BBL™ Brucella Agar with 5% Horse Blood
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Rx Only

I INTRODUCTION
BD BBL™ Brucella Agar with 5% Horse Blood is a culture medium which is used for the isolation and growth of both fastidious and nonfastidious bacterial species.

II PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with the cultures diluted to contain 10³–10⁴ CFU per 0.01 mL.
2. Incubate at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
3. Include a Chocolate II Agar plate as a control for the Haemophilus strain only.
4. Examine plates after 18–24 h for amount of growth, colony size and hemolytic reactions.
5. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>19615</td>
<td>Moderate to heavy growth, beta hemolysis</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>6305</td>
<td>Moderate to heavy growth, alpha hemolysis</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>Moderate to heavy growth. Colonies may or may not be hemolytic.</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Moderate to heavy growth</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12022</td>
<td>Moderate to heavy growth. Colonies large, shiny and gray and may or may not be hemolytic.</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>10211</td>
<td>Moderate to heavy growth. Colonies nonhemolytic, grayish, small and translucent with a distinct “mousy” odor.</td>
</tr>
</tbody>
</table>

*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 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

IV INTENDED USE
BD BBL Brucella Agar with 5% Horse Blood is used in qualitative procedures for the isolation and cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

V SUMMARY AND EXPLANATION
Brucella Agar was developed for the cultivation of Brucella species from diagnostic specimens such as blood, and from foods and other potentially contaminated material. BD BBL Brucella Agar with 5% Horse Blood plates are particularly useful for the cultivation of the more fastidious aerobic and anaerobic microorganisms including streptococci, pneumococci, Listeria, Neisseria meningitidis and Haemophilus influenzae.

VI PRINCIPLES OF THE PROCEDURE
This medium supports the growth of fastidious microorganisms due to its content of peptones, dextrose, yeast extract and blood. The peptones supply organic nitrogen. The yeast extract is a potent source of the B vitamins. Dextrose is utilized as an energy source.

Sodium Chloride and Sodium Bisulfite are added to the preparation to provide a source of sodium and to act as buffer agents. The defibrinated horse blood supplies both the X and V factors which are growth requirements for certain organisms; e.g., Haemophilus influenzae. Sheep blood is not suitable for this purpose in that it contains enzymes which inactivate the nicotinamide adenine dinucleotide (NAD) which is the V factor.

Defibrinated horse blood may give hemolytic reactions different from sheep blood. Some enterococci give beta-hemolytic reactions on horse blood but non-beta-hemolytic reactions on sheep blood. This may result in the isolate being mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a BD Taxo™ A disc and it also should be grouped serologically.

Beta-hemolytic streptococci and Haemophilus hemolyticus may be differentiated by performing a Gram stain on a smear prepared from the colony.

VII REAGENTS
BD BBL Brucella Agar with 5% Horse Blood

Approximate 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>10.0 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.9 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Bisulfite</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Horse Blood, defibrinated</td>
<td>5%</td>
</tr>
</tbody>
</table>

*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”5-8 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.
Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.  

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

**IX PROCEDURE**

**Material Provided:** BD BBL Brucella Agar with 5% Horse 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; then streak from this inoculated area.
- Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5% CO₂.
- Incubate plates at 35 ± 2 °C for 18–24 h 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 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. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

**XI LIMITATION 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.

**XII AVAILABILITY**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221547</td>
<td>BD BBL™ Brucella Agar with 5% Horse Blood, Pkg. of 20 plates</td>
</tr>
<tr>
<td>221548</td>
<td>BD BBL™ Brucella Agar with 5% Horse Blood, Ctn. of 100 plates</td>
</tr>
</tbody>
</table>

**XIII REFERENCES**


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