INSTRUCTIONS FOR USE

BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR BASE

BCSA SELECTIVE SUPPLEMENT

Dehydrated medium and selective supplement

BCSA: lactose/sucrose oxidizing and not oxidizing *B.cepacia* strains

1-INTENDED USE

In vitro diagnostic. Dehydrated medium and selective supplement 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.

2-COMPOSITION

BCSA BURKOLDERIA CEPACIA SELECTIVE AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Casein peptone	10 g
Yeast extract	1.5 g
Lactose	10 g
Sucrose	10 g
Sodium chloride	5 g
Phenol red	0.08 g
Crystal violet	0.002 g
Agar	11.5 g

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

BCSA SELECTIVE SUPPLEMENT
VIAL CONTENTS

Vancomycin	1.25 mg
Gentamicin	5 mg
Polymyxin B	300,000 UI

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 genomovars[1].
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 products[7].
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 formulations[8]. 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 Burkolderia Cepacia Selective Agar is prepared according to the formula described by Heny in 1997[9] and meets the USP <60> requirements[8].
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 method[8] 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-DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and, under aseptic conditions, add the contents of one vial of BCSA Selective Supplement (4240073), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

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5-PHYSICAL CHARACTERISTICS

Dehydrated medium

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	limpid, red orange
Final pH at 25 °C	6.8 ± 0.3
Selective supplement	
Lyophilized pellet appearance	short, dense, white pastille
Solution appearance	limpid, colourless

6-MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
BCSA Burkolderia Cepacia Selective Agar Base	Dehydrated medium	4011532	500 g (10.4 L)
BCSA Burkolderia Cepacia Selective Agar Base	Dehydrated medium	4011534	5 kg (104 L)
BCSA Selective Supplement	Lyophilized supplement	4240073	10 vials, each for 500 mL of medium

7-MATERIALS REQUIRED BUT NOT PROVIDED

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

8-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]

Good laboratory practices for collection, storage and transport to the laboratory should be applied.

9-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-SIFC[12] 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 57[13] recommendation: incubation at 35-37°C for 5 days with daily cultures reading.

10-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.

11-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
B.cepacia	ATCC	25416	35°C / 48 h / A	good growth
B. cenocepacia ATCC BAA-485 or *B.multivorans* ATCC BAA-487			35°C / 48 h / A	good growth
P.aeruginosa	ATCC	9027	35°C / 72 h / A	growth inhibited
S.aureus	ATCC	6538	35°C / 72 h / A	growth inhibited

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

12-PERFORMANCES CHARACTERISTICS

Performance was evaluated with an in-house study, by preparing BCSA plates (REF 541153) with dehydrated BCSA Base (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^{BCSA}/CFU^{CBA} x 100) and found to be higher than 0,5.

Prior to release for sale a representative sample of all lots of dehydrated BCSA Base and BCSA Selective Supplement is 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* ATCC 25608, *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 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}.

13-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, Plonga[14] 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 *Burkhoderia* 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.

14-PRECAUTIONS AND WARNINGS

- The medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement must be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- 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.
- Apply Good Manufacturing Practice in the preparation process of plated, tubed, bottled media.
- The selective supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as powder medium and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet are available in the website www.biolifeitaliana.it.

- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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.

15-STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium: upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method applied (temperature and packaging).
Selective supplement: upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16-REFERENCES

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BCSA SELECTIVE SUPPLEMENT **4240073**
SDS rev 1
Regulation (EU) 2020/878

Contains: gentamicin sulphate, vancomycin HCl

Classification

Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling
Pictogram

Signal word Warning

Hazard statement(s)
H317 May cause an allergic skin reaction.
Precautionary statements:
P280 Wear protective gloves.
P261 Avoid breathing dust / fume / gas / mist / vapours / spray.
P333+P313 If skin irritation or rash occurs: Get medical advice / attention.
P362+P364 Take off contaminated clothing and wash it before reuse.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/02
Revision 4	Removal of obsolete classification	2023/04

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

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