

Salt Tolerance (6.5% Broth)

I. PRINCIPLE

Brain Heart Infusion Broth is a general purpose medium for the cultivation of both fastidious and nonfastidious organisms. With the addition of 6.5% sodium chloride, the medium can be used to differentiate between salt tolerant and salt intolerant organisms. It is especially useful for distinguishing *Enterococcus* spp., which are salt tolerant, from non-enterococcal group D streptococci, such as *S. bovis* and *S. equinus*.

II. REAGENTS AND MATERIALS

- A. Brain heart infusion broth (store at 4°C with stated expiration date)
- B. Brain heart infusion broth with 6.5% sodium chloride (store at 4°C until stated expiration date)
- C. Inoculating loops

III. QUALITY CONTROL

- A. Quality control is performed with each new lot and shipment and includes the following organisms and their expected reactions:
 - 1. Positive (growth): *Enterococcus faecalis* ATCC 29212
 - 2. Negative (no growth): *Streptococcus pyogenes* ATCC 19615
- B. A brain heart infusion broth tube without sodium chloride is always inoculated along with each salt broth tube to serve as a growth control.

IV. PROCEDURE

- A. Using a sterile inoculating loop, emulsify 1 to 3 colonies of the organism in brain heart infusion broth.
- B. Using a sterile inoculating loop, emulsify 1 to 3 colonies of the organism in a tube containing 6.5% sodium chloride
- C. Incubate both tubes at 35°C in non-CO₂ for 24-48 h.

V. INTERPRETATION

- A. Positive: visible growth (turbidity) in BHI broth and salt broth tubes.
- B. Negative: growth in BHI broth, no growth in salt broth.

VI. LIMITATIONS

- A. If the medium is inoculated too heavily, the inoculum may be interpreted as growth, resulting in a false positive reaction.
- B. To avoid false negative interpretations, shake each tube gently prior to reading, since the growth may settle out during incubation.
- C. A bile esculin plate should be set up along with the BHI and salt broths for the identification of *Enterococcus*.

- D. Other gram-positive cocci besides *Enterococcus*, such as *Aerococcus*, *Pediococcus*, *Staphylococcus*, and up to 80% of group B *Streptococcus*, can grow in 6.5% salt broth. In addition, *Aerococcus* may also be bile esculin positive.

VII. REFERENCES

- A. BBL Quality Control and Product Information Manual for Tubed Media: Brain Heart Infusion Broth. BD Microbiology Systems, Cockeysville, MD.
- B. Isenberg, H.D. 1992. Clinical Microbiology Procedures Handbook. Vol 1. ASM, Washington, DC, p. 1.20.270-1.20.28.
- C. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn. 1992. Diagnostic Microbiology. 4th ed. J.B. Lippincott Company, Philadelphia, p. 46.

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Updates and Revisions: