BD™ Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood

INTENDED USE

BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood is a selective medium for the isolation of strictly anaerobic bacteria from clinical specimens. Due to the amikacin, most facultative organisms are inhibited.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Wilkins-Chalgren medium has been described for the susceptibility testing of anaerobic bacteria.[1] Since it is a semi-synthetic medium that contains supplements such as arginine, pyruvate, hemin and vitamin K1, it is suitable for the growth of many strict anaerobes belonging to various metabolic types, including *Bacteroides, Prevotella, Eubacterium, Clostridium, Veillonella* and others.[2] It has been noted that this medium, when used without blood, will not so well support the growth of *Porphyromonas* and *Peptostreptococcus*. With supplemented blood and vitamin K, this medium is a universal growth and isolation medium for all anaerobic bacteria involved in human infections. Aminoglycosides have been shown to inhibit the growth of most facultatively anaerobic bacteria (e.g. *Enterobacteriaceae*) whereas many strictly anaerobic bacteria, such as *Bacteroides,* are completely resistant to this group of antimicrobials.[3,4] In the early years, neomycin or gentamicin have been added to various media to render them selective for strict anaerobes. Amikacin, a more recent aminoglycoside, has a better inhibitory effect and a slightly broader spectrum of activity compared to gentamicin.

In **BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood**, the peptones and other compounds contained in the base medium together with the sheep blood provide the necessary nutrients. The amikacin concentration has been adjusted to allow sufficient inhibition of most facultative anaerobes without significant inhibition of most clinically frequent strict anaerobes.

REAGENTS

BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g
Pancreatic Digest of Gelatin	10.0
Yeast Extract	5.0
Glucose	1.0
Sodium Chloride	5.0
L-Arginine	1.0
Sodium Pyruvate	1.0
Hemin	5.0
Amikacin	0.05
Agar	15.0
Vitamin K1	0.5 mg
Sheep Blood, defibrinated	7%

pH 7,3 +/- 0,2

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

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., by using the **BD GasPak™** Anaerobic System) at 35 to 37° C.

Strains	**Growth Results**
Bacteroides fragilis ATCC™ 25285	Good to excellent growth; grey-white colonies
Porphyromonas levii ATCC 29147	Good to excellent growth; grey-brown small to medium-sized colonies
Clostridium perfringens ATCC 13124	Good to excellent growth; white to grey, lobate or confluent colonies, with beta hemolysis (double zone)
Peptostreptococcus anaerobius ATCC 27337	Good to excellent growth
Staphylococcus aureus ATCC 25923	Inhibition partial to complete
Proteus mirabilis ATCC 12453	Inhibition partial to complete
Escherichia coli ATCC 25922	Inhibition complete
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood (90 mm **Stacker™** plates) Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used for the primary isolation of strict anaerobes from 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] 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 onto **BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood**. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.
Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak for isolation from this inoculated area.
In order to provide detection of all anaerobic agents present in clinical specimens, an anaerobic non-selective agar such as **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** must be included. Incubate these plates anaerobically for 48 to 72 hours at 35 to 37° C. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak** anaerobic systems. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as the **BD GasPak** disposable anaerobic indicator.
Also, facultative anaerobes might be present in the specimen that are resistant to aminoglycosides and hence are not inhibited on **BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood**. Therefore, it is recommended to always include an aerobic medium (such as **BD Columbia Agar with 5% Sheep Blood**) when the primary cultures are set up.

This plate is incubated aerobically enriched with carbon dioxide together with the anaerobic cultures.[3] It allows the detection of facultative organisms in the specimen.

Results
After incubation, the plates are inspected for growth. Colonies which grow on this medium are suspected to be strict anaerobes. Eventually, and If aerobically incubated media have not been included, this should be confirmed by subculturing typical colonies onto aerobically incubated **BD Columbia Agar with 5% sheep blood**. Further microscopic and biochemical examination is necessary for the identification of the genera and species of the strict anaerobes. Since the number and types of strict anaerobes involved in human infections are large, respective references should be consulted.[3]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

This medium is used for the selective isolation of many strictly anaerobic bacteria resistant to aminoglycosides, e.g., the *Bacteroides fragilis group, Fusobacterium, Peptostreptococcus*, clinically frequent species of the genus *Prevotella,* e.g., *P. bivia,* and others.

Performance Results[6]
The medium was evaluated internally with clinical isolates and collection strains of the following strictly anaerobic species and compared to **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** as a nonselective growth reference medium:

Test strains	Results on BD Wilkins-Chalgren Agar with Amikacin and 7% Sheep Blood
Bacteroides fragilis, B. thetaiotaomicron, B. distasonis, B. ovatus, B. caccae, B. uniformis, B. vulgatus; Prevotella bivia, P. disiens, P. denticola; Fusobacterium varium, F. nucleatum Porphyromonas levii, Peptostreptococcus anaerobius, Clostridium perfringens	Growth good to excellent
Prevotella buccae, P. intermedia; Porphyromonas gingivalis, Mobiluncus mulieris, Campylobacter (Bacteroides) gracilis, Eggerthella lenta (Eubacterium lentum)	No growth or reduced growth

Limitations of the Procedure
Due to the various metabolic types among the strict anaerobes, there exist certain organisms, e.g. *Mobiluncus* spp., *Campylobacter (Bacteroides) gracilis, Porphyromonas (Bacteroides) gingivalis, Eggerthella lenta, Veillonella,* and others which are sensitive to amikacin or other aminoglycosides and, therefore, do not grow or grow only weakly on this medium.[3] Therefore, a nonselective anaerobic medium must be also inoculated with the specimen.
The number and types of bacterial species occurring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated or newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.
There exist strains of facultative anaerobes that resistant to aminoglycosides and hence are not inhibited on this medium.

REFERENCES

1. Wilkins, T.D., and S. Chalgren. 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. 10: 926-928.
2. MacFaddin, J.F. 1985. Media for isolation cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
3. Engelkirk, P.G. et al.: Principles and Practice of Clinical Anaerobic Bacteriology; Star Publishing Comp., Belmont, 1992

4. Yao, J.D.C., and C. Moellering, Jr.1995. Antibacterial agents. *In:* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Miller, J.M., Holmes, H.T. 1995. Specimen Collection, transport, and storage. *In:* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. Data on file. BD Diagnostic Systems Europe, Heidelberg, Germany

PACKAGING/AVAILABILITY

BD Wilkins-Chalgren Agar with Amikacin and 5% Sheep Blood
Cat. No. 254479 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-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/