

INSTRUCTIONS FOR USE – READY-TO-USE PLATED MFDIA

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PA-256506.04

BD™ CDC Anaerobe Agar + 5% Sheep Blood

INTENDED USE

BD CDC Anaerobe Agar with 5% Sheep Blood is a nonselective medium for the isolation and cultivation of fastidious, obligately anaerobic bacteria from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

CDC Anaerobe Agar with 5% Sheep Blood was formulated by Dowell et al. of the Centers for Disease Control and Prevention as an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic micro-organisms, particularly those found in clinical materials.¹-⁴ The medium contains **Trypticase™** Soy Agar supplemented with additional agar as a nutrient base. Sodium chloride maintains osmotic equilibrium. Sheep blood, hemin, cystine and vitamin K1 provide growth factors required by certain obligate anaerobes.¹, ⁵-⁻¹ 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.² Furthermore, less smooth to rough colonial variation of *Bacteroides fragilis* has been reported on this medium than on Schaedler Blood Agar.⁵

REAGENTS

BD CDC Anaerobe Agar with 5% Sheep Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Agar	20.0
Yeast Extract	5.0
Hemin	0.005
Vitamin K1	0.01
L-Cystine	0.4
Sheep Blood, defibrinated	5%

 $pH 7.5 \pm 0.2$

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.

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

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	Growth Results
Bacteroides fragilis ATCC™ 25285	Growth good to excellent
Clostridium perfringens ATCC 13124	Growth good to excellent
Fusobacterium nucleatum ATCC 25586	Growth good to excellent
Peptostreptococcus anaerobius ATCC 27337	Growth good to excellent
Porphyromonas levii ATCC 29147	Growth fair to good
Uninoculated	Red to dark red (blood color)

PROCEDURE

Materials Provided

BD CDC Anaerobe Agar with 5% Sheep Blood (90 mm Stacker™ plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a non-selective medium for the isolation and cultivation of strict anaerobes from all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe approved techniques for selection, collection and transport of anaerobic specimens.⁸⁻¹¹ Suitable transport media, e.g., **BD Port-A-Cul**TM, 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.

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.

For isolation of strictly anaerobic bacteria the use of at least two media is recommended for all specimens. One plate, **BD CDC Anaerobe Agar with 5% Sheep Blood**, is incubated anaerobically after inoculation. The second plate, e.g. **BD Columbia Agar with 5% Sheep Blood**, must be incubated aerobically with 5 to 10% carbon dioxide for isolation of aerobic pathogens that may be present. Additionally, a selective anaerobic medium for Gram negative strict anaerobes, such as **BD Schaedler Kanamycin-Vancomycin Agar with 5% Sheep Blood**, should also be inoculated. 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. For further details on specimen processing, consult the references. 8-10,12,13

Incubate plates in the appropriate atmosphere at 35 to 37°C for at least 48 h and up to 7 days before considering them negative.

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 **BD CDC Anaerobe Agar with 5% Sheep Blood**, all strict and all facultative anaerobes will grow. The growth on this anaerobic 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 **BD Schaedler Kanamycin-Vancomycin 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.^{8-10,14}

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

On **BD CDC Anaerobe Agar with 5% Sheep Blood** which is one of the nonselective standard media 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. 4,9,10,14-16

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 or longer 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 one, two and eventually three weeks of incubation.

This medium is not specifically selective for strict anaerobes. When incubated anaerobically, facultative organisms will also grow on this medium. Therefore, it is important to compare the result of the anaerobic culture with that of an aerobically incubated plate if mixed cultures are obtained.

BD CDC Anaerobe Agar with 5% Sheep Blood does not contain glucose or other sugars. Therefore, strongly saccharolytic organisms, such as lactobacilli and certain saccharolytic clostridia, will grow rather slowly on this medium. **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** is the preferred medium for the nonselective isolation of these organisms.

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 microorganisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.

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PACKAGING/AVAILABILITY

BD CDC Anaerobe Agar with 5% Sheep Blood

Cat. No. 256506 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.



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