QUALITY CONTROL PROCEDURES

I INTRODUCTION

Enterococcosel Agar is a selective medium for the detection and enumeration of enterococci from clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. For *S. pyogenes* and *E. coli*, use 18–24 h broth cultures diluted to yield 10^4–10^5 CFU/plate. For the remaining organisms, use 18–24 h broth cultures diluted to yield 10^3–10^4 CFU/plate.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine the plates after 18–24 and 42–48 h for amount of growth, colony size, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC™	Recovery	Reaction
Additional Organism			
Enterococcus hirae	10541	Moderate to heavy growth.	Colonies translucent with brownish-black to black zones

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Enterococcosel Agar, a modified esculin bile agar, is used for the rapid, selective detection and enumeration of enterococci.

V SUMMARY AND EXPLANATION

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.[1] 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.[2] 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.[3] Facklam and Moody performed a comparative study of tests used to presumptively identify group D streptococci and found that the bile-esculin test provides a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.[4] According to current nomenclature, the group D antigen is considered non-specific since it is produced by the genera *Enterococcus*, *Pediococcus* and by certain streptococci.[5]

Isenberg et al. modified the Bile Esculin Agar formulation by reducing the bile concentration from 40 to 10 g/L and by adding sodium azide.[6] This modification is supplied as **BBL Enterococcosel** Agar.

Consult the text for a list of specimens for which this medium is recommended for primary isolation.[7]

VI PRINCIPLES OF THE PROCEDURE

Enterococci hydrolyze the glycoside, esculin, to esculetin and dextrose. Esculetin reacts with an iron salt, ferric ammonium citrate, to form a dark brown or black complex.[8] Oxgall is used to inhibit gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative microorganisms.

VII REAGENTS

Enterococcosel™ Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	17.0 g	Esculin	1.0 g
Peptic Digest of Animal Tissue	3.0 g	Ferric Ammonium Citrate	0.5 g
Yeast Extract	5.0 g	Sodium Azide	0.25 g
Oxgall	10.0 g	Sodium Citrate	1.0 g
Sodium Chloride	5.0 g	Agar	13.5 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.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[9-12] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates 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, cracking 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.[13,14] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Enterococcosel™ 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.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge and streak from this inoculated area.

Incubate plates 24–48 h at 35 ± 2°C in an aerobic atmosphere.

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.

X RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Enterococci produce translucent colonies with brownish-black to black zones.

XI LIMITATIONS OF THE PROCEDURE

Listeria monocytogenes, *Streptococcus bovis* Group, pediococci and staphylococci may also grow on this medium; however, staphylococci do not produce black zones. Other organisms; i.e., micrococci, *Candida*, corynebacteria and gram-negative bacteria, may appear as small colonies or trace growth.

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.[8,13-17]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221492	**BBL™ Enterococcosel™** Agar, Pkg. of 20 plates
221493	**BBL™ Enterococcosel™** Agar, Ctn. of 100 plates

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