

INSTRUCTIONS FOR USE

BCSA

BURKHOLDERIA CEPACIA SELECTIVE AGAR

Ready-to-use plates

1-INTENDED USE

In vitro diagnostic device. Selective and differential medium for the determination of the absence of *Burkholderia cepacia* complex (Bcc), in non-sterile pharmaceutical products according to USP method and for the isolation of Bcc in clinical specimens mainly of respiratory origin.



BCSA: *B.cepacia* strains: on the left lactose/sucrose positive at the right lactose/sucrose negative.

2-COMPOSITION - TYPICAL FORMULATION *

| | |
|-----------------|------------|
| Casein peptone | 10 g |
| Yeast extract | 1,5 g |
| Lactose | 10 g |
| Saccharose | 10 g |
| Sodium chloride | 5 g |
| Phenol red | 0,08 g |
| Crystal violet | 0,002 g |
| Agar | 11,5 g |
| Vancomycin | 0,0025 g |
| Gentamicin | 0,010 g |
| Polymyxin B | 600.000 UI |
| Purified water | 1000 ml |

*the formulation may be adjusted and/or supplemented to meet the required performances criteria.

3-PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Burkholderia cepacia complex (Bcc) is a group of aerobic, Gram-negative, oxidase and catalase positive rods, actually comprising 20 species which are phenotypically nearly indistinguishable and can be sub grouped into nine genomovars¹.

Bcc has high metabolic versatility, wide environmental distribution, and variable virulence; furthermore, members of the *B. cepacia* complex have the ability to form biofilms in pharmaceutical water systems as well as the capability of overcoming antimicrobial preservative systems and being resistant to disinfectants.²

The species of Bcc group are opportunistic pathogens in mechanically ventilated patients, immunosuppressed, infants, the elderly and those with serious underlying disease.² Bcc causes serious infections in patients with cystic fibrosis and chronic granulomatous disease; two Bcc members, *B. cenocepacia* and *B. multivorans*, account for greater than 85% of the cystic fibrosis infections.³

A 2012 survey analyzed the reported recalls from the U.S. market of non sterile pharmaceutical products, cosmetics, medical devices and dietary supplements for microbiologically related issues for a 7-year period: the majority of these recalls (72%) were associated with objectionable microorganisms and the presence of *B.cepacia* represented the most frequent event (34%).⁴

Several recalls of non-sterile pharmaceutical products have also been reported in more recent years.^{5,6}

The FDA was sufficiently concerned in 2017 to issue an advisory notice of the dangers of Bcc contamination of aqueous, non-sterile drug products⁷.

In response to stakeholder requests, a test method for the determination of absence of *Burkholderia cepacia* complex was published in 2019 in the chapter <60> of USP, for defining test procedures and media formulations⁸. Among the various culture media described for the isolation of *B.cepacia*, namely MAST, BCA, OFPBL, BCSA, the choice fell on the latter because of its ability to support the faster growth of Bcc isolates and to suppress other respiratory organisms.^{2,9,10,11}

BCSA Burkholderia Cepacia Selective Agar is prepared according to the formula described by Heny in 1997⁹ and meets the USP <60> requirements⁸.

BCSA contains peptones that supply nutrients for the growth of *Burkholderia cepacia* and other microorganisms; lactose and sucrose are oxidized by the majority of Bcc isolates and the acid end-products result in the medium changing from orange to yellow due to the presence of the pH indicator, phenol red. Crystal violet is added to inhibit growth of Gram-positive organisms; antimicrobials vancomycin, gentamicin and polymyxin B are incorporated to inhibit organisms other than Bcc.

BCSA is intended for the determination of the absence of *Burkholderia cepacia* complex (Bcc), in non-sterile pharmaceutical products according to USP method⁸ and for the isolation of Bcc in clinical specimens mainly of respiratory origin in patients with cystic fibrosis and other respiratory diseases.^{12,13}

4-PHYSICAL CHARACTERISTICS

| | |
|-------------------|--------------------|
| Medium appearance | red-orange, limpid |
| Final pH at 25 °C | 6.8 ± 0.3 |

5-MATERIALS PROVIDED

| Product | Type | REF | Pack |
|--|---------------------|--------|--|
| BCSA Burkholderia Cepacia Selective Agar | Ready-to-use plates | 541153 | 2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box |





6-MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipments as required, ancillary culture media and reagents for the identification of the colonies.

7-SPECIMENS

Pharmaceutical samples: non-sterile products for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use; follow the procedure described by USP for the sample preparation.⁸

Clinical specimens: BCSA agar is used to detect *Burkholderia cepacia* complex from expectorated sputum, deep pharyngeal swab and aspirates, bronchoalveolar lavages. Specimens should be submitted directly to the laboratory without delay. If there is to be a delay in processing, store the specimens for no more than 2 hours in the refrigerator.^{9,10,12} Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, storage and transport to the laboratory should be applied.

8-TEST PROCEDURE

Pharmaceutical samples

Before performing the test for the determination of the absence of *Burkholderia cepacia* complex (Bcc), the ability of the method to detect Bcc in the presence of the product to be tested must be established (Suitability of the Test Method). The details of the procedure are described in USP <60>.⁸

Prepare a 1:10 dilution of the product to be examined using no less than 1 g of product. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in Suitability of the Test Method) of Tryptic Soy Broth or an appropriate dilution of Tryptic Soy Broth as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Mix and incubate at 30–35 °C for 48–72 h.

Subculture by streaking on a plate of BCSA, and incubate at 30–35°C for 48–72 h.

Clinical Specimens

Inoculate 100µL of the liquefied sputum or bronchoalveolar lavages onto a BCSA plate and spread inoculum over the entire surface of the agar plate. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate at 35-37°C for 48-72 hours.

AMCLI-SIFC¹² recommendation: incubation at 37°C for 3 days followed by an incubation at room temperature for one week and quantitative detection of CFUs. UK SMI B 57¹³ recommendation: incubation at 35-37°C for 5 days with daily cultures reading.

9-READING AND INTERPRETATION

The possible presence of Bcc is indicated by the growth of greenish–brown colonies with yellow halos, or white colonies surrounded by a pink–red zone on BCSA. Any growth on BCSA, typical or atypical should be confirmed by identification tests with biochemical, immunological, molecular, mass spectrometry techniques after colonies purification on a suitable medium.

10-USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.⁸

| CONTROL STRAINS | | INCUBATION T°/t / ATM | EXPECTED RESULTS |
|-----------------------|---|-----------------------|------------------|
| <i>B.cepacia</i> | ATCC 25416 | 35°C / 48 h / A | good growth |
| <i>B. cenocepacia</i> | ATCC BAA-485 or <i>B.multivorans</i> ATCC BAA-487 | 35°C / 48 h / A | good growth |
| <i>P.aeruginosa</i> | ATCC 9027 | 35°C / 72 h / A | growth inhibited |
| <i>S.aureus</i> | ATCC 6538 | 35°C / 72 h / A | growth inhibited |

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11-PERFORMANCES CHARACTERISTICS

Performance was evaluated with an in-house study, by preparing BCSA plates (REF 541153) with dehydrated BCSA (REF 4011532) supplemented with BCSA Selective Supplement (REF 4240073)

Performance was evaluated by qualitative ecometric technique incubating at 35°C for 24-72 hours, using 40 bacterial strains, 18 clinical isolates and 22 ATCC derivatives: *B. cepacia* 11, *B.cenocepacia* 2, *B.multivorans* 1, *P.aeruginosa* 15, *P.fluorescens* 2, *A.baumannii* 2, other Gram negative bacteria 4, Gram positive bacteria 2, yeast 1.

Productivity: the 14 strains of *Burkholderia* spp., grew at 24 hours and the morphology and colour changes were complete after 72 hours.

Selectivity: the other 25 bacterial strains and the yeast were totally inhibited within 72 hours with the exception of *Providencia stuartii* that is not inhibited on BCSA.

Productivity performance was evaluated also by quantitative spread plate technique using as reference medium Columbia Blood Agar (CBA) plates with 2 strains of *B.cepacia*, 1 strain of *B.cenocepacia*, 1 strain of *B.multivorans*. After incubation at 35°C for 48 hours, productivity ratio has been calculated (CFU^{B_{CSA}}/CFU^{C_{BA}} x 100) and found to be higher than 0,5.

Prior to release for sale a representative sample of all lots of BCSA ready-to-use plates and of the raw materials used for plates preparation (dehydrated BCSA Base, REF 401153 and BCSA Selective Supplement, REF 4240073) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative ecometric technique with the following target-strains: *B.cepacia* ATCC 25416, *B.cepacia* clinical isolate, *B.cenocepacia* ATCC BAA-245, *B.multivorans* ATCC BAA-247, *B.multivorans*, clinical isolate. After incubation at 35°C for 18-24 hours the colour of the medium and the colonies and the amount of growth are observed and recorded. The chromatic characteristics and the test strains growth shall be in compliance with specifications and comparable in both batches.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10⁻¹ to 10⁻⁴ of a 0.5 McFarland suspension of the non-target strains *P.aeruginosa* ATCC 9027, *P.fluorescens* ATCC 13525, *S.aureus* ATCC 6538, *E.faecalis* ATCC 29212, *B.subtilis* ATCC 6633, *C.albicans* ATCC 10231. After incubation at 35°C for 72 hours, the growth of non-target strains is inhibited at the dilution 10⁻¹.





12-LIMITATIONS OF THE METHOD

- The yellow color change of the medium indicates the degradation of sucrose and/or lactose occurred producing acidification; this degradation may not be present in all Bcc strains. Therefore it is recommended that any type of colony grown on BCSA be subjected to identification tests.
- There are reports that strains of *Burkholderia gladioli* and *Pseudomonas* spp. can be isolated on BCSA.¹³
- Although the superiority of the BCSA medium for the isolation of Bcc is recognized, Plonga¹⁴ reports the failure to grow on a marketed BCSA of 7 strains out of 43 inoculated (sensitivity 86%). It is therefore possible that there are Bcc strains that may be sensitive to antibiotics present in the medium.
- Rapidly growing mycobacteria (RGM) could be recovered from routine cultures of samples from patients with cystic fibrosis by extending incubation of BCSA to 7 days.¹⁵ However this strategy for the isolation of RGM still provides lower results than the use of more specific media.¹⁴
- The identification of Bcc members can be problematic since *B. cepacia* has a diverse genetic composition making accurate identification using phenotypic tests difficult. Many biochemical identification test systems have difficulty differentiating between the genera *Ralstonia*, *Burkholderia*, *Cupriavidus*, *Pandoraea*, *Achromobacter*, *Brevundimonas*, *Comamonas* and *Delftia*; this is compounded when attempting to differentiate within the *Burkholderia* genera (the species members are phylogenetically very closely related with little differences in the way of phenotypic characteristics). For example, *B. cepacia* is closely related to the bacterial species *B. gladioli*.¹
- The testing time of a pharmaceutical sample needs to be considered. The microbial growth kinetics of many Bcc organisms, due to their recovery from low-nutrient conditions, can often result in an extended lag phase; moreover, certain product ingredients can have an impact on microbial growth kinetics: by testing too early there may be insufficient bacterial cells for a Bcc contaminant to be detected.¹
- The ability of the USP test to detect Bcc in the presence of the product to be tested must be established. The incubation time for the method suitability should not exceed the shortest incubation period specified.⁸
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

13-PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostics, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana S.r.l. for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available in the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. This also applies in relation to any third party rights. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14-STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture)..

15-REFERENCES








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15. Esther CR Jr, Hoberman S, Fine J, Allen S, et al. Detection of rapidly-growing mycobacteria in routine cultures of samples from patients with cystic fibrosis. J Clin Microbiol 2011; 49:1421–1425.

TABLE OF APPLICABLE SYMBOLS

| | | | | |
|--|---|--|--|---|
| REF or REF Catalogue number | LOT Batch code | IVD <i>In vitro</i> Diagnostic Medical Device |  Manufacturer |  Use by |
|  Temperature limitation |  Contents sufficient for <n> tests |  Consult Instructions for Use |  For single use only |  Fragile, handle with care |

REVISION HISTORY

| Version | Description of changes | Date |
|---|---|---------|
| Instructions for Use (IFU) - Revision 1 | Updated layout and content in compliance with IVDR 2017/746 | 2020/05 |
| Revision 2 | Removal of obsolete classification | 2023/03 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

