# HBT BILAYER MEDIUM
## (Human Blood-Polysorbate 80)

### INTENDED USE
Remel HBT Bilayer Medium is a solid medium recommended for use in qualitative procedures for selective isolation and presumptive identification of *Gardnerella vaginalis*.

### SUMMARY AND EXPLANATION
*Gardnerella vaginalis* is a venereally transmitted bacterium that is associated with a clinical syndrome called bacterial vaginosis.[1] This is a condition characterized by a lack of inflammatory cells, an increase in the numbers of *G. vaginalis* and various obligate anaerobes, and a concomitant decrease in lactobacilli observed on gram-stained smears. Media used by previous investigators to recover *G. vaginalis* from clinical specimens include chocolate agar, peptone starch dextrose (PSD) agar, and Columbia colistin-nalidixic acid agar.[2-4] Greenwood and Pickett developed V agar and demonstrated that *G. vaginalis* is beta-hemolytic on agar containing human blood but not on sheep blood agar.[5,6] HBT Bilayer Medium, formulated by Totten et al., consists of a bottom layer of Columbia colistin-nalidixic acid agar supplemented with peptone, amphotericin B, and polysorbate 80 together with a top layer of the same media with 5% human blood added.[7] In further testing, they demonstrated significantly higher isolation rates for *G. vaginalis* than had been obtained with other media.

### PRINCIPLE
Casein and meat peptones supply nutrients, a phosphate buffer maintains pH, and cornstarch neutralizes toxic fatty acids. Colistin, nalidixic acid, and amphotericin B are selective agents inhibitory to most gram-negative rods, yeast, and diphtheroids. Polysorbate 80 enhances the diffusion of beta-hemolysis. The top layer contains the same medium with 5% human blood added. Because the zones of beta-hemolysis are viewed through a shallow layer of blood-containing medium, hemolysis is more distinct and easier to detect than with a single layer medium.

### REAGENTS (CLASSICAL FORMULA)*

| | | | | | |
|---|---|---|---|---|---|
| Casein Peptone | 12.0 | g | Colistin | 10.0 | mg |
| Meat Peptone | 5.0 | g | Nalidixic Acid | 10.0 | mg |
| Sodium Chloride | 5.0 | g | Amphotericin B | 3.0 | mg |
| Beef Extract | 3.0 | g | Polysorbate 80 | 0.33 | ml |
| Yeast Extract | 3.0 | g | Human Blood (added to top layer only) | 5 | % |
| Cornstarch | 1.0 | g | Agar | 10.0 | g |
| | | | Demineralized Water | 1000.0 | ml |

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

### PRECAUTIONS
**WARNING!** This medium contains human blood. Each unit of blood used in the preparation of this product was tested by FDA-licensed procedures and found to be nonreactive for the presence of antibodies to human immunodeficiency virus (anti-HIV), hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis C virus (anti-HCV), and antibody to human T-cell lymphotropic virus, type 1 (anti-HTLV-1). Because no test can guarantee the absence of every infectious agent, all human specimens should be considered potentially infectious and handled accordingly. Information on handling human blood is provided in the CDC/NIH manual, *Biosafety in Microbiology and Biomedical Laboratories*.[8]

### PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If specimen is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plate in 5-10% $CO_2$ at 33-37°C for 24-48 hours.
4. Examine plate for typical colony morphology. On HBT Bilayer Medium, *G. vaginalis* produces small, opaque colonies surrounded by a diffuse zone of beta-hemolysis.

### QUALITY CONTROL
All lot numbers of HBT Bilayer Medium have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| *Gardnerella vaginalis* ATCC® 14018 | $CO_2$, 24-48 h @ 33-37°C | Growth with beta hemolysis |
| *Candida albicans* ATCC® 10231 | Aerobic, 24-48 h @ 33-37°C | Inhibition (partial to complete) |
| *Escherichia coli* ATCC® 25922 | Aerobic, 24-48 h @ 33-37°C | Inhibition (partial to complete) |
| *Proteus mirabilis* ATCC® 12453 | Aerobic, 24-48 h @ 33-37°C | Inhibition (partial to complete) |

### LIMITATIONS
1. Obligately anaerobic strains of *G. vaginalis* have been reported, but studies have demonstrated that anaerobic isolates occur with such infrequency that routine anaerobic incubation of genital cultures is unwarranted.[9]
2. Organisms other than *G. vaginalis* may grow on HBT Bilayer Medium. Additional tests required for presumptive identification include: a Gram-stain demonstrating characteristic morphology (i.e., gram-positive, gram-negative, or gram-variable coccobacilli) and negative catalase and oxidase.[1]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.




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