BD™ Gardnerella Selective Agar with 5% Human Blood

INTENDED USE
BD Gardnerella Selective Agar with 5% Human Blood is a partially selective and differential medium for the isolation of Gardnerella vaginalis from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
Gardnerella vaginalis is considered to be one of the organisms causing vaginitis.1-4 Although the organism may be present in a high percentage of normal women in the vaginal flora, its importance as a cause of non-specific vaginitis (also called bacterial vaginosis) has never been questioned. In symptomatic women, G. vaginalis frequently is associated with anaerobes such as Prevotella bivia, P. disiens, Mobiluncus, Peptostreptococcus, and/or others which are a regular part of the urethral or intestinal, but not vaginal flora. In non-specific vaginitis, normal Lactobacillus flora is reduced or absent. Gardnerella vaginalis is considered to be the indicator organism for non-specific vaginitis which, in fact, is a polymicrobial infection.3,4 Although non-culture methods such as a direct Gram stain have been recommended in recent years for genital specimens, culture is still preferred by many laboratories.1,5 G. vaginalis may also be responsible for a variety of other diseases such as preterm birth, chorioamnionitis, urinary tract infections, newborn infections, and septicemia.6
The detection of the organism on routinely used media is difficult since Gardnerella and other bacteria such as lactobacilli and streptococci may produce alpha hemolysis on sheep blood containing media. On media containing human blood, however, Gardnerella vaginalis produces a characteristic beta-hemolysis.1-7 Since Gardnerella vaginalis, although often considered Gram negative, is a Gram positive bacterium, it may be selected on media containing colistin and nalidixic acid.1,10,11
BD Gardnerella Selective Agar with 5% Human Blood is based on Columbia CNA Agar which provides nutrients and the inhibitors colistin and nalidixic acid which inhibit Gram negative but not Gram positive bacteria. The medium is supplemented with Proteose Peptone to improve the growth of Gardnerella. Amphotericin B is added to reduce the growth of yeasts (e.g. Candida) which are also frequently present in vaginal specimens. Human blood is added as a nutrient and to detect the characteristic diffuse beta hemolysis of the organism.

REAGENTS
BD Gardnerella Selective Agar with 5% Human Blood
Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0</td>
</tr>
<tr>
<td>Proteose Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.01</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>0.01</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.004</td>
</tr>
<tr>
<td>Human Blood, defibrinated</td>
<td>5%</td>
</tr>
<tr>
<td>pH 7.3 +/- 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.
PRECAUTIONS
For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

WARNING:
Contains potentially biohazardous material. The human blood used for the preparation of this medium has been tested for the presence of HIV, HBsAg and other types of hepatitis using the criteria and methods presently used by blood banks to screen human blood used for transfusion and was found to be nonreactive. Because no test method currently available can offer complete assurance that HIV, hepatitis and other infectious agents are absent, specimens and this medium should be handled as though capable of transmitting an infectious disease. Therefore, it is recommended to handle this medium according to Biosafety Level 2.

STORAGE AND SHELF LIFE
On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL
Inoculate representative samples with the following strains (for details, see GENERAL INSTRUCTIONS FOR USE document). Incubate in a carbon dioxide enriched atmosphere for 48 to 72 hours at 35 to 37° C. Alternatively, the inoculated medium may be incubated in a microaerobic atmosphere, e.g., by using the BD CampyPak™ system.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardnerella vaginalis</td>
<td>Growth good to excellent; small grey white colonies surrounded by a diffuse beta-hemolysis</td>
</tr>
<tr>
<td>ATCC 14018</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Inhibition complete</td>
</tr>
<tr>
<td>ATCC 25922</td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Inhibition partial to complete; no swarming</td>
</tr>
<tr>
<td>ATCC 14153</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>ATCC 10231</td>
<td></td>
</tr>
<tr>
<td>Uninoculated Red</td>
<td>Red (blood color)</td>
</tr>
</tbody>
</table>

PROCEDURE
Materials Provided
BD Gardnerella Selective Agar with 5% Human Blood (90 mm Stacker™ plates).
Microbiologically controlled.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
Vaginal swabs or other specimens suspected to contain G. vaginalis are suitable. Ideally, two swabs should be collected, one for the culture and one for a direct Gram stain. A transport medium (e.g., BD Port-A-Cul™) must be used if the specimens are not immediately processed. (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).
Test Procedure
Streak the specimens onto **BD Gardnerella Selective Agar with 5% Human Blood** by an approved dilution technique. Also inoculate **BD Columbia Agar with 5% Sheep Blood**. Incubate both plates in a CO₂ enriched aerobic atmosphere at 36 +/- 2° C for 48 to 72 hours. Also, a direct Gram stain should be prepared from all vaginal specimens.¹²₅

Results
After the incubation, inspect **BD Gardnerella Selective Agar with 5% Human Blood** for the presence of small to medium colonies, surrounded by a diffuse beta-hemolysis. Compare this result with the growth obtained on the **BD Columbia Agar with 5% Sheep Blood** plate. If beta hemolytic colonies appear only on the selective **Gardnerella** medium, the presence of **G. vaginalis** is highly probable. If beta hemolytic colonies appear on both media, make sure by a Gram stain that the organism on the selective **Gardnerella** medium is a Gram variable, small, diphtheroid rod. The presence of cocci in the Gram stain from a beta hemolytic colony on **BD Gardnerella Selective Agar with 5% Human Blood** indicates that the isolated organisms are beta hemolytic streptococci or staphylococci, but not **G. vaginalis**. Alpha and non-hemolytic colonies can be disregarded. On media containing sheep blood, **Gardnerella** produces small alpha- or non-hemolytic colonies. In case of a mixed culture with other beta-hemolytic organisms, eventually subculture isolated colonies from **BD Gardnerella Selective Agar with 5% Human Blood** again on both media and incubate as indicated above. Further biochemical tests are necessary for the final identification of the isolates. Consult the references.¹²₇₉

In the direct Gram stain of vaginal specimens, “clue cells” (= vaginal epithelial cells loaded with masses of short, Gram variable rods) will be visible if the patient suffers from bacterial vaginosis.¹₅

Interpretation of the Results
If **G. vaginalis** has been isolated from vaginal specimens, the resulting diagnosis must be carefully compared with the subjective and objective clinical symptoms. Typically, if **Gardnerella** is the cause of bacterial vaginosis, the patients complain of an increased, malodorous discharge. The pH of the secretions is >4.5, and “clue cells” are present in the Gram stain. For details, consult the references.¹³₅

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
**BD Gardnerella Selective Agar with 5% Human Blood** is a partially selective medium for the isolation of **Gardnerella vaginalis**.⁴¹¹ **G. vaginalis** will produce beta-hemolytic colonies on the medium with human blood, and alpha-hemolytic colonies on media containing sheep blood. **BD Gardnerella Selective Agar with 5% Human Blood** is not inhibitory to other Gram positive organisms such as streptococci, staphylococci, or **Listeria** which may or may not produce beta hemolysis on media containing human blood and may also occur in vaginal specimens. Therefore, microscopic and biochemical tests are required to confirm the identity of an isolate as **G. vaginalis**.

The presence of **G. vaginalis** in a vaginal specimen does not necessarily indicate that the isolated organism is the cause of an infection.

Do not use **BD Gardnerella Selective Agar with 5% Human Blood** for the determination of hemolytic reactions of organisms other than **Gardnerella vaginalis**.

REFERENCES


PACKAGING/AVAILABILITY
BD Gardnerella Selective Agar with 5% Human Blood
Cat. No. 254094 Ready-to-use Plated Media, cpu 20
Cat. No. 257664 Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION
For further information please contact your local BD representative.

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