BD BBL™ CDC Anaerobe 5% Sheep Blood Agar

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QUALITY CONTROL PROCEDURES

I. INTRODUCTION

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. Inoculate representative samples with dilutions of the culture listed below.
   a. Streak the plates for isolation. Use cultures diluted to yield to 10^3-10^5 CFU /Plate.
   b. Include **BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA5%SB) plates as controls for all organisms and plates of a previously tested lot of CDC Anaerobe 5% Sheep Blood Agar as controls for the obligate anaerobes.
   c. Incubate the TSA5%SB plate controls aerobically at 35 ± 2°C and all other plates anaerobically (BD **GasPak** EZ anaerobic system) at 35 ± 2°C.
3. Examine all inoculated plates at 24-48 h for amount of growth, colony size, pigmentation and hemolytic reactions.
4. Expected Results

CLSI Organisms	**ATCC®**	**Recovery**
**Bacteroides fragilis*	25285	Growth
**Clostridium perfringens*	13124	Growth, beta hemolysis
**Fusobacterium nucleatum*	25586	Growth
**Peptostreptococcus anaerobius*	27337	Growth
**Prevotella melaninogenica*	25845	Growth

*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 7.5 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1°C for 84 h and examine for microbial contamination.

PRODUCT INFORMATION

IV. INTENDED USE

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.

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 **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

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

CDC Anaerobe 5% Sheep Blood Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	20.0 g
Yeast Extract	5.0 g
Hemin	0.005 g
Vitamin K_1	0.015g
L-Cystine	0.4 g
Sheep Blood, defibrinated	5%

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions:

For *in vitro* Diagnostic Use in Taiwan and Singapore

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: 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."

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 (formerly NCCLS) 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 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 pigmenting *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 ± 2°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
251733	BD **BBL™** CDC Anaerobe 5% Sheep Blood Agar, Pkg. of 20 plates
252467	BD **BBL™** CDC Anaerobe 5% Sheep Blood Agar, Ctn. of 100 plates

XIII. REFERENCES

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XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

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