



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Brain Heart Infusion with PABA and 0.1% Agar is used for the examination of blood from patients who have received sulfonamide therapy.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - For *B. fragilis*, prepare dilutions using 24- to 48-h Chopped Meat Medium cultures. For all other organisms prepare dilutions using 5-h **Trypticase™** Soy Broth cultures.
 - Inoculate tubes of the test samples with dilutions of each culture (0.1 mL/tube). The dilution must contain 1000 or less CFU.
 - Incubate tubes with loosened caps at $35 \pm 2^\circ\text{C}$ in an aerobic or anaerobic atmosphere depending upon the organism being cultured.
- Examine tubes at intervals up to 7 days for growth.
- Expected Results

Organisms	ATCC™	Recovery
Anaerobic Incubation		
* <i>Streptococcus pneumoniae</i>	6305	Growth
* <i>Bacteroides fragilis</i>	25285	Growth
Aerobic Incubation		
* <i>Pseudomonas aeruginosa</i>	27853	Growth
* <i>Streptococcus pneumoniae</i>	6305	Growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under "Product Deterioration."
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Incubate uninoculated representative tubes at $20\text{--}25^\circ\text{C}$ and $35 \pm 2^\circ\text{C}$ and examine after 72 h for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brain Heart Infusion (BHI) with PABA is a medium used for the examination of blood from patients who have received sulfonamide therapy. The addition of agar has been found to improve growth of anaerobes.

V SUMMARY AND EXPLANATION

PABA (para-aminobenzoic acid) has been incorporated into the formulation of Brain Heart Infusion to enable the detection of microorganisms in the blood of patients who are undergoing sulfonamide therapy. The addition of 0.1% agar results in a medium with improved ability to support the growth of certain microorganisms (e.g., anaerobes and microaerophiles).

VI PRINCIPLES OF THE PROCEDURE

Unsupplemented BHI broth supports the growth of a broad spectrum of microorganisms, including bacteria and fungi, due to its content of nutritive ingredients, including brain heart infusion, peptones and dextrose. Sodium chloride maintains osmotic equilibrium. PABA neutralizes, by competitive inhibition, the effect of sulfonamide in the inoculum. The inclusion of agar minimizes oxygen distribution by restricting convection currents.

VII REAGENTS

Brain Heart Infusion with PABA and 0.1% Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin.....	15.0 g	Sodium Chloride	5.0 g
Brain Heart, Infusion from (solids).....	6.0 g	Disodium Phosphate.....	2.0 g
Peptic Digest of Animal Tissue.....	6.0 g	p-Aminobenzoic Acid.....	0.05 g
Dextrose.....	3.0 g	Agar.....	1.0 g

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

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

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"¹⁻⁴ 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 $2\text{--}25^\circ\text{C}$. 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.^{5,6} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brain Heart Infusion with PABA and 0.1% Agar

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 1 to 2 drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

Liquid tubed 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 systems.

Alternatively, liquid media may be reduced immediately prior to use by boiling for 10 min with caps loosened and cooling to room temperature before inoculation.

Incubate tubes with loosened caps at $35 \pm 2^\circ\text{C}$ in an aerobic or anaerobic atmosphere depending upon the organism being cultured.

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

Examine tubes at intervals up to 7 days for growth, which 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 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 **BD GasPak EZ** anaerobic system.

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.⁵⁻¹⁰

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.

XII AVAILABILITY

Cat. No.	Description
220842	BBL™ Brain Heart Infusion with PABA and 0.1% Agar, 20 mL, Pkg. of 10 size A tubes

XIII REFERENCES

1. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
2. 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.
3. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
4. 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.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.). 2003. *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11th ed. Mosby, Inc., St. Louis.
7. 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.
8. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
9. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
10. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152 USA
800-638-8663

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