

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for not more than 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to approximately 45-50°C. Swirl to disperse the insoluble material and pour into plates.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.²⁻⁵ The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora. When using slants, streak the surface of the slant with a sterile inoculating loop needle using two to three isolated colonies.

Incubate plates in an inverted position (agar side up) for up to 5 days at 25 ± 2°C.

Expected Results

Within 5 days of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Slants should show evidence of growth.

Examine plates and slants for colonies showing characteristic growth patterns and morphology. The following table summarizes typical *Candida* colonial morphology.⁶

SPECIES OF CANDIDA	COLONIAL MORPHOLOGY
<i>C. albicans</i>	Smooth, circular or hemispherical brown-black colonies; may have slight mycelial fringe; no color diffusion into surrounding medium; no metallic sheen.
<i>C. tropicalis</i>	Smooth, discrete, dark brown to black colonies (may have black-colored centers); slight mycelial fringe; diffuse blackening of medium after 72 hours; metallic sheen.
<i>C. krusei</i>	Large, flat, wrinkled silvery brown-black colonies with brown peripheries; yellow to brown halo diffusion into medium; metallic sheen.
<i>C. kefyr</i>	Medium size, flat, dark reddish-brown glistening colonies; may have slight mycelial fringe; no diffusion.

References

1. Nickerson. 1953. J. Infect. Dis. 93:43.
2. Haley, Trandel and Coyle. 1980. Cumitech 11, Practical methods for culture and identification of fungi in the clinical mycology laboratory. Coord. ed., Sherris. American Society for Microbiology, Washington, D.C.
3. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
5. Reisner, Woods, Thompson, Larone, Garcia and Shimizu. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ BiGGY Agar

Cat. No. 211027 Dehydrated – 500 g

United States and Canada

Cat. No. 297254 Prepared Plates – Pkg. of 20*
297255 Prepared Slants – Pkg. of 10*

Europe

Cat. No. 255002 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 252563 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.

Bacteroides Bile Esculin Agar (BBE)

Intended Use

Bacteroides Bile Esculin Agar (BBE) is recommended as a primary isolation medium for the selection and presumptive identification of the *B. fragilis* group.^{1,2}

Summary and Explanation

Among the most frequently encountered anaerobes in human clinical infections are members of the “*Bacteroides fragilis* group”. Rapid detection and identification of these organisms is important since they have been found to be more resistant to antimicrobial therapy than other anaerobes.³ *B. fragilis* and *B. thetaiotaomicron* are the species of greatest clinical significance.³ Other species in the group include: *B. caccae*, *B. distasonis*, *B. eggerthii*, *B. merdae*, *B. ovatus*, *B. stercoris*, *B. uniformis* and *B. vulgatus*.

Frequently these pathogens occur in a mixture of microorganisms which may overgrow the primary isolation medium. Selective media, such as CDC Anaerobe 5% Sheep Blood Agar

with Kanamycin and Vancomycin, have been recommended as appropriate for primary isolation.⁴ However, limited evidence for the presumptive identification of the *B. fragilis* group was provided. In 1978, Livingston et al. described a primary plating medium (BBE) which was found to provide selective recovery of the *B. fragilis* group and also evidence for presumptive identification.¹

Principles of the Procedure

Bacteroides Bile Esculin Agar is a primary plating medium for the selective isolation and presumptive identification of the *B. fragilis* group. Selective inhibition of facultative anaerobes and most gram-negative anaerobic organisms is obtained by the presence of gentamicin and oxgall. Differentiation of the *B. fragilis* group is based on esculin hydrolysis, which produces esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a dark brown to black complex that appears in the medium surrounding colonies of members of the *B. fragilis* group.

Procedure

As some strains of the *B. fragilis* group may not grow well due to the selective properties of the medium, it is advisable to include a nonselective blood agar medium, such as CDC Anaerobe 5% Sheep Blood Agar. All media should be pre-reduced. Incubate immediately under anaerobic conditions (BBL™ GasPak™ EZ anaerobic systems or alternative anaerobic system) for at least 48 hours at $35 \pm 2^\circ\text{C}$.

Bacteroides fragilis
ATCC™ 25285



Expected Results

After 48 hours of incubation, colonies of the *B. fragilis* group should be greater than 1 mm in diameter and appear gray, circular, entire and raised. Most anaerobes other than the *B. fragilis* group are inhibited. Esculin hydrolysis is indicated by a blackening of the medium around the colonies.

Limitation of the Procedure

B. vulgatus may not hydrolyze esculin.^{2,3}

References

- Livingston, Kominos and Yee. 1978. J. Clin. Microbiol. 7:448.
- Reischelderfer and Mangels. 1992. In Isenberg (ed.), Clinical microbiology procedure handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Chapin and Murray. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta, Ga.

Availability

BBL™ Bacteroides Bile Esculin Agar (BBE)

BS10 CMPH MCM7

United States and Canada

Cat. No. 221836 Prepared Plates – Pkg. of 10*

Japan

Cat. No. 251972 Prepared Plates – Pkg. of 10*

BBL™ Bacteroides Bile Esculin Agar (BBE)// CDC Anaerobe Laked Sheep Blood Agar with KV

CMPH MCM7

Cat. No. 297022 Prepared I Plate™ Dishes – Pkg. of 20*

297260 Prepared I Plate™ Dishes – Ctn. of 100*

*Store at 2-8°C.

Baird-Parker Agar Base • Baird-Parker Agar EY Tellurite Enrichment

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Baird-Parker Agar Base is used with EY (Egg Yolk) Tellurite Enrichment in the preparation of Egg-Tellurite-Glycerine-Pyruvate Agar (ETGPA) for selective isolation and enumeration of coagulase-positive staphylococci from food, skin, soil, air and other materials. It may also be used for identification of staphylococci on the basis of their ability to clear egg yolk.

Summary and Explanation

A number of culture media had been utilized for the recovery of staphylococci from foods prior to the development of a new formulation by Baird-Parker in 1962.^{1,2} This scientist subsequently published additional results on the efficacy of the medium for the recovery of coagulase-positive staphylococci.^{3,4} In 1971, Tardio and Baer⁵ and Baer⁶ reported on the results of a study comparing 18 staphylococcal isolation media in which they concluded that Baird-Parker Agar should be substituted for Vogel and Johnson Agar in the official AOAC

procedure for the isolation and enumeration of *Staphylococcus aureus*. In this study, it was shown that Baird-Parker Agar was less inhibitory than Vogel and Johnson Agar for selected strains of *S. aureus* and that it possesses a diagnostic aid (egg yolk reaction) not present in Vogel and Johnson Agar. The use of Baird-Parker Agar subsequently was officially adopted by AOAC International⁷ and is recommended in the *USP* for use in the performance of Microbial Limit Tests.⁸

Principles of the Procedure

Baird-Parker Agar Base contains peptone, beef extract and yeast extract as sources of nitrogenous compounds, carbon, sulfur, vitamins and trace minerals. Sodium pyruvate is incorporated in order to stimulate the growth of *S. aureus* without destroying the selectivity. The tellurite additive is toxic to egg yolk-clearing strains other than *S. aureus* and imparts a black color to the colonies. The egg yolk additive, in addition to being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). Glycine and lithium chloride have inhibitory action for organisms other than *S. aureus*.