BBL™ Schaedler Agar with Vitamin K₁ and 5% Sheep Blood

I INTRODUCTION

Schaedler Agar with Vitamin K₁ and 5% Sheep Blood is a highly nutritious medium for the isolation and cultivation of fastidious anaerobic microorganisms. The addition of vitamin K₁ enables the growth of certain strains of the pigmenting Prevotella and Porphyromonas species, for which it is a growth requirement, and also enhances the growth of other Bacteroides species and gram-positive nonsporeformers.

II PERFORMANCE TEST PROCEDURE

1. Reduce all Schaedler Sheep Blood plates overnight at room temperature in a BD GasPak™ EZ anaerobic system.
2. Preparation of inocula
   a. Prepare the anaerobe test cultures by swabbing the growth from a 2–5 day CDC anaerobe 5% Sheep Blood Agar plate into a tube containing 5 mL of reduced Enriched Thioglycollate Medium containing vitamin K₁ and hemin. Mix well and adjust turbidity comparable to a 0.5 McFarland standard.
   b. Use a 4- to 5-h BD Trypticase™ Soy Broth culture of S. pneumoniae diluted to yield 10³–10⁴ CFU/plate.
3. Inoculation of the plates
   a. Using a volumetric pipettor or equivalent method, deliver 0.05 mL of the appropriate inoculum to each plate or sector and streak for isolation. For S. pneumoniae using a 0.01 mL loop inoculate and streak the plates.
   b. Include BD Trypticase Soy Agar with 5% Sheep Blood (TSA II) plates for all organisms use plates of a previously tested lot of CDC Anaerobe 5% SB Agar as a control for obligate anaerobes.
4. Incubate all plates anaerobically (BD GasPak EZ system) at 35 ± 2 °C.
5. Examine all inoculated plates at 24 and 48 h for amount of growth, colony size, pigmentation and hemolytic reactions.
6. Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Bacteroides fragilis</td>
<td>25285</td>
<td>Growth</td>
</tr>
<tr>
<td>*Clostridium perfringens</td>
<td>13124</td>
<td>Growth, beta hemolysis</td>
</tr>
<tr>
<td>*Fusobacterium nucleatum</td>
<td>25586</td>
<td>Growth</td>
</tr>
<tr>
<td>*Peptostreptococcus anaerobius</td>
<td>27337</td>
<td>Growth</td>
</tr>
<tr>
<td>Additional Organisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyromonas levii</td>
<td>29147</td>
<td>Growth</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>6305</td>
<td>Moderate to heavy growth. Colonies small, translucent, grayish and flat. May be alpha-hemolytic.</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

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. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.6 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Schaedler Agar with Vitamin K₁ and 5% Sheep Blood is used for the isolation and cultivation of fastidious aerobes and anaerobes from a variety of clinical and nonclinical specimens. It is especially useful for the recovery of the fastidious anaerobic bacteria such as Bacteroides, Prevotella and Porphyromonas species.

V SUMMARY AND EXPLANATION

In the first half of the 20th century, the majority of the culture media used for the recovery of anaerobic microorganisms from clinical specimens contained sodium thioglycollate as the reducing agent which provided the required atmosphere for replication of obligate anaerobes. Special Petri dishes were designed to create anaerobiosis. With improved culturing techniques and culture media formulations, anaerobes are recovered with greater frequencies and particular genera are recognized as important contributors to disease processes.

In 1965, Schaedler, Dubos and Costello¹ reported on the bacterial flora of the gastrointestinal tract of mice. In these studies several new media formulations were introduced. The majority of these contained inhibitors of specific bacterial species or groups since the authors indicated the need for selective media when processing specimens which contain large numbers of a heterogeneous bacterial population. The basal medium, without inhibitors, is the original version of the medium designated as Schaedler Agar. It was formulated to support the growth of fastidious anaerobic microorganisms such as lactobacilli, streptococci, clostridia and Bacteroides. Mata and coworkers², studying the fecal microflora in healthy persons in Central America, modified Schaedler Agar to produce a number of new formulations. The modifications in the basal medium of Schaedler included adjustments in the peptone content, since BD Trypticase Soy Broth (BBL™) was substituted for the BD Trypticase peptone component of the original formulation, and increase in the sodium chloride content. Additionally, the dextrose concentration was reduced to avoid interference with hemolytic reactions and the yeast extract level lowered to avoid darkening of the medium.³

The inclusion of vitamin K₁ is an additional modification and was added since it is a growth requirement for some strains of Prevotella melaninogenica⁴ and is reported to enhance the growth of some strains of Bacteroides and gram-positive nonsporeformers.⁵
VI PRINCIPLES OF THE PROCEDURE

The combination of three peptones derived from both animal and vegetable sources, dextrose and yeast extract render the basic formulation highly nutritious by providing nitrogenous growth factors, carbohydrates as energy sources and vitamins. The sheep blood and hemin also are important in stimulating the growth of fastidious microorganisms. As discussed above, the vitamin K₁ additive is crucial for the recovery of certain anaerobes.

Fastidious aerobes and anaerobes grow luxuriantly on this medium and the type of organisms recovered is dependent on the environment utilized in the incubation process (aerobic, aerobic supplemented with carbon dioxide or anaerobic conditions).

VII REAGENTS

**Schaeleder Agar with Vitamin K₁ and 5% Sheep Blood**

Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>8.2 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Papaic Digest of Soybean Meal</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.8 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>1.7 g</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Hemin</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Vitamin K₁</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Tris (hydroxymethyl) aminomethane</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
</tr>
<tr>
<td>Sheep Blood, defibrinated</td>
<td>5%</td>
</tr>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>8.2 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>2.5 g</td>
</tr>
</tbody>
</table>

*Dipotassium Phosphate 0.8 g, Sodium Chloride 1.7 g, Yeast Extract 5.0 g, Agar 13.5 g, Sheep Blood, defibrinated 5% *

**Warning 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"6-9 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

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

**Material Provided:** Schaeleder Agar with Vitamin K₁ and 5% Sheep Blood

**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.

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 from this inoculated area.

For isolation of anaerobic bacteria the use of at least two plates prepared with 5% defibrinated sheep blood is recommended for all specimens. One plate should be incubated aerobically with added carbon dioxide for isolation of aerobic pathogens that may be present. The other plate, previously reduced by placing under anaerobic conditions for 6–24 h, should be immediately incubated under anaerobic conditions or placed in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates are accumulated to activate an anaerobic jar (but no longer than 3 hours). An efficient and easy way to obtain suitable anaerobic conditions is through the use of BD GasPak EZ anaerobic systems.

Inoculate plates at 35 ± 2 °C for 18–24 h. Plates incubated under anaerobic conditions should be held at 35 ± 2 °C for at least 48 h and up to 7 days before considering them negative. Regardless of the anaerobic system used, it is important to include an indicator of anaerobiosis such as the BD GasPak disposable anaerobic indicator.

**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 incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms 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 semi-quantitatively scored on the basis of growth in each of the streaked areas.
In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, inoculate the following media:  

1. One anaerobe blood agar plate to be incubated anaerobically.
2. One aerobic blood agar (or chocolate agar) plate to be incubated in an aerobic atmosphere enriched with carbon dioxide. The chocolate agar is particularly needed to distinguish nutritionally-fastidious *Haemophilus* species and other bacteria which will grow on anaerobe blood agar incubated anaerobically and on chocolate agar under increased carbon dioxide tension but which fail to grow on blood agar in the presence of carbon dioxide or in air.
3. One aerobic blood agar plate to be incubated aerobically without added carbon dioxide.
4. Tubes of Enriched Thioglycollate Medium and/or Cooked Meat Medium and a tube of Peptone Yeast Extract Glucose Broth. Incubate all cultures at 35 ± 2 °C for a minimum of 24 h and up to 7 days. 

Record the relationship to oxygen as either obligate anaerobe or nonanaerobe (aerotolerant anaerobe, microaerophilic, or facultative anaerobe).  

Colonies of the type(s) which prove to be obligate anaerobes can be further studied using the corresponding broth cultures. Organisms failing to grow on the aerobic subculture plates may be presumed to be obligately anaerobic in terms of their oxygen requirements.

### XI LIMITATIONS OF THE PROCEDURE

Schaedler Agar contains a high concentration of dextrose, which supports the rapid growth of saccharolytic organisms but may compromise the viability of organisms exposed to the acids accumulated during bacterial metabolism. Also, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha-hemolysis.

For identification, the organisms must be in pure culture. Biochemical tests may be performed for complete identification. Appropriate texts should be consulted for further information.

### XII AVAILABILITY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221539</td>
<td>BD BBL™ Schaedler Agar with Vitamin K₁ and 5% Sheep Blood, Pkg. of 20 plates</td>
</tr>
</tbody>
</table>

### XIII REFERENCES


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

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