

Bile Esculin Agar Plate

I. PRINCIPLE

Group D streptococci (including *Enterococcus* spp.) and a few other bacteria, such as *Listeria* spp., can grow in the presence of 40% bile and also hydrolyze esculin to esculetin. Esculetin reacts with ferric ions, supplied by ferric citrate in the agar medium, to form a diffusible black complex. Most strains of viridans streptococci that are capable of hydrolyzing esculin will not grow in the presence of 40% bile.

II. REAGENTS AND MATERIALS

- A. Bile esculin agar plate (store at 4°C until stated expiration date)
- B. Inoculating loop

III. QUALITY CONTROL

- A. Quality control testing is not required. Selective media for enterococci are listed as exempt in CLSI document M22-A3 (2004).

IV. PROCEDURE

- A. Streak the surface of the bile esculin agar plate with several colonies of the organism to be tested.
- B. Incubate at 35°C in non-CO₂ for 24 to 48 h.

V. INTERPRETATION

- A. Positive: diffuse blackening of more than half of the plate within 24 to 48 h.
- B. Negative: no growth or growth without blackening of the medium after 48 h (i.e. no esculin hydrolysis).

VI. LIMITATIONS

- A. If the inoculum is too heavy, viridans streptococci may give a false positive test result.
- B. Approximately 3% of viridans streptococci are able to hydrolyze esculin in the presence of bile.
- C. *Lactococcus*, *Leuconostoc*, and *Pediococcus* that give a positive bile-esculin reaction have been reported.
- D. Soy and salt broths need to be setup with the bile esculin plate in order to differentiate enterococci from non-enterococcal group D streptococci.

VII. REFERENCES

- A. BBL Quality Control and Product Information Manual for Tubed Media: Bile Esculin Agar. BD Microbiology Systems, Cockeysville, MD. 2001.
- B. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 1992. Vol 1. ASM, Washington, DC, p. 1.20.18-1.20.19.
- C. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn. Diagnostic Microbiology. 4th ed. J.B. Lippincott Company, Philadelphia, 1992, p. 459-460.

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Updates and Revisions: