PRODUCT INFORMATION AND QUALITY CONTROL SHEET
BILE ESCULIN AGAR

I. INTENDED USE
Bile Esculin Agar is used for the selective isolation and differentiation of group D streptococci.

II. SUMMARY AND EXPLANATION
Bile Esculin Agar is based on the formulation described by Swan and further evaluated by Facklam and Moody.[1,2] Rochaix first noted the value of esculin hydrolysis in the identification of enterococci.[3] Meyer and Schöenfeld added bile to the esculin medium and demonstrated 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while other streptococci could not.[4]

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.[5] Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,[1] and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.

Bile Esculin Agar is in standard procedures for the microbiological examination of food products.[6-8]

III. PRINCIPLES OF THE PROCEDURE
Organisms positive for esculin hydrolysis hydrolyze the esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit gram-positive bacteria other than enterococci. Beef Extract and Enzymatic Digest of Gelatin are the carbon and nitrogen sources used for general growth requirements in Bile Esculin Agar. Agar is the solidifying agent.

IV. TYPICAL FORMULA AND APPEARANCE
Appearance = grey-yellow, slightly opalescent
(Approximate formula* per liter of processed water)

Beef Extract	11.0 g
Enzymatic Digest of Gelatin	34.5 g
Esculin	1.0 g
Oxbile	2.0 g
Ferric Ammonium Citrate	0.5 g
Agar	15.0

*adjusted and/or supplemented to meet performance criteria.
Final pH: 6.6 ± 0.2 @ 25^{0}C

V. PRECAUTIONS
This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

VI. STORAGE/SHELF LIFE
Plated media should be stored at 2-8^{0}C (36-46^{0}F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used. Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

VII. SPECIMEN COLLECTION
The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Consult appropriate references for information about the processing and inoculation of specimens bacterial culture. Sterile swabs and collection containers should be used. Plates should be inoculated promptly after specimen collection.

VIII. MATERIALS PROVIDED
Bile Esculin Agar Plates (10/pkg)

IX. MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 35-37^{0}C.
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE
Inoculate the specimen as soon as possible after it is received in the laboratory. Streak the specimen with a sterile inoculating loop to obtain isolated colonies. Incubate the inoculated plates at 35-37^{0}C, agar side for 18-24 hours.

XI. EXPECTED RESULTS
NCCLS CONTROL ORGANISMS (ATCC STRAINS)

Microorganism	Response	Reaction Esculin Hydrolysis
Enterococcus faecalis ATCC 19433	growth	+ (black colonies)
Enterococcus faecalis ATCC 29212	growth	+ (black colonies)
Enterococcus faecalis ATCC 33186	growth	+ (black colonies)
Escherichia coli ATCC 25922	growth	—
Streptococcus pyogenes ATCC 19615	inhibited	—

XII. LABORATORY RESULTS
This medium is intended to be used as a primary isolation medium. Identification of organisms may be made on the basis of typical gross colony morphology, microscopic characteristics, and physiologic and pathologic characteristics. Additional test procedures should be used to confirm findings.

XIII. LIMITATIONS
The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

XIV. REFERENCES
1. Swan, A. 1954. The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. 7:160.
2. Facklam, R.R., and M.D. Moody. 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. 20: 245.
3. Rochaix, A. 1924. Millieux a leculine pour le diagnostid differentieldes bacteries du grojps strepto-entero-pneumocoque. Comt. Rend. Soc. Biol. 90:771-772.
4. Meyer, K., and H. Schönfeld. 1926. Über die Unterscheidund des Enterococcus vom Streptococcus viridans und die Beziehunger beider zum Streptococcus lactis. Zentralb. Bakteriol Parasitenkd. Infektionskr. Hyg. Abt. I orig. 99:402-416.
5. Schleifer, K.H., and R. Klipper-Balz. 1987. Molecular and

chemotaxonomic approaches to the classification of streptococci. Enterococci and lactococci: a review. Syst. Appl. Microbiol. 10:1-19.

6. Vanderzant, C., and D.F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. Bacteriological Analytica Manual, 1995. 8th ed. AOAC International, Gaithersburg, MD.
8. Marshall, R.T. (ed.). 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

I. QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within 2-8^{0}C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 35-37^{0}C.

II. QUALITY CONTROL

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory:

Inspect plates according to instructions contained in the Section VI "STORAGE/SHELF LIFE"

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

HealthLink
3611 St. Johns Bluff Rd. So. Ste. 1
Jacksonville, FL 32224

1-800-638-2625

January, 2003

Product No. 1012 Rev. No. New