



BBL™ Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV)

8807321 • Rev. 03 • November 2015

QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV) is used for the selective isolation of fastidious and slow growing gram-negative obligately anaerobic bacteria.

II PERFORMANCE TEST PROCEDURE

1. Reduce plates at room temperature for 18–24 h prior to use in a **BD GasPak™** EZ anaerobic system.
2. Inoculate representative samples with the cultures listed below.
 - a. For *E. coli*, streak inoculate 1 µL (0.001 mL) from a 4–5 h culture of **BD Trypticase™** Soy Broth diluted to yield 10^6 – 10^7 CFU/mL.
 - b. For obligate anaerobes, streak inoculate with 10^3 – 10^4 CFU from a 48–72 h culture of Chopped Meat Broth that has incubated for 2 days at 35–37 °C.
 - c. Incubate plates at $36 \pm 1^\circ\text{C}$ in an anaerobic atmosphere.
 - d. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for inhibited strains.
3. Examine plates after 48 h for growth, colony color and selectivity.
4. Expected Results

Organisms	ATCC®	Recovery	Colony Color
* <i>Bacteroides fragilis</i>	25285	Fair to heavy growth	Gray
* <i>Clostridium perfringens</i>	13124	Inhibition (partial to complete)	N/A
<i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A
<i>Porphyromonas levii</i>	29147	Fair to heavy growth	Brown-black

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User Quality Control testing according to CLSI M22-A3 Table 1B.

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. Note the firmness of plates during the inoculation procedure.
4. Incubate uninoculated representative plates at 33–37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV) is used for the selective isolation and cultivation of anaerobic microorganisms.

V SUMMARY AND EXPLANATION

Brucella Agar was developed for the cultivation of *Brucella* species from diagnostic specimens, such as blood, and from foods and other potentially contaminated material. Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV) plates are particularly useful for the cultivation of fastidious, obligately anaerobic, gram-negative bacilli from clinical materials containing mixed populations. The combination of kanamycin and vancomycin for use in selective isolation of gram-negative anaerobes, especially *Bacteroides*, was first described by Finegold et al.¹ Vancomycin, however, may inhibit *Porphyromonas asaccharolytica* (*Bacteroides asaccharolyticus*).² The blood has been laked for improved pigmentation of the *Prevotella melaninogenica*-*P. asaccharolytica* (*B. melaninogenicus*-*B. asaccharolyticus*) group.³

VI PRINCIPLES OF THE PROCEDURE

The digests of casein and animal tissue supply organic nitrogen. The yeast extract is a source of the B vitamins. Dextrose is utilized as an energy source. Sodium chloride maintains osmotic equilibrium. The sheep blood, hemin and vitamin K₁ provide essential nutrients for certain obligate anaerobes. The laked blood improves pigmentation of the *P. melaninogenica*-*P. asaccharolytica* group.

Kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.⁴

VII REAGENTS

Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV)

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein.....	10.0 g	Agar.....	15.0 g
Peptic Digest of Animal Tissue	10.0 g	Hemin.....	0.005 g
Dextrose.....	1.0 g	Vitamin K ₁	0.01 g
Yeast Extract.....	2.0 g	Kanamycin	0.1 g
Sodium Chloride.....	5.0 g	Vancomycin.....	0.0075 g
Sodium Bisulfite	0.1 g	Sheep Blood, defibrinated, laked	5%

*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"⁵⁻⁸ 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

Refer to appropriate texts for details of specimen collection and handling procedures.⁹⁻¹⁵

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brucella Laked Blood Agar with Kanamycin and Vancomycin (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.

This medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18–24 h.¹¹ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak** anaerobic systems.¹⁶

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 sterile inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Inoculate an enrichment broth, such as **BBL 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).¹⁷ Protect from light. Incubate for at least 48 h, and if no growth occurs, continue incubation for up to 7 days. An indicator such as the **BD GasPak** disposable anaerobic indicator should be used to detect anaerobiosis.

Examine the plates 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."

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 at least 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, follow established procedures.¹⁸ The colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.^{9-11,15,19,20}

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
297840	BD BBL™ Brucella Laked Blood Agar with Kanamycin and Vancomycin (KV), Pkg. of 20 plates

XIII REFERENCES

1. Finegold, S.M., A.B. Miller, and D.J. Posnick. 1965. Further studies on selective media for *Bacteroides* and other anaerobes. *Ernährungsforschung* 10:517-528.
2. Van Winklehoff, A.J., and J. de Graaff. 1983. Vancomycin as a selective agent for isolation of *Bacteroides*. *J. Clin. Microbiol.* 18:1282-1284.
3. Finegold, S.M., and D.M. Citron. 1980. Gram-negative, nonsporeforming anaerobic bacilli, p. 431-439. *In* E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Estevez, E.G. 1984. Bacteriologic plate media: review of mechanisms in action. *Lab. Med.* 15:258-262.
5. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
6. 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.
7. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
8. 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.
9. 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.
10. 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.
11. Dowell, V.R., and T.M. Hawkins. 1987. *Laboratory methods in anaerobic bacteriology*. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta.
12. Isenberg, H.D., F.D. Schoenknecht, and A. von Graevenitz. 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coordinating ed., S.J. Rubin. American Society for Microbiology, Washington, D.C.
13. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis.
14. Miller, J.M., and H.T. Holmes. 1995. Specimen collection, transport, and storage, p. 19-32. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
15. Rodloff, A.C., P.C. Applebaum, and R.J. Zabransky. 1991. Cumitech 5A, Practical anaerobic bacteriology. Coordinating ed., A.C. Rodloff. American Society for Microbiology, Washington, D.C.
16. Seip, W.F., and G.L. Evans. 1980. Atmospheric analysis and redox potentials of culture media in the GasPak system. *J. Clin. Microbiol.* 11:226-233.
17. Martin, W.J., 1971. Practical method for isolation of anaerobic bacteria in the clinical laboratory. *Appl. Microbiol.* 22:1168-1171.
18. 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 of Microbiology, Washington, D.C.
19. Murray, P.R. E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.). 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington D.C.
20. 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.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

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

ATCC is a trademark of the American Type Culture Collection.

BD, BD Logo, and all other trademarks are property of Becton, Dickinson and Company. © 2015 BD