QUALITY CONTROL PROCEDURES

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 Trypticase™ Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
   c. Include 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
   - *Enterococcus faecalis* 29212: Growth, Blackening around colonies (blackening of half or more of the medium)
   - *Streptococcus pyogenes* 19615: Inhibition, No blackening (partial to complete)
   - *Streptococcus bovis* 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.

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

<table>
<thead>
<tr>
<th>Approximate Formula* Per Liter Purified Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Gelatin: 5.0 g</td>
</tr>
<tr>
<td>Beef Extract: 3.0 g</td>
</tr>
<tr>
<td>Oxnall: 20.0 g</td>
</tr>
<tr>
<td>Ferric Citrate: 0.5 g</td>
</tr>
<tr>
<td>Esculin: 1.0 g</td>
</tr>
<tr>
<td>Agar: 14.0 g</td>
</tr>
</tbody>
</table>

*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.
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.”

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

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.

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

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

AVAILABILITY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221409</td>
<td>BBL™ Bile Esculin Agar Slants, Pkg. of 10 size K tubes</td>
</tr>
<tr>
<td>221410</td>
<td>BBL™ Bile Esculin Agar Slants, Ctn. of 100 size K tubes</td>
</tr>
</tbody>
</table>

REFERENCES


