BBL™ Enterococcosel™ Broth

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Enterococcosel™ Broth is a selective medium for the cultivation and differentiation of enterococci.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. For *Escherichia coli*, use a 24-h **Trypticase™** Soy Agar Slant culture to prepare a suspension in sterile purified water equal to a 0.5 McFarland Standard. Inoculate tubes using a 0.01 mL calibrated loop. For the remaining organisms, inoculate each tube with growth from 24- to 48-h **Trypticase** Soy Agar Slant cultures, using a 0.01 mL calibrated loop.
   b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase** Soy Broth tubes as controls for the *Streptococcus pyogenes* and *Escherichia* strains.
2. Examine tubes after 2, 4 and 18 – 24 h for growth, selectivity and reactions.
3. Expected Results

Organisms	ATCC®	Recovery	Reaction
**Enterococcus faecalis*	29212	Growth	Blackening of medium within 2 h
Enterococcus faecalis	10741	Growth	Blackening of medium within 2 h
**Streptococcus pyogenes*	19615	Inhibition (partial)	If growth, no blackening reaction
**Escherichia coli*	25922	Inhibition (partial)	If growth, no blackening reaction

*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

Enterococcosel Broth is recommended for use in the differentiation of enterococci.

V SUMMARY AND EXPLANATION

This enterococcus selective broth has the same formula as **Enterococcosel™** Agar with the agar omitted. Colonies suspected of being *Enterococcus faecalis* can be emulsified in 1 – 2 mL of broth and incubated at 35°C. The combination of esculin and a rather low concentration of bile in the presence of azide permits the selection of enterococci and differentiation by esculin hydrolysis (blackening of the medium) within 2 h.[1]

VI PRINCIPLES OF THE PROCEDURE

Enterococci hydrolyze esculin to produce esculetin which reacts with the ferric ammonium citrate to form a dark brown or black complex.[2] Oxgall inhibits gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative microorganisms.

VII REAGENTS

Enterococcosel™ Broth

Approximate Formula* Per Liter Purified Water

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

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

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

Medium contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

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 – 25°C. Avoid freezing and overheating. Do not open until ready to use. 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. Minimize exposure to light.

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.[3,4] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Enterococcosel Broth

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

Test Procedure: Observe aseptic techniques.

Colonies, from a primary isolation plate, suspected of being enterococci can be emulsified in 2 mL of **Enterococcosel** Broth and incubated 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 guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Enterococci turn the medium black within 2 h when a heavy inoculum is used. Other organisms are inhibited or do not turn the medium black. For additional information, consult an appropriate text.[5]

XI LIMITATIONS OF THE PROCEDURE

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.[2,4,6]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of **Enterococcosel** Broth are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are inoculated with **Trypticase™** Soy Broth cultures of *Enterococcus faecalis* (ATCC 29212), *E. faecalis* (ATCC 10741) and *Streptococcus pyogenes* (ATCC 19615) diluted 10^{-1} and a **Trypticase** Soy Agar culture of *Escherichia coli* (ATCC 25922) diluted to a 0.5 McFarland standard. Inoculated tubes are incubated at 35 ± 2°C with loosened caps. Tubes are read for growth and reaction after 2 – 4 h and 18 – 24 h. Within 2 h enterococci exhibit blackening of the medium indicating the hydrolyzation of the glycoside esculetin; no blackening is exhibited by *S. pyogenes* and *E. coli*. Growth of *E. faecalis* is moderate to heavy within 24 h; growth of *S. pyogenes* and *E. coli* is partially to completely inhibited.

XIII AVAILABILITY

Cat. No.	Description
221383	**BD BBL™ Enterococcosel™** Broth

XIV REFERENCES

1. Isenberg, H.D., D. Goldberg, and J. Sampson. 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. 20:433-436.
2. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott, Williams & Wilkins, Baltimore.
3. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
4. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Baily & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
5. Facklam, R.R., D.F. Sahm, and L.M. Teixeira. 1999. Enterococcus, p. 297-305. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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