



BD BBL™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁

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QUALITY CONTROL PROCEDURES (Optional)

R_x Only

I INTRODUCTION

BD BBL™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ (Brucella Agar) is a highly nutritious medium for the isolation and cultivation of strict anaerobes from clinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Reduce all Brucella Agar plates overnight at room temperature in a **BD GasPak™** EZ anaerobic system.
2. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak the plates for isolation. Use an 18–24 h broth culture diluted to yield 10⁴–10⁵ CFU per plate.
NOTE: Cultures must be handled quickly to avoid prolonged exposure to oxygen. Total exposure time should not exceed 20 min.
 - b. Incubate strains at 35 ± 2 °C in an anaerobic atmosphere for 48–72 h (**BD GasPak** EZ anaerobic system).
 - c. Include Columbia Agar with 5% Sheep Blood plates as controls for all organisms and plates of a previously tested lot of Brucella Agar as controls.
 - d. Incubate the Columbia Agar with 5% Sheep Blood plate controls aerobically at 35 ± 2 °C and all other plates anaerobically at 35 ± 2 °C.
3. Examine all inoculated plates at 48 and 72 h for amount of growth, colony size and hemolytic reactions.
4. Expected Results

| Organisms | ATCC® | Recovery |
|--------------------------------------|-------|--|
| * <i>Bacteroides fragilis</i> | 25285 | Growth fair to heavy, grey colonies |
| * <i>Clostridium perfringens</i> | 13124 | Growth fair to heavy, large, lobate, grey-white colonies; beta hemolysis |
| <i>Fusobacterium nucleatum</i> | 25586 | Growth fair to heavy |
| <i>Peptostreptococcus anaerobius</i> | 27337 | Growth fair to heavy |

*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 7.0 ± 0.2.
4. Note the firmness of the plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BD BBL Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ is used for the isolation and cultivation of fastidious, obligately anaerobic organisms.

V SUMMARY AND EXPLANATION

BD BBL Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁ is an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms. Nonselective media are used to isolate organisms present in low numbers and to provide an indication of the numbers and types of organisms present in the specimen or sample.

VI PRINCIPLES OF THE PROCEDURE

Brucella Agar supports the growth of fastidious microorganisms due to its content of peptones, dextrose and yeast extract. The sheep blood constituents, hemin and vitamin K₁ provide growth factors required by certain obligate anaerobes.^{1,2}

VII REAGENTS

BD BBL Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁

Approximate Formula* Per Liter Purified Water

| | | | |
|--------------------------------------|--------|---------------------------------|---------|
| Pancreatic Digest of Casein | 10.0 g | Sodium Bisulfite..... | 0.1 g |
| Peptic Digest of Animal Tissue | 10.0 g | Agar..... | 15.0 g |
| Dextrose | 1.0 g | Hemin | 0.005 g |
| Yeast Extract | 2.0 g | Vitamin K ₁ | 0.01 g |
| Sodium Chloride..... | 5.0 g | Sheep 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.

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

Refer to appropriate texts for details of specimen collection and handling procedures.²⁻¹¹

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC-NIH recommendations, CLSI guidelines and local institution guidelines for any potentially infectious human serum, blood or other body fluids. Prior to discarding sterilize specimen containers and other contaminated materials by autoclaving.

IX PROCEDURE

Material Provided: **BD BBL** Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K₁

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.

This medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18–24 h.⁸ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak EZ** anaerobic systems.¹²

Streak the specimen as soon as possible after it is received in the laboratory. Minimize exposure to air. Plated media should be inoculated using the streak plated method in order to obtain pure cultures from specimens containing mixed flora. An enrichment broth such as **BD BBL**™ Enriched Thioglycollate Medium should be inoculated at the same time as the primary isolation plates.

With liquid specimens media should be inoculated with one drop of the specimen. Tissue specimens should be minced and then ground in sterile broth such as **BD BBL** Enriched Thioglycollate Medium before inoculation. Inoculation is then performed as for liquid specimens. Swab specimens may be rolled onto the first quadrant of a plated medium and then used to inoculate a liquid medium. Alternatively, the swab may be “scrubbed” in a small volume of reduced broth and the broth used to inoculate media as performed with liquid specimens.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 h).¹³ Incubation should be at 35 ± 2 °C for at least 48 h and up to 7 days. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as the **BD GasPak** anaerobic indicator.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

X RESULTS

After sufficient incubation, the 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.

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Bacteroides* group should fluoresce orange to brick-red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, follow established procedures.¹⁴ Those colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical and serological procedures. Consult appropriate texts for detailed information and recommended procedures.^{6-9,11,15}

XII AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 297848 | BD BBL ™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K ₁ , Pkg. of 20 plates |
| 297716 | BD BBL ™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K ₁ , Pkg. of 100 plates |

XIII REFERENCES

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Technical Information: In the United States contact BD Technical Service and Support at 1.800.638.8663 or www.bd.com.

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