, sugars, and other nutrients to promote bacterial growth. Selective agents and crystal violet are added to inhibit the growth of Gram-positive organisms, as well as organisms other than members of the Bcc group that may be present in the sample. Chromogenic substrates allow for differentiation of members of the Bcc group due to colony color. Sodium chloride helps to maintain osmotic balance and agar is the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Carbohydrate Mixture	20.0g
Selective Agents	19.5ml
Casein Peptone	10.0g
Sodium Chloride	5.0g
Yeast Extract	1.5g
Chromogenic Mixture	1.07g
Crystal Violet	2.0mg
Agar	14.0g

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store away from direct light at 2-8 ºC. 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 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.

PROCEDURE

Perform membrane filtration or the plate-count method as required. For method suitability, growth promotion, and enumeration see the reference method.[1]

Incubate media using appropriate atmospheric, temperature, and duration conditions as outlined by the test method.

Sample Preparation and Pre-Incubation:

1. Prepare a sample using a 1:10 dilution containing 1.0g or 1.0ml of the product inoculated into 10.0ml of Tryptic Soy Broth, also known as Soybean Casein Digest Broth (e.g. Cat. no. K82).

2. Alternatively, follow method suitability as outlined in USP <60> to create an appropriate dilution of the sample in a suitable volume of Tryptic Soy Broth (TSB).[1]

3. Mix and incubate at 30-35°C for 48-72hr.

Method of Use: The medium should be warmed to room temperature and the surface dry prior to inoculating. Refer to the listed reference for more information on inoculating the medium.[1]

1. Subculture the pre-incubated sample by streaking on a HardyCHROM™ Bcc plate to obtain isolated colonies.

2. Incubate plates inverted in a darkened incubator at 30-35°C for 48-72hr.

3. Observe colonies for chromogenic reaction.

QC Performance Testing and Preparation of Test Strains: Use stable standardized suspensions of test strains per reference method. Use appropriate diluent for making test suspensions and use suspensions within the specified time period or maintain under appropriate storage practices.[1]

INTERPRETATION OF RESULTS

Growth and the formation of pink to purple colonies are indicative of *Burkholderia cepacia* or members of the Bcc group. Growth should be confirmed by identification methods following USP <1113> or alternative reference method.

For environmental monitoring procedures, consult USP<1116> and count the number of colonies: report as the number of colony forming units (CFU).

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.

USP Method Suitability states the ability of the product to detect *Burkholderia cepacia* complex in the presence of the product must be established.[1] It is advisable that a sample preparation be performed to confirm.

If further biochemical or confirmatory testing involves a colorimetric reaction, it is recommended users validate the test method of using a colony direct from HardyCHROM™ Bcc or subculture on isloated colony to a non-selective plate to ensure chromgens from the medium do not interfere with the results of the test.

Accurate counting may be difficult with spreading colonies.

Rare, fastidious strains may fail to grow or grow poorly on this medium.

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 (e.g. Cat. no. K82), 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; pink to purple colonies
Burkholderia cenocepacia ATCC® BAA-245	J	48-72hr	30-35°C	Aerobic	Growth; pink to purple colored colonies
Burkholderia multivorans ATCC® BAA-247	J	48-72hr	30-35°C	Aerobic	Growth; pink to purple colonies
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>.[1]

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

HardyCHROM™ Bcc should appear opaque and white with a purple hue.

REFERENCES

1.USP <60> Microbiological Examination of Nonsterile Products - Tests for Burkholder ia cepacia complex. *United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

2. The Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States. 2013. U.S. Department of Health and Human Services. The Centers for Disease Control and Prevention.

3. Rao, T.V. 2017. Facts on Ten Most Dangerous Antibiotic Resistant Bacteria.

4. Devanga Ragupathi, K. and B. Veeraraghavan. 2019. Accurate Identification and Epidemiological Characterization of Burkholderia cepacia complex: an update. *Ann. Clin Microbiol Antimicrob.* 18:7.

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

HDQA 2207H [D]