HD Heart Infusion Agar with 5% Sheep Blood

INTENDED USE
BD Heart Infusion Agar (=HIA) with 5% Sheep Blood is a general purpose medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. The formula for HIA contains beef heart infusion and Tryptose, which is better suited to the nutritional requirements of pathogenic bacteria than peptones. Sodium chloride maintains the osmotic balance of the medium. The addition of 5% sheep blood provides additional growth factors and is used to determine hemolytic reactions. Due to its low carbohydrate content, BD Heart Infusion Agar with 5% Sheep Blood provides excellent hemolytic reactions of streptococci, staphylococci, and others, but colonies are often smaller than on media containing other blood agar bases, e.g., Columbia Agar.

REAGENTS
BD Heart Infusion Agar with 5% Sheep Blood
Formula* Per Liter Purified Water
<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Heart, Infusion from</td>
<td>500.0 g</td>
</tr>
<tr>
<td>Bacto™ Tryptose</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Sheep Blood, defibrinated</td>
<td>5%</td>
</tr>
<tr>
<td>pH 7.4 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS
IVD. 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.

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 the inoculated plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Examine plates after 18 to 24 h for amount of growth, colony size and hemolytic reactions.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC™ 25922</td>
<td>Growth; may be beta hemolytic</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>Growth</td>
</tr>
</tbody>
</table>
**Staphylococcus aureus ATCC 25923**
Growth; beta hemolysis

**Streptococcus pneumoniae ATCC 6305**
Growth; alpha hemolysis

**Streptococcus pyogenes ATCC 19615**
Growth; beta hemolysis

**Uninoculated**
Red (Blood color)

**PROCEDURE**

**Materials Provided**
BD Heart Infusion Agar with 5% Sheep Blood (90 mm Stacker™ plates). Microbiologically controlled.

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

**Specimen Types**
This is a universal isolation medium and can be used for all types of aerobically incubated bacteriological specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

**Test Procedure**
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 for isolation from this inoculated area. Appropriate selective media for detection of specific pathogens, e.g., **BD MacConkey II Agar** for the isolation of **Enterobacteriaceae**, should be included.

Since many pathogens require carbon dioxide on primary isolation, **BD Heart Infusion Agar with 5% Sheep Blood** plates should be incubated in an aerobic atmosphere containing approximately 3 to 10% CO₂. Incubate plates at 35 ± 2°C for 18 to 72 h. Read for the first time after 18 to 24 hours and re-incubate if necessary.

**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 micro-organisms 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.

The number of species growing on this medium is very large. Therefore, no comprehensive information on the appearance of the organisms on this medium can be given here. Consult the appropriate references for information on appearance and further differential tests of the organisms isolated.⁴-⁵

Typical colonial morphology of frequently isolated organisms on **BD Heart Infusion Agar with 5% Sheep Blood** is as follows:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococci (non-group D)</strong></td>
<td>Tiny to small, white to grayish. Beta or alpha hemolysis</td>
</tr>
<tr>
<td><strong>Enterococci (Group D)</strong></td>
<td>Small, but larger than group A streptococci, grayish. Alpha (rarely beta) hemolysis</td>
</tr>
<tr>
<td><strong>Staphylococci</strong></td>
<td>Medium-sized to large, white to gray or cream to yellow, with or without hemolysis</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>Small, white to grayish, with weak beta hemolysis</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>Medium-sized to large, grey colonies, with or without hemolysis</td>
</tr>
<tr>
<td><strong>Candida spp.</strong></td>
<td>Tiny to small, white</td>
</tr>
</tbody>
</table>

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**
**BD Heart Infusion Agar with 5% Sheep Blood** is used for the isolation and cultivation of many aerobically growing micro-organisms, such as **Enterobacteriaceae, Pseudomonas** and other non-fermenting Gram negative rods, streptococci, staphylococci, **Candida** species, and many
others. Due to its low carbohydrate content, it provides excellent hemolytic reactions of streptococci, staphylococci, and others, but colonies are often smaller and organisms may need a longer incubation than on media containing other blood agar bases, e.g., Columbia Agar which, on the other hand, often shows a greenish beta-hemolysis of streptococci.

**BD Heart Infusion Agar with 5% Sheep Blood** lacks V factor (nicotinamide adenine dinucleotide, NAD) since sheep blood contains NADase which destroys the NAD. For this reason, *Haemophilus influenzae* which requires both the X and V factors, will not grow on this medium. *Neisseria gonorrhoeae* does not grow well on this medium. Instead, **BD Chocolate Agar (GC II Agar with IsoVitaleX)** should be used for the recovery of this species. Also, the medium is not suitable for the isolation and growth of *Mycobacterium, Legionella, Bordetella* and other organisms with highly specific nutritive requirements. The number and types of bacterial species occurring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated or newly described microorganisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.4,5

**REFERENCES**


**PACKAGING/AVAILABILITY**

**BD Heart Infusion Agar with 5% Sheep Blood**
Cat. No. 257026 Ready-to-use Plated Media, cpu 20

**FURTHER INFORMATION**

For further information please contact your local BD representative.