BD™ Brucella Agar with 5% Horse Blood

INTENDED USE

BD Brucella Agar with 5% Horse Blood is used for the isolation and growth of fastidious and nonfastidious bacterial species, including *Brucella*, from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Brucellosis is a zoonotic disease with a domestic-animal reservoir. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1-3]

Brucella Agar was developed for the cultivation of *Brucella* species from diagnostic specimens such as blood, and from foods and other potentially contaminated material. Brucella Agar is prepared according to the APHA formula for Albimi Broth, which is used for the isolation of *Brucella* species.[4-7] **BD Brucella Agar with 5% Horse Blood** is particularly useful for the cultivation of all more fastidious aerobic, microaerophilic, and anaerobic microorganisms including streptococci, pneumococci, *Listeria*, *Brucella, Neisseria meningitidis, Haemophilus influenzae* and *Helicobacter pylori*.[1,6-10]

This medium supports the growth of fastidious micro-organisms 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. Glucose is utilized as an energy source. Horse blood supplies both the X and V factors which are growth requirements for certain organisms; e.g. *Haemophilus influenzae*.

It should be noted that beta-hemolytic reactions depend on the type of blood added; as an example, enterococci which only very rarely hemolyse sheep blood, will produce a well visible beta hemolysis on horse blood. *Staphylococcus aureus* which is usually beta hemolytic on sheep blood, will often be non-hemolytic on horse blood. Beta-hemolytic streptococci and *Haemophilus haemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.

REAGENTS

BD Brucella Agar with 5% Horse Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g
Peptic Digest of Animal Tissue	10.0
Glucose	1.0
Yeast Extract	2.0
Sodium Chloride	5.0
Sodium Bisulfite	0.1
Agar	15.0
Horse Blood, defibrinated	5%

pH 7.0 +/- 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.

Laboratory procedures involving *Brucella* require special equipment and techniques to minimize biohazards.[1,9] Biosafety Level 3 is required for handling of specimens and cultures.

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**
Streptococcus pyogenes ATCC 19615	Growth good to excellent, beta hemolysis
Streptococcus pneumoniae ATCC 6305	Growth good to excellent, alpha hemolysis
Staphylococcus aureus ATCC 25923	Growth good to excellent; may or may not be beta hemolytic
Haemophilus influenzae ATCC 10211	Growth good to excellent; small to medium transparent colonies, no hemolysis
Shigella flexneri ATCC 12022	Growth good to excellent, colonies large, shiny and gray
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Brucella Agar with 5% Horse Blood (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD Brucella Agar with 5% Horse Blood can be used for all types of specimens if fastidious and slow-growing organisms are suspected to be involved in an infection. Optimal specimens for the diagnosis of brucellosis include blood and bone marrow. For collection and transport of such specimens, consult the references.[1,8,9] Specimens from patients with suspected brucellosis should be labelled appropriately so that laboratory exposures to this agent can be minimized. This medium should not be used as a universal primary isolation medium (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 from this inoculated area.
Since many pathogens require carbon dioxide on primary isolation, plates should be incubated in an atmosphere containing approximately 5% CO_2.
Incubate plates at 35± 2°C for 18 to 24 h in an aerobic atmosphere supplemented with carbon dioxide. For the isolation of *Brucella*, incubation at 35 to 37° C for 3 to 7 days or longer may be necessary. Refer to the appropriate texts.[1,8]

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. For the isolation and identification of *Brucella*, consult the references.[1,2,8]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Brucella Agar with 5% Horse Blood is a standard formulation used for the isolation of fastidious bacteria, streptococci, pneumococci, *Listeria*, *Brucella*, *Neisseria meningitidis,* and *Haemophilus influenzae*.[1,2,6-8] Also, it is recommended as a primary nonslective isolation medium for *Helicobacter pylori*.[10] Since it is not selective, many fastidious and nonfastidious microorganisms will grow on the medium. For the isolation of specific micro-organisms from heavily contaminated specimens, appropriate selective media must also be used. The medium may also be used as a subculture medium for blood cultures, e.g., in cases of suspected brucellosis.[1,3,9]

This medium is not generally used as a universal primary isolation medium. Formulations based on Columbia or Trypticase™ Soy Agar, supplemented with blood, are usually preferred for this purpose.[2,6,8] Although this medium may also be used for strict anaerobes, enriched media, e.g., **BD Brucella Blood Agar with Hemin and Vitamin K1** are preferred for this purpose.[6]

Further differentiation and identification procedures are needed to identify the organisms isolated on this medium.[1,8]

REFERENCES

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PACKAGING/AVAILABILITY

BD Brucella Agar with 5% Horse Blood

Cat. No. 255027 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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