BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (Biplate)

INTENDED USE

BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (Biplate) is used for the non-selective isolation of anaerobes and for the selective isolation of Gram-negative anaerobic rods, especially *Bacteroides* and *Prevotella* species and a variety of other Gram-negative anaerobes from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Schaedler Agar with 5% Sheep Blood is a highly nutritious medium, specifically developed for the growth of obligate anaerobes such as lactobacilli, streptococci, clostridia and *Bacteroides*. [1-3] With the addition of vitamin K1 and hemin, it is the base for several selective media including Schaedler KV Agar with 5% Sheep Blood.

In Schaedler Agar with 5% Sheep Blood three peptones provide nutrients. Glucose is an energy source. Tris buffer is included to avoid an extreme decrease of the pH during glucose fermentation. Yeast extract is a rich source of the vitamins. The hemin and sheep blood supply heme needed by a variety of strict anaerobes and additional growth promoting substances. The inclusion of vitamin K is an additional modification and was added since it is a growth requirement for some strains of *Prevotella melaninogenica* (*Bacteroides melaninogenicus*) and is reported to enhance the growth of some strains of *Bacteroides* and Gram-positive nonsporeformers. [4,5] Sodium chloride provides essential electrolytes.

Today, the medium is widely used as a non-selective, highly nutritious medium for the isolation strict anaerobes.

Schaedler KV Agar with 5% Sheep Blood contains the same base medium as Schaedler Agar but 5% of lysed Sheep Blood is added. In addition, the medium is supplemented with kanamycin and vancomycin. Kanamycin inhibits Gram-negative facultatively anaerobic rods and several other facultative bacteria, while vancomycin inhibits Gram-positive bacteria. The addition of these antimicrobials renders the medium selective for Gram-negative strict anaerobes, such as *Bacteroides* and *Prevotella*.[3, 5-8] The addition of lysed blood provides improved growth and allows easy differentiation of the two media included in this biplate. Note that hemolytic reactions cannot be read on this medium.

BD Schaedler Agar / Schaedler KV Agar with 5% Sheep Blood (Biplate) is used for primary isolation of strict anaerobes and of Gram-negative strictly anaerobic rods from clinical specimens.[5-8]

REAGENTS

BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (Biplate)

Formula* Per Liter Purified Water

Schaedler Agar with 5% Sheep Blood			
Pancreatic Digest of Casein	8.2 g	L-Cystine	0.4
Peptic Digest of Animal Tissue	2.5	Hemin	0.01
Papaic Digest of Soybean Meal	1.0	Vitamin K 1	0.01
Glucose	5.8	Tris (hydroxymethyl) aminomethane	3.0
Yeast Extract	5.0	Agar	13.5
Sodium Chloride	1.7	Sheep Blood, defibrinated	5%
Dipotassium Phosphate	0.8		

pH 7.6 +/- 0.2

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

Schaedler KV Agar contains 5% lysed Sheep Blood, and, in addition to the ingredients listed above, contains 0.1 g/l kanamycin and 0.0075 g/l vancomycin.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 48 to 72 hours in an anaerobic atmosphere (e.g. **BD GasPak™** Anaerobic System).

Strains	**Schaedler Agar with 5% Sheep Blood**	**Schaedler KV Agar with 5% Sheep Blood**
Bacteroides fragilis ATCC™ 25285	Growth good to excellent; grey-white colonies, beta hemolysis	Growth good to excellent; grey-white colonies
Bacteroides thetaiotaomicron ATCC 29741	Growth good to excellent; grey-white colonies, beta hemolysis	Growth good to excellent; grey-white colonies
Clostridium perfringens ATCC 13124	Growth good to excellent; large, lobate, grey-white colonies; beta (double-zone) hemolysis	Inhibition partial to complete
Fusobacterium nucleatum ATCC 25586	Growth good to excellent; grey–white colonies, surrounded by dark-grey zones	Inhibition partial to complete
Peptostreptococcus anaerobius ATCC 27337	Growth good to excellent; whitish colonies	Inhibition complete
Porphyromonas levii ATCC 29147	Growth fair to good; dirty whitish to grey-brown colonies	Inhibition partial to complete
Escherichia coli ATCC 25922	Growth	Inhibition complete
Staphylococcus aureus ATCC 25923	Growth	Inhibition complete
Proteus mirabilis ATCC 12453	Growth; swarming	Inhibition partial to complete; swarming inhibited
Uninoculated	Red to dark red, opaque	Red, transparent

PROCEDURE

Materials Provided

BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (90 mm **Stacker™** biplates). Microbiologically controlled. For differentiation of the two media of this biplate, Schaedler KV Agar contains lysed sheep blood which renders the medium transparent while Schaedler Agar is opaque.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
This biplate contains two media used for primary isolation of strict anaerobes and can be used for all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe approved techniques for collection and transport of anaerobic specimens. [5-12] Suitable transport media, e.g., **BD Port-A-Cul™,** must be used.

Test Procedure
Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.
To inoculate this biplate with specimens from swabs, first roll the swab over a small area of Schaedler Agar with 5% Sheep Blood, and afterwards over a small area of Schaedler KV Agar with 5% Sheep Blood. With a loop, streak from the inoculated area, first on Schaedler Agar with 5% Sheep Blood, then on Schaedler KV Agar with 5% Sheep Blood. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak** anaerobic systems. Regardless of the anaerobic system used, it is important to include an indicator of anaerobiosis such as the **GasPak** disposable anaerobic indicator.
Incubate plates in an anaerobic atmosphere at 35 to 37°C for at least 48 h and up to 7 days before considering them negative.
As a reference medium for the aerobically growing bacteria, the specimen should be streaked onto **BD Columbia Agar with 5% Sheep Blood** which is incubated aerobically with 5 to 10% carbon dioxide.

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 micro-organisms 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.
On Schaedler Agar with 5% Sheep Blood, all strict and all facultative anaerobes will grow. The growth on this medium is compared to that on the aerobically incubated **BD Columbia Agar with 5% Sheep Blood** plate which will contain only the facultative anaerobes. Finally, growth on Schaedler KV Agar with 5% Sheep Blood is compared to the growth on the other two media. If mixed cultures of strict and facultative anaerobes are present, appropriate subcultures on non-selective media, incubated aerobically and anaerobically, must be made from the anaerobic media to confirm that the isolate is a strict anaerobe.
For further differentiation and identification procedures, consult appropriate texts.[7-13]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (Biplate) provides two standard isolation media, one for strictly anaerobic bacteria, and the other one for strictly anaerobic Gram-negative rods.[5-8,10-13]
On Schaedler Agar with 5% Sheep Blood, which is a standard medium for the isolation of strict anaerobes, *Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Clostridium, Peptostreptococcus,* strictly anaerobic non-sporeforming rods (e.g., the former genus *Eubacterium), Mobiluncus, Actinomyces* and many others will grow.
Note that the growth rates of strict anaerobes vary considerably: While *Bacteroides fragilis* will grow well after 24 hours, *Mobiluncus* or strains of *Porphyromonas* need 4 to 5 days, and *Actinomyces* may need 1 to 3 weeks to produce well visible colonies. If cultures are negative after 2 or 3 days of incubation, re-incubate anaerobically for an additional 2 to 3 days. If *Actinomyces* was suspected, special cultures should be inoculated that are inspected after 1, 2 and eventually 3 weeks of incubation.

This medium is not specifically selective for strict anaerobes; facultative organisms will also grow. Therefore, it is important to compare the result of the anaerobic culture with that of an aerobically incubated plate if mixed cultures are obtained.
Schaedler Agar with 5% Sheep blood contains a high concentration of glucose, which supports the rapid growth of saccharolytic organisms but may compromise the viability of organisms exposed to the acids accumulated during bacterial metabolism.[8]
On Schaedler KV Agar with 5% Sheep blood, all species of the *Bacteroides fragilis* group, *Prevotella* species such as *P. bivia, P. disiens, P. denticola, P. buccae,* the *Prevotella melaninogenica* group and several other Gram-negative strict anaerobes will grow. Consult the reference for the recent taxonomy.[12]
Bacterial beta hemolysis cannot be evaluated on Schaedler KV Agar since this medium contains lysed blood. However, beta hemolysis is not used for diagnosis of the organisms growing on this medium.

Fusobacterium spp. may or may not grow, depending on the sensitivity of individual strains to the antimicrobials.[7].
The concentration of vancomycin (7.5 mg/ml) usually is inhibitory to *Porphyromonas* species and on fusobacteria.[7,13]
Facultative anaerobes exhibiting resistance to aminoglycosides may grow on the medium.

Although certain diagnostic tests may be performed directly on **BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood** (Biplate), biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.

REFERENCES

1. Schaedler, R.W., R. Dubos, and R. Costello. 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. 122:59-66.
2. Mata, L.J., C. Carrillo, and E. Villatoro. 1969. Fecal microflora in healthy persons in a preindustrial region. Appl. Microbiol. 17:596-602.
3. MacFaddin, J.F. 1985. Media for isolation cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
4. Gibbons, R.J., and J.B. MacDonald. 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bacteriol. 80:164-170.
5. Finegold, S.M., V.L. Sutter, H.R. Attebery, and J.E. Rosenblatt. 1974. Isolation of anaerobic bacteria, p. 368. In E.H. Lennette, E.H. Spaulding, and J.P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
6. 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 for Micro biology, Washington, D.C.
7. Murray, P.R., and D.M. Citron. 1991. General processing of specimens for anaerobic bacteria. *In:* A. Balows, W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
8. 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.
9. Engelkirk, P.G., J. Duben-Engelkirk, and V.R. Dowell, Jr. 1992. Principles and practice of clinical anaerobic bacteriology. Star Publishing Co., Belmont, Calif.
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, CA, USA.
11. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8thed. American Society for Microbiology, Washington, D.C.
12. Jousimies-Somer, H.R., et al. 2003. *Bacteroides*, *Porphyromonas, Prevotella, Fusobacterium*, and other anaerobic gram-negative bacteria. *In:* Murray, P. R., E. J. Baron,

J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
13. van Winkelhoff, A.J., and J. de Graaff. 1983. Vancomycin as a selective agent for isolation of Bacteroides. J. Clin. Microbiol. 18:1282-1284.

PACKAGING/AVAILABILITY

BD Schaedler Agar/Schaedler KV Agar with 5% Sheep Blood (Biplate)

Cat. No. 254476	Ready-to-use Plated Media, cpu 20
Cat. No. 257589	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/