



BBL™ CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV)



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QUALITY CONTROL PROCEDURES

I INTRODUCTION

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

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 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 organisms 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 the plated media samples and streak for isolation. For *Proteus mirabilis*, use a 10⁻³ dilution of the broth culture as the inoculum.
 - b. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as controls for all organisms and CDC Anaerobe 5% Sheep Blood Agar plates as controls for the obligate anaerobes.
 - c. Incubate the TSA II plate controls aerobically at 35 ± 2°C and all other plates anaerobically (**GasPak** EZ anaerobic system) at 35 ± 2°C.
4. Examine all inoculated plates at 48 and 72 h for amount of growth, colony size, pigmentation and hemolytic reactions. Observe the *Porphyromonas* and *Prevotella* strains under UV light (365 nm) for fluorescence.

5. Expected Results

Organisms	ATCC™	Recovery
* <i>Bacteroides fragilis</i>	25285	Growth
* <i>Clostridium perfringens</i>	13124	Inhibition (complete)
* <i>Fusobacterium mortiferum</i>	25557	Fair to heavy growth at 72 h. Colonies are off-white to yellow-tan, circular with scalloped to erose edges, umbonate often with bumpy, rigid, uneven surface. Nonhemolytic to trace alpha or beta hemolysis.
<i>Porphyromonas levii</i>	29147	Trace to heavy growth at 72 h. Colonies are tan to medium brown to black, circular, entire, convex, opaque. Bright pink to orange to brick-red fluorescence under UV light.**
<i>Prevotella intermedia</i>	25611	Trace to heavy growth at 72 h. Colonies are tan to medium brown to black, circular, entire, convex, opaque. Bright pink to orange to brick-red fluorescence under UV light.**
<i>Escherichia coli</i>	25922	No growth to fair growth. Colonies are gray, low convex, moist, semi-opaque to opaque with shiny surface.
* <i>Proteus mirabilis</i>	12453	No growth to moderate growth. Colonies are light gray, circular, translucent to opaque. Slight spreading is evident.
<i>Staphylococcus aureus</i>	25923	Complete inhibition

*Recommended organism strain for User Quality Control.

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

NOTE: This medium is exempt from User QC testing 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 7.5 ± 0.2.
4. Note the firmness of 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

CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin is used for the selective isolation of fastidious and slow-growing, obligately anaerobic gram-negative bacteria from a variety of clinical and nonclinical materials.

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 sample. Selective media are employed to facilitate recovery of the desired organisms present in mixed populations.

CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin was formulated by Dowell et al. of the Centers for Disease Control and Prevention as an enriched, selective medium for the isolation and cultivation of *Prevotella melaninogenica*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum* and other fastidious, obligately anaerobic, gram-negative bacilli, from clinical materials containing mixed populations.^{2,3} The medium employs **BBL Trypticase Soy Agar** supplemented with additional agar, yeast extract, vitamin K₁, hemin, cystine, 5% sheep blood, kanamycin and vancomycin. The combination of kanamycin and vancomycin for use in selective isolation of gram-negative anaerobes was first described by Finegold et al.⁴ Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.⁵ This medium is similar to CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin except that the blood has been laked, by subjecting it to three freeze-thaw cycles, for improved pigmentation of the *P. melaninogenica*-*P. asaccharolytica* group.⁴

VI PRINCIPLES OF THE PROCEDURE

CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin 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 other trace ingredients. Yeast extract is an important source of B vitamins. Sodium chloride maintains osmotic equilibrium. The sheep blood, hemin, cystine and vitamin K₁ provide essential nutrients for certain obligate anaerobes.^{3,6-9} The laked blood improves pigmentation of the *Prevotella* and *Porphyromonas*.

The addition of the antimicrobial agents, kanamycin and vancomycin, renders the medium selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.¹⁰

VII REAGENTS

CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0	g	Vitamin K ₁	0.01	g
Papaic Digest of Soybean Meal	5.0	g	L-Cystine	0.4	g
Sodium Chloride	5.0	g	Kanamycin	0.1	g
Agar	20.0	g	Vancomycin	0.0075	g
Yeast Extract	5.0	g	Sheep Blood, defibrinated, laked	5%	
Hemin	0.005	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 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"¹¹⁻¹⁴ 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 time. 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 or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as **BBL** specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.^{15,16}

The laboratory must be furnished with sufficient clinical information to enable the microbiologist to select the most suitable media and appropriate techniques.

IX PROCEDURE

Material Provided: CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin

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 **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.¹⁷ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **GasPak EZ** anaerobic 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 **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).¹⁸ Protect from light. 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 a **GasPak** 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 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, inoculate the following media:¹⁷

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.)¹⁷

Organisms failing to grow on the aerobic subculture plates may be presumed to be obligately anaerobic in terms of their oxygen requirements.

Colonies of the type(s) which prove to be obligate anaerobes can be further studied using the corresponding broth cultures.

Consult appropriate texts for further information, including identification procedures.^{8,9,19-23}

XI LIMITATIONS OF THE PROCEDURE

The concentration of vancomycin (7.5 µg/mL) may be inhibitory to asaccharolytic *Porphyromonas* species.^{5,23}

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for detailed information and recommended procedures.^{22,24-27}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. 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 PERFORMANCE CHARACTERISTICS

Prior to release, all lots of CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) are tested for performance characteristics. Representative samples of the lot are streak-inoculated with 0.05 mL of the following cultures: *Bacteroides fragilis* (ATCC 25285), *Clostridium perfringens* (ATCC 13124), *Fusobacterium mortiferum* (ATCC 25557), *Porphyromonas levii* (ATCC 29147), *Prevotella intermedia* (ATCC 25611), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453) and *Staphylococcus aureus* (ATCC 25923). The inocula for *B. fragilis*, *C. perfringens*, *F. mortiferum*, *P. levii* and *P. intermedia* are taken from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted to a 0.5 McFarland standard in reduced Thioglycollate Medium, Enriched. The inocula for *E. coli* and *S. aureus* are taken from a broth culture and diluted 10⁻¹; the inoculum for *P. mirabilis* is taken from a broth culture and diluted 10⁻³. After inoculation, the plates are incubated at 35 ± 2°C in a complete **GasPak™** system. Plates are read after 48 h incubation. *B. fragilis*, *F. mortiferum*, *P. levii* and *P. intermedia* show trace to heavy growth with typical colonial morphology and hemolytic reactions, while *E. coli* and *P. mirabilis* show trace to heavy growth. Compared to a nonselective control, the colony size of *E. coli* is reduced and swarming is reduced with *P. mirabilis*. *C. perfringens* and *S. aureus* are inhibited. When viewed under UV light (365 nm), *P. levii* and *P. intermedia* exhibit bright pink to bright orange to brick red fluorescence.


XIII AVAILABILITY


Cat. No.	Description
221846	BBL™ CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV), Pkg. of 20 plates

XIV REFERENCES

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