BD BBL™ Phenylethyl Alcohol Agar with 5% Sheep Blood

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QUALITY CONTROL PROCEDURES

I. INTRODUCTION

Phenylethyl Alcohol Agar with 5% Sheep Blood is a selective medium for the isolation of gram-positive organisms from clinical and nonclinical materials.

II. PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using a volumetric pipettor or equivalent method, deliver 0.1 mL of a dilution yielding 30 - 300 CFU to each plate and spread-inoculate using a sterile glass spreader.
   b. Incubate the plates at 35 ± 2°C in an aerobic atmosphere supplemented with 3-5% carbon dioxide.
   c. Include **Trypticase** Soy Agar with 5% Sheep Blood (TSA5%SB) plates as nonselective controls for all organisms.
2. Examine plates after 18-24 h for growth, colony size, hemolytic reactions and selectivity.
3. Expected Results

CLSI Organisms	**ATCC™**	**Recovery**
**Streptococcus pyogenes*	19615	Growth
**Staphylococcus aureus*	25923	Growth
**Proteus mirabilis*	12453	Inhibition (partial)
Additional Organism		
Streptococcus pneumoniae	6305	Growth

*Recommended organism strain for User Quality Control.

III. ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 30 ± 1°C for 84 h and examine for microbial contamination.

PRODUCT INFORMATION

IV. INTENDED USE

Phenylethyl Alcohol Agar with 5% Sheep Blood is a selective medium for the isolation of gram-positive organisms, particularly gram-positive cocci, from specimens of mixed gram-positive and gram-negative flora. The medium should not be used for determination of hemolytic reactions since atypical reactions may be observed.

V. SUMMARY AND EXPLANATION

After having noted that phenylethyl alcohol exhibited a marked inhibitory effect on gram-negative bacteria with only slight effect on gram-positive organisms, Lilley and Brewer incorporated the chemical in an infusion agar base as a selective agent for the isolation of gram-positive bacteria.[1] Phenylethyl Alcohol with 5% Sheep Blood is used in the microbiology laboratory to inhibit gram-negative bacteria, particularly *Proteus*, in specimens containing a mixed bacterial flora.

VI. PRINCIPLES OF THE PROCEDURE

Phenylethyl Alcohol Agar with 5% Sheep Blood supports the growth of gram-positive bacterial species, due

to its content of peptones, which supply nitrogen, carbon, sulfur and trace nutrients. Sodium chloride maintains osmotic equilibrium. Sheep blood is a source of many growth factors. Phenylethyl alcohol is bacteriostatic for gram-negative bacteria since it selectively and reversibly inhibits DNA synthesis.[2]

VII. REAGENTS

Phenylethyl Alcohol Agar with 5% Sheep Blood

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
ß-Phenylethyl Alcohol	2.5 g
Agar	15.0 g
Sheep Blood, defibrinated	5%

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

Warnings and Precautions:

For *in vitro* Diagnostic Use in Taiwan

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[3-6] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2-8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.[7,8]

Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX. PROCEDURE

Material Provided: Phenylethyl Alcohol Agar with 5% Sheep Blood

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge and streak from this inoculated area.

Incubate plates 24-48 h at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.

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

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the

specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on Phenylethyl Alcohol Agar with 5% Sheep Blood is as follows.

Streptococci.	Small, white to gray. May exhibit alpha or beta hemolysis but hemolysis may be atypical.
Enterococci (group D).	Small, but larger than group A streptococci, and blue-gray. May exhibit alpha or beta hemolysis.
Staphylococci.	Large, white to gray or cream to yellow. Hemolytic reactions are variable.
Micrococci	Large, white to gray or yellow to orange. Hemolytic reactions are variable.
Corynebacteria	Small to large, white to gray to yellow. Hemolytic reactions are variable.
Candida	Small, white
Listeria monocytogenes	Small to large, blue-gray and beta-hemolytic
Gram-negative bacteria	No growth to moderate growth; swarming inhibited.

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.[7-12]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII. AVAILABILITY

Cat. No.	Description
212086	BD **BBL™** Phenylethyl Alcohol Agar with 5% Sheep Blood, Pkg. of 20 plates

XIII. REFERENCES

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XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

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