

Difco™ & BBL™ Manual

Manual of Microbiological Culture Media



Second Edition



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CDC Anaerobe 5% Sheep Blood Agar

CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV) • CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA)

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

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.

CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV), CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA) and CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) are used for the selective isolation of fastidious and slow-growing, obligately anaerobic bacteria from a variety of clinical and nonclinical materials.

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 Center for Disease Control (currently named the Centers for Disease Control and Prevention CDC) 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 BBL™ 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

CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin was formulated as an enriched, selective medium for the isolation of obligately anaerobic, gram-negative bacilli from clinical materials.² The medium employs 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.⁷ Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.⁸

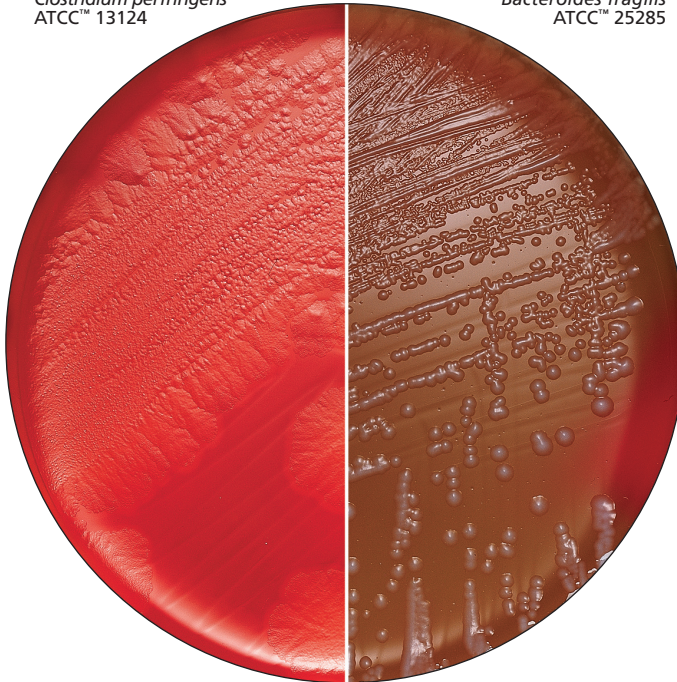
CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol was formulated as an enriched selective medium for the isolation and cultivation of obligately anaerobic bacteria from clinical materials containing rapidly growing, facultatively anaerobic bacteria such as *Proteus* and other members of the family *Enterobacteriaceae*.² The medium employs Trypticase Soy Agar supplemented with additional agar, yeast extract, vitamin K₁, hemin, cystine, 5% sheep blood and phenylethyl alcohol.

CDC Anaerobe Laked Blood Agar with Kanamycin and Vancomycin was formulated 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.^{1,3} The medium employs 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.⁷ Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.⁸ This medium is similar to CDC Anaerobe 5% Sheep Blood Agar and 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.⁹

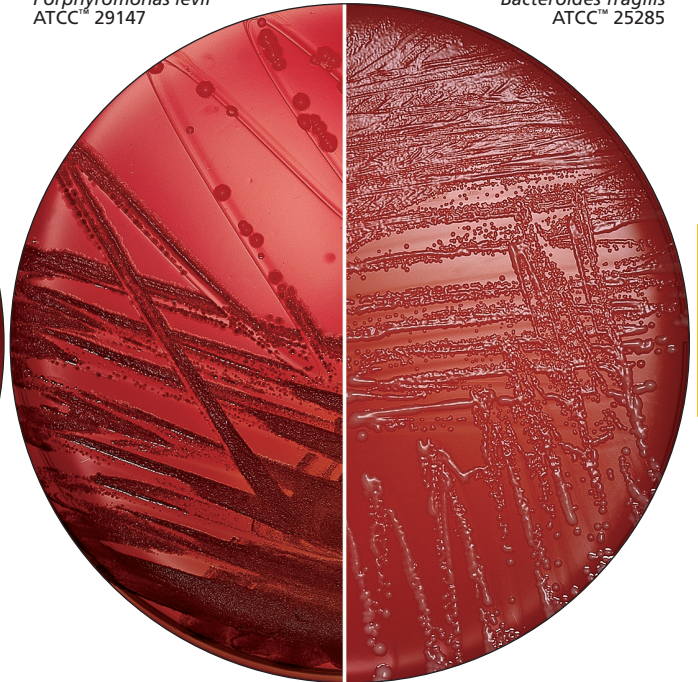
Principles of the Procedure

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

CDC Anaerobe 5% Sheep Blood Agar
Clostridium perfringens
ATCC™ 13124



CDC Anaerobe Agar with Laked Sheep Blood and KV
Porphyromonas levii
ATCC® 29147



CDC Anaerobe 5% Sheep Blood Agar
with Phenylethyl Alcohol (PEA)
Peptostreptococcus
anaerobius
ATCC™ 27337



maintains osmotic equilibrium. Sheep blood constituents, hemin, cystine and vitamin K₁ provide growth factors required by certain obligate anaerobes.^{3-6,10,11}

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.¹²

Selectivity is achieved through the addition of phenylethyl alcohol which reduces the growth of facultatively anaerobic, gram-negative bacteria without inhibiting growth of obligately anaerobic bacteria.¹³

The addition of laked sheep blood to CDC Anaerobe Agar improves pigmentation of the *Prevotella*, *Porphyromonas*, and pigmented *Bacteroides* species.¹²

Divided Petri dishes containing CDC Anaerobe 5% Sheep Blood Agar in each half enable two specimens to be streaked on one plate. Combinations of selective media are also provided in divided dishes.

Procedure

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 one 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.

These media should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.³ An efficient and easy way to obtain suitable anaerobic conditions is through the use of BD GasPak™ EZ anaerobic systems or an alternative anaerobic system.¹⁴

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 hours).¹⁴ Incubation should be at 35 ± 2°C for at least 48 hours and up to 7 days.

Expected Results

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 the 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 hours 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 proved 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.

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Availability

BBL™ CDC Anaerobe 5% Sheep Blood Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 221733 Prepared Plates – Pkg. of 20*
221734 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 256506 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251733 Prepared Plates – Pkg. of 20*
251584 Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV)

CMPH2 MCM9

Cat. No. 221735 Prepared Plates – Pkg. of 20*
221736 Prepared Plates – Ctn. of 100*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA)

BS12 CMPH2 MCM9

Cat. No. 221739 Prepared Plates – Pkg. of 20*

BBL™ CDC Anaerobe Agar with Laked Sheep Blood and KV

CMPH2 MCM9

Cat. No. 221846 Prepared Plates – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar// CDC Anaerobe 5% Sheep Blood Agar with PEA

Cat. No. 297646 Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with KV// CDC Anaerobe 5% Sheep Blood Agar with PEA

Cat. No. 297004 Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with PEA// Anaerobe Laked Sheep Blood Agar with KV

Cat. No. 299611 Prepared I Plate™ Dishes – Pkg. of 20*

*Store at 2-8°C

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