



BD™ Schaedler CNA Agar with 5% Sheep Blood

INTENDED USE

BD Schaedler CNA Agar with 5% Sheep Blood is a partially selective medium for the isolation of strictly anaerobic Gram positive cocci and other anaerobic Gram positive bacteria from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Schaedler Agar with 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. Ellner et al. found that a medium containing 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia agar base, enriched with 5% sheep blood, supports the growth of many Gram positive organisms while inhibiting the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species.⁴ Today, colistin and nalidixic acid (=CNA) are used in a variety of media to inhibit Gram negative bacteria. When incubated anaerobically, **BD Schaedler CNA Agar with 5% Sheep Blood** is used for the selective isolation of Gram positive cocci, especially *Peptostreptococcus* and *Peptococcus*.^{3,5} Additionally, other Gram positive anaerobes, e.g. clostridia, *Eggerthella lenta* (= *Eubacterium lentum*), *Mobiluncus* and (although they are Gram negative) several species of the *Bacteroides fragilis* group, such as *B. fragilis*, *B. thetaiotaomicron*, and *B. vulgatus* will grow. The medium is especially useful for specimens containing mixed flora.

In **BD Schaedler CNA 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. Colistin and nalidixic acid are inhibitors of facultatively anaerobic Gram negative bacteria, especially *Enterobacteriaceae*.

REAGENTS

BD Schaedler CNA Agar with 5% Sheep Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	8.2 g	Hemin	0.01 g
Peptic Digest of Animal Tissue	2.5	Vitamin K1	0.01
Papaic Digest of Soybean Meal	1.0	Tris (hydroxymethyl) aminomethane	3.0
Glucose	5.8	Colistin	0.01
Yeast Extract	5.0	Nalidixic Acid	0.01
Sodium Chloride	1.7	Agar	13.5
Dipotassium Phosphate	0.8	Sheep Blood, defibrinated	5%
L-Cystine	0.4		

pH 7.6 +/- 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., **BD GasPak™** Anaerobic System) at 35 to 37° C.

Strains	Growth Results
<i>Peptostreptococcus anaerobius</i> ATCC™ 27337	Growth good to excellent
<i>Clostridium perfringens</i> ATCC 13124	Growth fair to good; beta hemolysis (double zone)
<i>Bacteroides fragilis</i> ATCC 25285	Growth good to excellent
<i>Proteus mirabilis</i> ATCC 12453	Inhibition complete
Uninoculated	Red to dark red (blood color)

PROCEDURE

Materials Provided

BD Schaedler CNA 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 selective medium for the isolation and cultivation of Gram positive strict anaerobes from all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). It is especially helpful for processing specimens containing mixed flora of facultative Gram negative bacteria.

Observe approved techniques for collection and transport of anaerobic specimens. ⁶⁻⁸ Suitable transport media, e.g., **BD Port-A-Cul™**, must be used.

Test Procedure

Streak the specimen onto **BD Schaedler CNA Agar with 5% Sheep Blood** 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 from this inoculated area.

In order to recover all possible pathogens involved, a non-selective anaerobic medium, such as **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** must be included. The use of a selective medium for Gram negative anaerobes, such as **BD Schaedler Kanamycin-Vancomycin-Agar with 5% Sheep Blood**, is recommended. Incubate plates under anaerobic conditions at 35 to 37°C for at least 48 hours up to 7 days before considering them negative. 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.

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.

The presence of growth on **BD Schaedler CNA Agar with 5% Sheep Blood**, in the absence of corresponding colonies on the aerobically incubated **BD Columbia Agar with 5% Sheep Blood** plate, indicates the presence of anaerobic bacteria. If growth is obtained on **BD Schaedler CNA Agar with 5% Sheep Blood** and no growth occurs on **BD Schaedler Kanamycin-Vancomycin-Agar with 5% Sheep Blood**, the isolates are probably strictly anaerobic Gram positive bacteria. If the same type of colonies is detected on **BD Schaedler Kanamycin-Vancomycin-Agar with 5% Sheep Blood** plate, the isolates may belong to the *B. fragilis* group. In both cases, a Gram stain will reveal the type of bacteria that has been isolated. Growth on **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** without growth on both selective anaerobic media indicates the presence of strict anaerobes sensitive to the inhibitors of these media, such as Gram negative anaerobic cocci or others. 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.

Further differentiation steps, including Gram stains, are necessary to identify the isolated organisms. Consult appropriate texts for further information, including identification procedures.⁶⁻¹¹

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Schaedler CNA Agar with 5% Sheep Blood when incubated anaerobically, is a medium for the selective isolation of many anaerobic Gram positive micro-organisms.^{3,5} On this medium *Peptostreptococcus*, *Peptococcus* and other Gram positive anaerobes, e.g. clostridia, *Eggerthella lenta* (= *Eubacterium lentum*), *Mobiluncus*, other anaerobic Gram positive nonsporeforming rods will grow. This medium is inhibitory to facultative Gram negative anaerobes, e.g., *Enterobacteriaceae*.

BD Schaedler CNA Agar with 5% Sheep Blood when incubated anaerobically, is not absolutely selective for strictly anaerobic Gram positive organisms. Facultative Gram positive organisms like *Staphylococcus spp.*, *Streptococcus spp.*, *Listeria*, or others may grow on this medium.

Since most of the species of the *Bacteroides fragilis* group and certain species of the genus *Prevotella*, e.g., *Prevotella bivia*, are resistant to the selective ingredients, they will regularly grow on this medium.

Occasionally, Gram positive anaerobes sensitive to the selective agents, e.g., certain *Clostridium spp.*, may be partially to completely inhibited on this medium. Therefore, specimens must also be streaked onto a nonselective medium.

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PACKAGING/AVAILABILITY

BD Schaedler CNA Agar with 5% Sheep Blood

Cat. No. 254485 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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