



BD BBL™ CDC Anaerobe 5% Sheep Blood Agar

L007357 • Rev. 11 • October 2016

QUALITY CONTROL PROCEDURES (Optional)

R_x Only

I INTRODUCTION

BD BBL™ CDC Anaerobe 5% Sheep Blood Agar is an enriched, nonselective culture medium particularly useful for the isolation and cultivation of obligate anaerobes from clinical specimens. It supports the growth of a wide variety of obligately anaerobic, facultatively anaerobic, microaerophilic and aerobic bacteria.

II PERFORMANCE TEST PROCEDURE

1. Reduce all anaerobic agar plates overnight at room temperature in a BD GasPak™ EZ anaerobic system.
2. Preparation of inocula
 - a. Prepare the obligate anaerobe test cultures by swabbing the growth from a 2- to 5-day BD BBL CDC Anaerobe 5% Sheep Blood Agar plate into a tube containing 5 mL of reduced Enriched Thioglycollate Medium containing vitamin K₁ and hemin. Mix well and adjust to a turbidity comparable to a 0.5 McFarland standard.

NOTE: Cultures must be handled quickly to avoid prolonged exposure to oxygen. Total exposure time should not exceed 20 min.
 - b. Use an 18- to 24-h broth culture of the facultatively anaerobic organism diluted 10⁻¹.
3. Inoculation of the plates
 - a. Using a volumetric pipettor or equivalent method, deliver 0.05 mL of the appropriate inoculum to each plate or sector and streak for isolation.
 - b. Include BD Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plates as controls for all organisms and plates of a previously tested lot of BD BBL CDC Anaerobe 5% Sheep Blood Agar as controls for the obligate anaerobes.
 - c. Incubate the TSA II plate controls aerobically at 35 ± 2 °C and all other plates anaerobically (BD GasPak EZ anaerobic system) at 35 ± 2 °C.
4. Examine all inoculated plates at 24–48 h for amount of growth, colony size, pigmentation and hemolytic reactions. Observe *Porphyromonas* and *P. intermedia* strains under UV light (365 nm) for fluorescence up to 72 h (if necessary).
5. Expected Results

CLSI Organisms	ATCC®	Recovery
* <i>Bacteroides fragilis</i>	25285	Growth
* <i>Clostridium perfringens</i>	13124	Growth, beta hemolysis
* <i>Fusobacterium nucleatum</i>	25586	Growth
* <i>Peptostreptococcus anaerobius</i>	27337	Growth
* <i>Prevotella melaninogenica</i>	25845	Growth
Additional Organisms		
<i>Prevotella intermedia</i>	25611	Fair to heavy growth at 72 h. Colonies are off-white to tan, circular, entire, convex, opaque. Bright pink to orange to brick-red fluorescence under UV light.**
<i>Staphylococcus aureus</i>	25923	Moderate to heavy growth at 72 h. Colonies are light gray, opaque, smooth, low convex, butyrous with entire edge. May be hemolytic.

*Recommended organism strain for User Quality Control.

**Fluorescence is seen only in young colonies. Pigment develops slowly and may obscure fluorescence.

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 7.5 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BD BBL CDC Anaerobe 5% Sheep Blood Agar is used for the isolation and cultivation of fastidious and slow growing, obligately anaerobic bacteria from a variety of clinical and nonclinical materials. It also supports good growth of most aerobic, facultatively anaerobic and microaerophilic bacteria if incubated appropriately.

V SUMMARY AND EXPLANATION

The isolation of obligately anaerobic bacteria from clinical and nonclinical materials requires the use of selective, nonselective and enrichment media.¹ The choice of media to be employed is based upon the type of material and the results of direct microscopic observation. 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. Selective media are employed to facilitate recovery of the desired organisms present in mixed populations.

BD BBL CDC Anaerobe 5% Sheep Blood Agar was formulated by Dowell et al. of the Centers for Disease Control and Prevention as an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms, particularly those found in clinical materials.²⁻⁵ The medium employs BD Trypticase Soy Agar supplemented with additional agar, yeast extract, vitamin K₁, hemin, cystine and 5% sheep blood. Improved growth of *Prevotella melaninogenica*, *Fusobacterium necrophorum*, *Clostridium haemolyticum*, as well as certain strains of *Actinomyces israelii* and *Bacteroides thetaiotaomicron*, has been demonstrated on this medium.³ Furthermore, less smooth to rough colonial variation has been reported on this medium than on Schaedler Blood Agar.⁶

VI PRINCIPLES OF THE PROCEDURE

BD BBL CDC Anaerobe 5% Sheep Blood Agar is a highly nutritious medium due to its content of peptones, yeast extract, hemin, vitamin K₁ and sheep blood. The peptones provide nitrogenous growth factors, carbon, sulfur and trace ingredients. Yeast extract is an important source of B vitamins. Sodium chloride maintains osmotic equilibrium. Sheep blood constituents, hemin, cystine and vitamin K₁ provide growth factors required by certain obligate anaerobes.^{2,6-8}

VII REAGENTS

BD BBL CDC Anaerobe 5% Sheep Blood Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0 g	Hemin	0.005 g
Papaic Digest of Soybean Meal	5.0 g	Vitamin K ₁	0.01 g
Sodium Chloride	5.0 g	L-Cystine	0.4 g
Agar	20.0 g	Sheep Blood, defibrinated	5%
Yeast Extract	5.0 g		

*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"⁹⁻¹² 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.

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.^{13,14} 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 CDC Anaerobe 5% Sheep Blood Agar

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. Minimize exposure to air. With liquid specimens, media should be inoculated with 1 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 plated media and then used to inoculate liquid media. 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.

This medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 6–24 h.¹⁵ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak EZ** gas generating systems.

Plated media should be inoculated using the streak plate 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.

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).¹⁶ 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** disposable 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 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 semiquantitatively scored on the basis of growth in each of the streaked areas.

Examine colonies using a dissecting microscope and a long-wave UV lamp (colonies of the pigmented *Porphyromonas-Prevotella* species should fluoresce orange to brick-red under UV light). Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, inoculate the following media:¹⁵

1. One anaerobe blood agar plate to be incubated anaerobically.
2. One aerobic blood agar (or chocolate agar) plate to be incubated in an aerobic atmosphere enriched with carbon dioxide. The chocolate agar is particularly needed to distinguish nutritionally-fastidious *Haemophilus* species and other bacteria which will grow on anaerobe blood agar incubated anaerobically and on chocolate agar under increased carbon dioxide tension but which fail to grow on blood agar in the presence of carbon dioxide or in air.
3. One aerobic blood agar plate to be incubated aerobically without added carbon dioxide.

4. Tubes of Enriched Thioglycollate Medium and/or Cooked Meat Medium and a tube of Peptone Yeast Extract Glucose Broth. Incubate all cultures at $35 \pm 2^\circ\text{C}$ for a minimum of 24 h and up to 7 days. Record the relationship to oxygen as either obligate anaerobe or nonanaerobe (aerotolerant anaerobe, microaerophilic, or facultative anaerobe).¹⁵ Colonies of the type(s) which prove to be obligate anaerobes can be further studied using the corresponding broth cultures. Organisms failing to grow on the aerobic subculture plates may be presumed to be obligately anaerobic in terms of their oxygen requirements.

XI LIMITATIONS 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.^{5,13-22}

XII AVAILABILITY

Cat. No.	Description
221733	BD BBL™ CDC Anaerobe 5% Sheep Blood Agar, Pkg. of 20 plates
221734	BD BBL™ CDC Anaerobe 5% Sheep Blood Agar, Ctn. of 100 plates

XIII REFERENCES

- Dowell, V.R., Jr. 1975. Wound and abscess specimens, p. 70-81. In A. Balows (ed.), *Clinical microbiology. How to start and when to stop*. Charles C. Thomas, Springfield, Ill.
- Dowell, V.R., Jr., G.L. Lombard, F.S. Thompson, and A.Y. Armfield. 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta.
- Dowell, V.R., Jr., and T.M. Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta.
- Rodloff, A.C., P.C. Appelbaum, and R.J. Zabransky. 1991. Cumitech 5A, Practical anaerobic bacteriology. Coordinating ed., A.C. Rodloff. American Society for Microbiology, Washington, D.C.
- Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, 2nd ed., vol. 1, 2, and 3. American Society for Microbiology, Washington, D.C.
- Starr, S.E., G.E. Killgore, and V.R. Dowell, Jr. 1971. Comparison of Schaedler agar and Trypticase soy-yeast extract agar for the cultivation of anaerobic bacteria. *Appl. Microbiol.* 22:655-658.
- Gibbons, R.J., and J.B. MacDonald. 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J. Bacteriol.* 80:164-170.
- Wilkins, T.D., S.L. Chalgren, F. Jimenez-Ulate, C.R. Drake, Jr., and J.L. Johnson. 1976. Inhibition of *Bacteroides fragilis* on blood agar plates and reversal of inhibition by added hemin. *J. Clin. Microbiol.* 3:359-363.
- National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
- 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.
- U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
- 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.
- 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.
- Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
- Allen, S.D., J.A. Siders, and L.M. Marler. 1985. Isolation and examination of anaerobic bacteria, p. 413-433. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
- Martin, W.J. 1971. Practical method for isolation of anaerobic bacteria in the clinical laboratory. *Appl. Microbiol.* 22:1168-1171.
- Holdeman, L.V., E.P. Cato, and W.E.C. Moore (ed.). 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
- Engelkirk, P.G., J. Duben-Engelkirk, and V.R. Dowell, Jr. 1992. Principles and practice of clinical anaerobic bacteriology. Star Publishing Co., Belmont, Calif.
- Summanen, P., E.J. Baron, D.M. Citron, C.A. Strong, H.M. Wexler, and S.M. Finegold. 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, Calif.
- Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
- MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
- Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.

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

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA

ATCC is a registered trademark of the American Type Culture Collection.
© 2016 BD. BD, the BD Logo and all other trademarks are property of Becton, Dickinson and Company.