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QUALITY CONTROL PROCEDURES

**I INTRODUCTION**

Brain Heart Infusion (BHI) is a general purpose liquid medium for the growth of a wide variety of bacterial and fungal species. Brain Heart Infusion with 6.5% Sodium Chloride is used to differentiate enterococci from nonenterococcal group D streptococci.

**II PERFORMANCE TEST PROCEDURE**

1. Inoculate representative samples with the cultures listed below.
  - a. From 24- to 48-h **Trypticase™** Soy Broth cultures, prepare dilutions for use as inocula.
  - b. Inoculation of media
    - 1) For BHI, inoculate tubes of the test samples with a dilution of each culture. The dilution must contain 1,000 or less CFU. Fill volumes of greater than 5 mL should be inoculated with 1.0 mL. Fill volumes of 5 mL or less should be inoculated with 0.1 mL.
    - 2) For BHI with 6.5% Sodium Chloride, inoculate tubes of the test samples using 10<sup>-1</sup> dilutions of 18- to 24-h **Trypticase** Soy Broth cultures using a 0.01 mL calibrated loop.
  - c. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
2. Examine tubes of Brain Heart Infusion at 24 and 48 h for growth. Examine tubes of BHI with 6.5% Sodium Chloride at 18 – 24 h for growth and selectivity.

3. Expected Results

**Brain Heart Infusion**

CLSI Organisms	ATCC™	Recovery
* <i>Escherichia coli</i>	25922	Growth
* <i>Staphylococcus aureus</i>	25923	Growth

**Additional Organisms**

<i>Pseudomonas aeruginosa</i>	27853	Growth
<i>Enterococcus faecalis</i>	29212	Growth
<i>Streptococcus pyogenes</i>	19615	Growth

**BHI with 6.5% Sodium Chloride**

Organisms	ATCC	Recovery
* <i>Enterococcus faecalis</i>	29212	Growth
* <i>Streptococcus gallolyticus</i>	9809	No Growth

\*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**

Brain Heart Infusion (BHI) is a general-purpose liquid medium used in the cultivation of fastidious and nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials.

The broth medium which contains 6.5% sodium chloride is used to differentiate the enterococci from nonenterococcal group D streptococci.

**V SUMMARY AND EXPLANATION**

BHI Broth is used for the cultivation of a wide variety of microorganisms, including bacteria, yeasts and molds.<sup>1</sup>

BHI with 6.5% Sodium Chloride is used to differentiate the enterococci (e.g., *E. faecalis*, *E. faecium*, *E. durans* and *E. avium*) from the nonenterococcal species (*S. gallolyticus* and *S. equinus*) by the 6.5% salt tolerance test.<sup>2</sup>

**VI PRINCIPLES OF THE PROCEDURE**

BHI Broth is a nutritious, buffered culture medium that contains infusions of brain and heart tissue and peptones to supply protein and other nutrients necessary to support the growth of fastidious and nonfastidious microorganisms. In the formulation containing 6.5% sodium chloride, the salt acts as a differential and/or selective agent by interfering with membrane permeability and osmotic and electrokinetic equilibria in salt-intolerant organisms.<sup>1</sup>

## VII REAGENTS

### Brain Heart Infusion

Approximate Formula\* Per Liter Purified Water

Brain Heart, Infusion from (solids) .....	6.0 g
Peptic Digest of Animal Tissue.....	6.0 g
Sodium Chloride .....	5.0 g
Dextrose.....	3.0 g
Pancreatic Digest of Gelatin .....	14.5 g
Disodium Phosphate.....	2.5 g

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

Brain Heart Infusion with 6.5% Sodium Chloride contains 60 g/L of sodium chloride in addition to the ingredients listed above.

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

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>3-6</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. 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. Minimize exposure to light. 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. Allow the medium to warm to room temperature before inoculation.

**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.<sup>7,8</sup> Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Brain Heart Infusion or Brain Heart Infusion with 6.5% Sodium Chloride

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

**Test Procedure:** Observe aseptic techniques.

With liquid specimens, tubed media should be inoculated with one or two drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

Liquid media for anaerobic incubation should be reduced prior to incubation by placing the tubes, with caps loosened, under anaerobic conditions for 18 – 24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™ EZ** anaerobic system.

Alternatively, liquid media may be reduced immediately prior to use by boiling in a water bath\* with caps loosened and cooling to room temperature with tightened caps before inoculation.

Inoculate the 6.5% NaCl broth lightly with one or two colonies of suspect bacteria. Incubate aerobically at 35 ± 2°C overnight. Examine for growth; reincubate negative tests for an additional 24 h.

\*NOTE: Use of a microwave oven is not recommended.

**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

Growth in the tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining and subculturing onto appropriate media, e.g., a **Trypticase Soy Agar** with 5% Sheep Blood (TSA II) and/or **Chocolate II Agar** plate, **EMB Agar** or **MacConkey II Agar** plates. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a **GasPak EZ** anaerobic system.

Enterococci will grow in the 6.5% NaCl broth within 24 – 48 h. Nonenterococcal group D streptococci fail to grow in the medium after 48 h of incubation.<sup>2</sup>

## 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.<sup>7-9</sup>

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

Strains of other catalase-negative gram-positive cocci; i.e., *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Vagococcus*, have been isolated from human infections. Therefore, the presumptive identification of enterococci based on the bile-esculin reaction and growth in 6.5% NaCl broth only cannot be made.<sup>10</sup>

## XII PERFORMANCE CHARACTERISTICS

### Brain Heart Infusion

Prior to release, all lots of Brain Heart Infusion are tested for performance characteristics. Using a sterile pipette, representative samples of the lot are inoculated with 0.1 mL (for fill volumes of 5 mL or less) or 1.0 mL (for fill volumes greater than 5 mL) of **Trypticase** Soy Broth or Thioglycollate Medium, Enriched cultures containing 1,000 Colony Forming Units (CFU) or less of *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The tubes are incubated with loosened caps at 35 ± 2°C and read after 18 – 24 h and 42 – 48 h for growth. All cultures exhibit moderate to heavy growth within 48 h.

### Brain Heart Infusion with 6.5% Sodium Chloride

Prior to release, all lots of Brain Heart Infusion with 6.5% Sodium Chloride are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are tested with **Trypticase** Soy Broth cultures diluted 10<sup>-1</sup> of *Enterococcus faecalis* (ATCC 29212) and *Streptococcus gallolyticus* (ATCC 9809). The tubes are incubated at 35 ± 2°C and read after 18 – 24 h and 42 – 48 h for growth. *E. faecalis* exhibits moderate to heavy growth while *S. gallolyticus* is completely inhibited.


Additionally, representative samples are tested chemically by silver nitrate titration for sodium chloride content. The calculated percent sodium chloride is 6.0 to 7.0.

## XIII AVAILABILITY

Cat. No.	Description
221778	<b>BBL™</b> Brain Heart Infusion, 0.5 mL, Ctn. of 100 size K tubes
297769	<b>BBL™</b> Brain Heart Infusion, 2 mL, Ctn. of 100 size K tubes
221812	<b>BBL™</b> Brain Heart Infusion, 5 mL, Pkg. of 10 size K tubes
221813	<b>BBL™</b> Brain Heart Infusion, 5 mL, Ctn. of 100 size K tubes
220837	<b>BBL™</b> Brain Heart Infusion, 8 mL, Ctn. of 100 size K tubes
221785	<b>BBL™</b> Brain Heart Infusion with 6.5% Sodium Chloride, Pkg. of 10 size K tubes

## XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for the isolation- cultivation-identification-maintenance of medial bacteria, vol. I. Williams & Wilkins, Baltimore.
2. Pratt-Rippin, K., and M. Pezzlo. 1992. Identification of commonly isolated aerobic gram-positive bacteria, p. 1.20.1-1.20.47. In H. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
5. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
7. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis.
9. 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.
10. 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. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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