BURKHOLDERIA CEPACIA SELECTIVE AGAR (BCSA)

INTENDED USE

Remel Burkholderia Cepacia Selective Agar (BCSA) is a solid medium recommended for use in qualitative procedures for the selective and differential isolation of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis.

SUMMARY AND EXPLANATION

B. cepacia complex has a wide geographic distribution and has been isolated from multiple environmental sources including soil, tap water, and distilled water.[1] It is capable of surviving for extended periods of time in hostile environments due to its innate resistance to many antiseptics and antibiotics.[2] Since the mid-1980's, *B. cepacia* complex has been recognized as an important pathogen affecting patients with cystic fibrosis (CF).[3,4] Colonization and pulmonary infection with this organism have been associated with a rapid decline in pulmonary function and poor patient prognosis. Isolation and identification of the organism is important to facilitate selection of proper treatment, ascertain carrier status, and maintain infection control.[5-7] The practice of cohorting *B. cepacia* complex-infected patients from all other CF patients to prevent the spread of this organism often results when a patient is identified as a carrier. This practice may lead to social isolation and exclusion as a candidate for lung transplantation, underscoring the need for accurate and timely laboratory procedures.[8]

B. cepacia complex consists of nine distinct genomic species (genomovars) all of which have been recovered from persons with CF.[9] Such phenotypic diversity, especially from patients with longstanding colonization, presents additional challenges in identifying this organism. *B. cepacia* complex tends to grow slowly and is easily obscured by overgrowth of mucoid *Pseudomonas aeruginosa* and other flora present in respiratory specimens from CF patients. In 1997, a highly selective and differential medium was formulated by Henry et al. to improve the speed and accuracy of isolation of *B. cepacia* complex, as well as provide quantitative recovery.[10,11] BCSA has been reported to be superior to other selective media for the recovery of *B. cepacia* complex and suppression of other respiratory pathogens and flora.

PRINCIPLE

This medium contains yeast extract and casein peptone as nutritive components. Sodium chloride maintains osmotic equilibrium and agar is added as a solidifying agent. Crystal violet, polymyxin B, gentamicin, and vancomycin are selective agents, which inhibit organisms commonly found in respiratory secretions other than *B. cepacia* complex. Sucrose and lactose are carbohydrates for enrichment and differentiation with phenol red as a pH indicator. *B. cepacia* complex colonies will be yellow on this medium. Colonies that absorb the crystal violet dye will appear purple to purple-gray. Acid production from carbohydrate oxidation will create a yellow zone in the medium surrounding the agar while peptone utilization will result in a pink zone.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	10.0	g	Gentamicin	10.0	mg
Lactose	10.0	g	Vancomycin	2.5	mg
Sucrose	10.0	g	Crystal Violet	2.0	mg
Sodium Chloride	5.0	g	Polymyxin B	600,000	U
Yeast Extract	1.5	g	Agar	14.0	g
Phenol Red	0.08	g	Demineralized Water	1000.0	ml

pH 7.0 ± 0.1 @ 25°C (Prior to terminal sterilization)

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If the material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plate aerobically at 33-37°C. Plates should be incubated a minimum of 72 hours before being discarded as negative.
4. Examine plate for typical colony morphology. On BCSA, *B. cepacia* complex colonies are smooth and slightly raised, small to large in size, yellow in color, and surrounded by a pink-yellow zone in the medium. Growth may require up to 72 hours of incubation. Identification of *B. cepacia* complex should be confirmed by conventional biochemical testing following established laboratory guidelines. Consult appropriate references for further instructions.[9,12]

QUALITY CONTROL

All lot numbers of Burkholderia Cepacia Selective Agar (BCSA) have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.[13] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Burkholderia cepacia* ATCC® 25416	Aerobic, up to 72 h @ 33-37°C	Good growth with pink to yellow zone
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 33-37°C	Inhibition (partial to complete)
**Pseudomonas aeruginosa* ATCC® 27853	Aerobic, up to 72 h @ 33-37°C	Inhibition (partial to complete)
**Staphylococcus aureus* ATCC® 25923	Aerobic, up to 72 h @ 33-37°C	Inhibition (partial to complete)

*CLSI recommended organism

(Continued on back)

LIMITATIONS

1. Isolates of *B. cepacia* complex produce a slow, weak-positive oxidase reaction.[9]
2. This formulation does not contain a selective agent against yeast or filamentous fungi.
3. BCSA can be used in quantitative procedures for the isolation of *B. cepacia* complex.[11,14] Refer to bibliography references 11 and 14 for stepwise instructions.
4. All first time isolates of *B.cepacia* complex from a CF patient should be sent to a referral laboratory for identification confirmation. The United States Cystic Fibrosis Foundation has established a reference laboratory at the University of Michigan for this purpose.[12]
5. Additional supplemental tests required for identification confirmation of *B. cepacia* complex include, at a minimum, maltose and lactose oxidation, lysine decarboxylase, and ONPG.[9,12]
6. While this medium is highly selective against gram-negative organisms, breakthrough growth of some gram-negative rods has been observed. In particular, *Burkholderia gladioli* and *Chryseobacterium indologenes* may be recovered demonstrating poor growth. Positive reactions for the oxidation of maltose and lactose can be used to separate *B. cepacia* complex from *B. gladioli*. A positive indole reaction can be used to separate *C. indologenes* (*B. cepacia* complex is negative).[9]
7. *Stenotrophomonas maltophilia* (colistin resistant strains) have been shown to grow on BCSA. A positive reaction on DNase Agar after a full 72 hours of incubation can be used to differentiate between *S. maltophilia* (positive) and *B. cepacia* complex (negative).[12]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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