



BD™ 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.¹ 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.^{2,3} 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

Beef Heart, Infusion from	500.0 g
Bacto™ Tryptose	10.0
Sodium Chloride	5.0
Bacto Agar	15.0
Sheep Blood, defibrinated	5%

pH 7.4 ± 0.2

*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.

Strains	Growth Results
<i>Escherichia coli</i> ATCC™ 25922	Growth; may be beta hemolytic
<i>Enterococcus faecalis</i> ATCC 29212	Growth

<i>Staphylococcus aureus</i> ATCC 25923	Growth; beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC 6305	Growth; alpha hemolysis
<i>Streptococcus pyogenes</i> 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:

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

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 micro-organisms, 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

1. Huntoon, F. M. 1918. "Hormone" Medium. A simple medium employable as a substitute for serum medium. J. of Infect. Dis. 23: 169-172.
2. MacFaddin, J.F. 1985. Media for the isolation – cultivation – maintenance of medical bacteria. Volume 1. Williams and Wilkins, Baltimore, London.
3. Downes, F.P., and K. Ito. 2001. Compendium of methods for the microbiological examination of foods. 4th edition. American Public Health Association (APHA). Washington, D.C. USA.
4. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol.1, p. 1.6.1-1.6.7. American Society for Microbiology, Washington, D.C.
5. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

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.



Becton Dickinson GmbH

Tullastrasse 8 – 12

D-69126 Heidelberg/Germany

Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16

Reception_Germany@europe.bd.com

<http://www.bd.com>

<http://www.bd.com/europe/regulatory/>

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