



QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

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

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the the cultures listed below.
 - Streak inoculate the plates for isolation with $10^3 - 10^4$ CFU using a 10 μ L (0.01 mL) loop.
 - Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
 - Include **BD Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 18–24 h for amount of growth, colony size, reaction and selectivity.
- Expected Results

CLSI Organisms	ATCC®	Recovery	Reaction
* <i>Enterococcus faecalis</i>	29212	Fair to heavy growth	Blackening around colonies
* <i>Streptococcus pyogenes</i>	19615	Inhibition (partial to complete)	
Additional Organisms			
<i>Enterococcus hirae</i>	10541	Fair to heavy growth	Blackening of the medium
<i>Streptococcus gallolyticus</i>	9809	Fair to heavy growth	Blackening

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine 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 The enterococci were able to split esculin, but other streptococci could not. 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

Approximate Formula* Per Liter Purified Water

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

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

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

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, 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. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: Bile Esculin Agar

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium with two or three colonies and incubate overnight at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere. Reincubation of negative tests for an additional 24 h is recommended.

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

The test is positive when any blackening of the medium occurs.

If no blackening occurs, 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.^{1,7}

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.^{6, 8-12}

XII AVAILABILITY

Cat. No.	Description
221838	BD BBL™ Bile Esculin Agar, Pkg. of 10 plates

XIII REFERENCES

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3. Rochar, A. 1924. Milleux a leucine pour le diagnostic differential des bacteries du groupe strept-entéro- pneumocoque. Compt. Rend. Soc. Biol. 90:771-772.
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