



QUALITY CONTROL PROCEDURES

I INTRODUCTION

HBT (Human Blood Tween™) Bilayer Medium is for the selective isolation and differentiation of *Gardnerella vaginalis*.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Streak the plates for isolation using 10⁻¹ dilutions of 18- to 24-h broth cultures; dilute the *Proteus mirabilis* culture 10⁻² prior to streaking.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with 3–10% carbon dioxide.
 - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls.
2. Examine plates after 18–24 and 42–48 h for growth, hemolysis and colony size.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Gardnerella vaginalis</i>	14018	Fair to heavy growth, colonies surrounded by diffuse, beta hemolysis
* <i>Candida albicans</i>	10231	Inhibition (partial to complete)
* <i>Proteus mirabilis</i>	12453	Inhibition (partial to complete)

*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 35 ± 2°C in an aerobic atmosphere supplemented with 3–10% carbon dioxide for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

HBT (Human Blood Tween) Bilayer Medium is a selective and differential medium used in the primary isolation and presumptive identification of *Gardnerella vaginalis* from clinical specimens.

V SUMMARY AND EXPLANATION

HBT Bilayer Medium was described in 1982 by Totten et al. for the selective isolation and detection of *G. vaginalis* from clinical specimens.¹ The basal medium consists of Columbia Agar supplemented with selective agents and Tween (polysorbate) 80. The top layer is made by adding human blood to the basal medium. Colonies of *G. vaginalis* may be differentiated by a diffuse, beta-hemolytic reaction they produce in the presence of human blood.²⁻⁵ Totten et al. reported that the rate of isolation of *G. vaginalis* was better on HBT Bilayer Medium than on a single layer human blood medium because the characteristic beta-hemolytic reaction used in differentiating the bacterium was more apparent.¹

VI PRINCIPLES OF THE PROCEDURE

Columbia Agar supplies the nutrients necessary to support bacterial growth. Enzymic digests of casein, meat and beef extract provide amino acids and other complex nitrogenous substances. Yeast extract primarily supplies the B-complex vitamins. Corn starch is incorporated to neutralize fatty acids that may be toxic to *G. vaginalis*. Sodium chloride maintains the osmotic equilibrium. Colistin, nalidixic acid (CNA) and amphotericin B are added to facilitate the selective recovery of *G. vaginalis* from clinical specimens. Colistin and nalidixic acid inhibit most gram-negative organisms. Amphotericin B is active against yeasts and filamentous fungi.

A thin layer of the medium with human blood enhances the detection of the characteristic diffuse beta hemolysis of *G. vaginalis*.

VII REAGENTS

HBT Bilayer Medium

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0	g
Peptic Digest of Animal Tissue	5.0	g
Yeast Extract	3.0	g
Beef Extract.....	3.0	g
Peptone	10.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	13.5	g
Polysorbate 80	0.075	g
Colistin.....	10.0	mg
Nalidixic Acid	20.0	mg
Amphotericin B.....	3.0	mg
Human Blood, Anticoagulated (top layer only)	10%	

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

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

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"⁶⁻⁹ 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.

Each donor unit of blood used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAg (hepatitis B surface antigen) and found to be nonreactive.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The FDA recommends such material be handled at a Biosafety Level 2. Biosafety Level 2 is referenced in the Centers for Disease Control/National Institutes of Health manual, *Biosafety in Microbiological and Biomedical Laboratories*.⁸

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.^{4,10} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: HBT Bilayer Medium

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, then streak from this inoculated area.

Incubate the plates in an inverted position (agar side up) at 35 ± 2°C in a CO₂-enriched atmosphere (3–10%) for 24–48 h.

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 sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

G. vaginalis produces small, white colonies surrounded by a beta-hemolytic zone with a diffuse edge.

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.^{4,10-14}

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
297884	BBL™ HBT Bilayer Medium, Pkg. of 10 plates

XIII REFERENCES

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