BD BBL™ Bacteroides Bile Esculin Agar (BBE) // CDC Anaerobe Laked Sheep Blood Agar with KV

111-252483-N-00 , April 2016

QUALITY CONTROL PROCEDURES

I. INTRODUCTION

Bacteroides Bile Esculin (BBE) Agar is used as a primary isolation medium for the selection and presumptive identification of the *B. fragilis* group.

CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) is an enriched, selective culture medium for the selective isolation of obligately anaerobic gram-negative bacilli from clinical and nonclinical materials.

II. PERFORMANCE TEST PROCEDURE

A. **Bacteroides Bile Esculin Agar**

1. Reduce all plates overnight at room temperature in the **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. Nonselective anaerobic blood agar as controls for the obligate anaerobes.
   c. Incubate plates at 35 ± 2°C in an anaerobic atmosphere.
3. Examine plates after 48 h for growth, esculin hydrolysis and selectivity.
4. Expected Results

CLSI Organisms	ATCC®	Recovery	Esculin Reaction
**Bacteroides fragilis*	25285	growth	+ (blackening)
Bacteroides thetaiotaomicron	29741	growth	+ (blackening)
**Clostridium perfringens*	13124	inhibition	N/A
Fusobacterium nucleatum	25586	inhibition	N/A
Peptostreptococcus anaerobius	27337	inhibition	N/A
**Proteus mirabilis*	12453	inhibition	N/A

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

B. **CDC Anaerobe Laked Sheep Blood Agar with KV**

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation. Use cultures diluted to yield to 10^3-10^5 CFU /Plate.
   b. CDC Anaerobe 5% Sheep Blood Agar as controls for the obligate anaerobes.
   c. Incubate plates at 35 ± 2°C in an anaerobic atmosphere.
2. Examine plates after 48 h for growth, color formation and selectivity.
3. Expected Results

CLSI Organisms	ATCC®	Recovery	Colony Color
**Bacteroides fragilis*	25285	growth	N/A
Bacteroides thetaiotaomicron	29741	growth	N/A
Escherichia coli	25922	Inhibition	N/A
Peptostreptococcus anaerobius	27337	Inhibition	N/A
**Prevotella melaninogenica*	25845	growth	black
**Proteus mirabilis*	12453	Inhibition	N/A
Streptococcus intermedius	27335	Inhibition	N/A
Veillonella parvula	10790	growth	N/A

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC test according to CLSI M22-A3. However, monitoring of exempt media used for anaerobes is strongly recommended.

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 ± 0.2 (Bacteroides Bile Esculin Agar) and 7.6 ± 0.2(CDC Anaerobe Laked Sheep Blood Agar with KV).
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.

IV. INTENDED USE

These media are used in qualitative procedures for the isolation and cultivation of obligately anaerobic microorganisms from clinical and nonclinical specimens. These media in two-sectored **I Plate™** dishes offer the ability to utilize the properties of the two media in one plate.

V. SUMMARY AND EXPLANATION

The members of the *Bacteroides fragilis* group are among the most frequently encountered anaerobes in human clinical infections. Rapid detection and identification of these organisms is important, since they tend to be more resistant to antimicrobial therapy than other anaerobes.[1,2] *B. fragilis* and *B. thetaiotaomicron* are the species of greatest clinical significance.[1] Other species in the group are: *B. caccae, B. distasonis, B. eggerthii, B. merdae, B. ovatus, B. stercoris, B. uniformis and B. vulgatus*.
BBE was developed by Livingston et al. as a primary plating medium.[3] This medium provides selective recovery of the *B. fragilis* group and also evidence for presumptive identification based on esculin hydrolysis.[3] CDC Anaerobe Blood Agar was developed at the Centers for Disease Control and Prevention as a nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms, particularly those found in clinical materials.[4,5]
The addition of the antimicrobial agents kanamycin and vancomycin enables the selective isolation of gram-negative, nonsporeforming anaerobic bacilli, particularly *Prevotella* species.[4,6]
CDC Anaerobe Laked Sheep Blood Agar with KV consists of CDC Anaerobe Blood Agar base supplemented with laked sheep blood, kanamycin and vancomycin.

VI. PRINCIPLES OF THE PROCEDURE

BBE is a primary plating medium for the selective isolation and presumptive identification of the *B. fragilis* group. Selective inhibition of facultative anaerobes and most gram-negative anaerobes is obtained by the presence of gentamicin and oxgall.[1,7] Differentiation of the *B. fragilis* group is based on esculin hydrolysis. Hydrolysis of the esculin by members of the *B. fragilis* group produces esculetin and glucose. The esculetin reacts with the iron salt contained in the medium to produce a dark brown to black complex that appears as zones around the colonies of organisms that hydrolyze esculin.
CDC Anaerobe 5% Sheep Blood Agar consists of **BD BBL™ Trypticase™** Soy Agar enriched with yeast extract to supply vitamins, amino acids and other essential nutrients. The medium is further enriched with hemin and vitamin K_1 to supply nutrients that are required by some strains of the pigmenting *Porphyromonas-Prevotella* (formerly Bacteroides) species and are reported to enhance the growth of some other species and some gram-positive nonsporeforming anaerobes.[5,8,9] Defibrinated sheep blood is added to supply nutrients and for the determination of hemolytic reactions and pigmentation. The use of laked blood improves the pigmentation of the *Prevotella* species.[6] Kanamycin and vancomycin inhibit most gram-positive facultative and obligate anaerobic microorganisms.[7]

VII. REAGENTS

Bacteroides Bile Esculin Agar (BBE)

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g
Hemin	0.01 g
Papaic Digest of Soybean Meal	5.0 g
Gentamicin	0.1 g
Sodium Chloride	5.0 g
Vitamin K_1	0.01 g
Esculin	1.0 g
Agar	14.0 g
Ferric Ammonium Citrate	0.5 g
Growth Factors	1.8 g
Oxgall	15.0 g

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

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.
CDC Anaerobe Laked Sheep Blood Agar with KV consists of CDC Anaerobe Blood Agar base with 5% laked defibrinated sheep blood, 100.0 mg/L kanamycin and 7.5 mg/L vancomycin.

Warnings and Precautions: For *in vitro* Diagnostic Use in Taiwan.
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.[1,2,5,10,11]
Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC-NIH recommendations, CLSI guidelines or 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:
Bacteroides Bile Esculin Agar // CDC Anaerobe Laked Sheep Blood Agar with KV
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.
These media should be reduced immediately prior to inoculation by placing them under anaerobic conditions for 6 – 24 h.[12] Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora. Inoculate an enrichment broth, such as Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes. Incubate plates and tubes immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35 ± 2°C, or place the media in a holding jar flushed with oxygen free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 h).[13] Incubate for at least 48 h, and, if no growth occurs, continue incubation for up to 7 days. An indicator should be used to detect anaerobiosis. Examine for growth after 48 h of incubation. Cultures should not be regarded as negative until after 7 days of incubation.
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 a minimum of 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.
Growth in liquid media is indicated by the presence of turbidity compared with an uninoculated control.
Isolates of the *B. fragilis* group cultured on BBE should be greater than 1 mm in diameter and appear gray, circular, entire and raised. Esculin hydrolysis is indicated by a blackening of the medium around the colonies.
Note: If the plates are to be examined after 24 h, examine quickly and reincubate under anaerobic conditions.
Examine colonies on the CDC medium using a dissecting microscope and with 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, follow established procedures.[12] Those colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.[1,2,10,11,14-17]

XI. limitations of the procedure

These prepared plated media are intended for primary isolation. Some diagnostic tests may be performed with the primary plate.
B. vulgatus may not hydrolyze esculin.[1] The concentration of vancomycin (7.5 µg/mL) may be inhibitory to asaccharolytic *Porphyromonas* species.[1] 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.[1,2,10,11,14-17]
A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit the growth of a

species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII. availability

Cat. No.	Description
252483	**BD BBL™** BBE // CDC Anaerobe Laked Sheep Blood Agar with KV, Pkg. of 20 plates

XIII. REFERENCES

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XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

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