

R<sub>x</sub> Only

## QUALITY CONTROL PROCEDURES

## 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  $10^3$ – $10^4$  CFU per 0.01 mL.
2. Incubate at  $35 \pm 2$  °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

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

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

## PRODUCT INFORMATION

## 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. 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.<sup>1</sup>

Defibrinated horse blood may give hemolytic reactions different from sheep blood.<sup>1</sup> Some enterococci give beta-hemolytic reactions on horse blood but non-beta-hemolytic reactions on sheep blood.<sup>2</sup> 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.<sup>1</sup> 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

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

\*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"<sup>3-6</sup> 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.<sup>5</sup>

**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.<sup>1,7</sup>

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% CO<sub>2</sub>.

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.<sup>1,7,8</sup>

## XII AVAILABILITY

Cat. No.	Description
221547	BD BBL™ Brucella Agar with 5% Horse Blood, Pkg. of 20 plates
221548	BD BBL™ Brucella Agar with 5% Horse Blood, Ctn. of 100 plates

## XIII REFERENCES

1. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Teixeira, L.M. and R.R. Facklam. 2003. *Enterococcus*, p. 422-433. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, PA.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
5. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
8. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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