QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Bile Esculin Agar is a medium for the presumptive identification of *Enterococcus* species and the *Streptococcus bovis* group of streptococci.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Streak-inoculate the slant surfaces with a 0.01 mL calibrated loop using 10^{-1} dilutions of 18- to 24-h **BD Trypticase™** Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
   c. Include **BD Trypticase** Soy Agar slants as nonselective controls for all organisms.
2. Examine tubes after 18–24 and 42–48 h for growth, selectivity and correct reactions.
3. Expected Results

CLSI Organisms	ATCC®	Recovery	Esculin Reaction
**Enterococcus faecalis*	29212	Growth	Blackening around colonies (blackening of half or more of the medium)
**Streptococcus pyogenes*	19615	Inhibition (partial to complete)	No blackening
Additional Organism			
Streptococcus gallolyticus	9809	Growth	Blackening of half or more of the medium

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Bile Esculin Agar is used to differentiate enterococci and the *Streptococcus bovis* group from other streptococci.[1,2]

V SUMMARY AND EXPLANATION

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.[3] Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.[4] Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.[5]

VI PRINCIPLES OF THE PROCEDURE

Enterococci and certain streptococci hydrolyze the glycoside, esculin, to esculetin and dextrose. Esculetin reacts with an iron salt to form a dark brown or black complex.[6] Ferric citrate is incorporated into the medium as an indicator of esculin hydrolysis and resulting esculetin formation. Oxgall is used to inhibit gram-positive bacteria other than enterococci.

VII REAGENTS

Bile Esculin Agar Slants

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	5.0 g	Ferric Citrate	0.5 g
Beef Extract	3.0 g	Esculin	1.0 g
Oxgall	20.0 g	Agar	14.0 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[7,8] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Bile Esculin Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Inoculate the medium with two or three colonies and incubate overnight at 35 ± 2 °C in an aerobic atmosphere.[9]

User Quality Control: See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

If more than half of the slant is blackened within 24–48 h, the test is positive. If less than half of the slant is blackened or no blackening occurs within 24–48 h, the test is negative.

XI LIMITATIONS OF THE PROCEDURE

Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* that give a positive bile-esculin reaction have been isolated from human infections.[1,9]

Occasional strains of viridans streptococci blacken the medium or display weakly positive reactions.[2]

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[7,8]

XII PERFORMANCE CHARACTERISTICS

Hussain et al. tested 194 streptococcal isolates, previously identified by serological testing, to determine the efficacy of various biochemical tests for identification of group A and B streptococci as well as differentiation between enterococcal and non-enterococcal group D streptococci. Twenty-two (22) strains of group D enterococci were identified. One hundred percent (100%) of the group D streptococci and 1 strain each of group R and a non-groupable streptococcus caused blackening of the Bile Esculin Agar slants. Using a heavy inoculum, hydrolysis of esculin was detectable within 4 h in 93% of the samples tested.[10]

XIII AVAILABILITY

Cat. No.	Description
221409	**BD BBL™** Bile Esculin Agar Slants
221410	**BD BBL™** Bile Esculin Agar Slants

XIV REFERENCES

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10. Hussain, Z., R. Lannigan, and L. Stoakes. 1984. A new approach for presumptive identification of clinically important streptococci. Zbl. Bakt. Hyg. A *258*:74-79.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA

EC REP Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland