Schaedler Media
Schaedler Agar • Schaedler Agar with Vitamin K_1 and 5% Sheep Blood • Schaedler K-V Agar with 5% Sheep Blood • Schaedler Broth • Schaedler Broth with Vitamin K_1

Intended Use

Schaedler Agar is a base for several media formulations used for the recovery of anaerobic microorganisms.

Schaedler Agar with Vitamin K_1 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. Schaedler K-V Agar with 5% Sheep Blood, containing kanamycin and vancomycin, is especially useful in the selective isolation of *Bacteroides* and *Prevotella* species.

Schaedler Broth and Schaedler Broth with Vitamin K_1 are media used for the cultivation of fastidious aerobic and anaerobic microorganisms.

Summary and Explanation

In 1965, Schaedler, Dubos and Costello[1] 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 coworkers,[2] 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 **Trypticase**™ Soy Broth was substituted for the **Trypticase** peptone component of the original formulation, and an 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 K_1, is an additional modification and was added since it is a growth requirement for some strains of *Prevotella melaninogenica* (*Bacteriodes melaninogenicus*)[4] and is reported to enhance the growth of some strains of *Bacteroides* and gram-positive nonsporeformers.[5]

The combination of kanamycin and vancomycin in Schaedler K-V Agar with 5% Sheep Blood is used for the selective isolation of gram-negative anaerobes.[6]

Schaedler Broth has the same formula as Schaedler Agar except that the agar is omitted. Stalons et al.[7] found Schaedler Broth to be the most effective medium of nine broth media tested for the growth of obligately anaerobic bacteria when incubated in an anaerobic atmosphere. The incorporation of vitamin K_1 broadens the spectrum of organisms that can be cultivated in Schaedler Broth.

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 K_1 additive is crucial for the recovery of certain anaerobes.

The addition of the antimicrobial agents kanamycin and vancomycin in the agar medium renders the medium selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.[8]

Using Schaedler media, fastidious aerobes and anaerobes grow well; however, the type of organisms recovered is dependent on the environment utilized in the incubation process (aerobic, aerobic supplemented with carbon dioxide or anaerobic conditions).

Formulae

BBL™ Schaedler Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	8.2	g
Peptic Digest of Animal Tissue	2.5	g
Papaic Digest of Soybean Meal	1.0	g
Dextrose	5.8	g
Yeast Extract	5.0	g
Sodium Chloride	1.7	g
Dipotassium Phosphate	0.8	g
L-Cystine	0.4	g
Hemin	0.01	g
Tris (hydroxymethyl) aminomethane	3.0	g
Agar	13.5	g

BBL™ Schaedler Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Schaedler Agar – 41.9 g;
   BBL™ Schaedler Broth – 28.4 g.
   Mix thoroughly.
2. If desired, add 1 mL of a 1% vitamin K_1 solution in absolute ethanol.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. For the agar medium, cool to approximately 45°C and add 5% sterile defibrinated sheep blood when required.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agars

These media should be reduced immediately prior to inoculation by placing them under anaerobic conditions for 18-24 hours.[9]

Use standard procedures to obtain isolated colonies from specimens. Inoculate an enrichment broth, such as Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate plates and tubes immediately after inoculation, with plates in an inverted position (agar side up) under anaerobic conditions at 35°C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 hours). Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days. It is recommended that an indicator of anaerobiosis be used.

Examine the selective medium for growth after 48 hours of incubation. Cultures should not be regarded as negative until after 7 days incubation. Since some anaerobes may be inhibited due to the selective nature of the medium, it is advisable to include a nonselective medium such as Schaedler Agar with Vitamin K_1 and 5% Sheep Blood.

Broths

Inoculate the specimen directly into the broth medium. Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours prior to use. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Incubate tubes at 35 ± 2°C in the appropriate atmosphere (aerobic, anaerobic, or supplemented with carbon dioxide) for up to 7 days.

User Quality Control

Identity Specifications

BBL™ Schaedler Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.19% solution, soluble in purified water upon boiling. Solution is medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Medium, tan to yellow, clear to slightly hazy.
Reaction of 4.19% Solution at 25°C:	pH 7.6 ± 0.2

BBL™ Schaedler Broth

Dehydrated Appearance:	Fine, homogeneous, may contain tan specks.
Solution:	2.84% solution, soluble in purified water upon boiling. Solution is light to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Light to medium, tan to yellow, clear to slightly hazy.
Reaction of 2.84% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

BBL™ Schaedler Agar

Prepare the medium per label directions without (plain) and with added vitamin K_1 and 5% sheep blood (SB). Inoculate and incubate anaerobically at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH VIT. K_1 AND SB
Bacteroides fragilis	25285	$\leq 10^6$	N/A	Good
Clostridium perfringens	13124	$\leq 10^6$	N/A	Good, beta hemolysis
Streptococcus pyogenes	19615	10^3-10^4	Good	N/A

BBL™ Schaedler Broth

Prepare the medium per label directions. Inoculate and incubate anaerobically at 35 ± 2°C for up to 7 days (incubate *S. pyogenes* aerobically).

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacteroides vulgatus	8482	$\leq 10^3$	Growth
Clostridium novyi A	7659	$\leq 10^3$	Growth
Streptococcus pyogenes	19615	$\leq 10^3$	Growth

Expected Results

Agars

In order to determine the relationship to oxygen of each colony type present on the medium, follow established procedures.[10] The colony types that prove to contain obligate anaerobes can be further studied.[11]

Broths

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control. If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media (e.g., a TSA II and/or Chocolate II Agar plate, etc.). If obligate anaerobes are suspected, subcultures should be incubated anaerobically.

References

1. Schaedler, Dubos and Costello. 1965. J. Exp. Med. *122*:59.
2. Mata, Carrillo and Villatoro. 1969. Appl. Microbiol. *17*:596.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
5. Finegold, Sutter, Attebery and Rosenblatt. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Finegold, Miller and Posnick. 1965. Ernahrungsforschung *10*:517.
7. Stalons, Thornsberry and Dowell. 1974. Appl. Microbiol. *27*:1098.
8. Estevez. 1984. Lab. Med. *15*:258.
9. Dowell. 1975. *In* Balows (ed.). Clinical microbiology. How to start and when to stop. Charles C. Thomas, Springfield, Ill.
10. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Schaedler Agar

Cat. No. 212189 Dehydrated – 500 g

BBL™ Schaedler Agar with Vitamin K_1 and 5% Sheep Blood

United States and Canada

Cat. No. 221539 Prepared Plates – Pkg. of 20*
221540 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254042 Prepared Plates – Pkg. of 20*
254084 Prepared Plates – Ctn. of 120*

BBL™ Schaedler K-V Agar with 5% Sheep Blood

United States and Canada

Cat. No. 221555 Prepared Plates – Pkg. of 20*
221556 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254023 Prepared Plates – Pkg. of 20*
254077 Prepared Plates – Ctn. of 120*

BBL™ Schaedler Broth

Cat. No. 212191 Dehydrated – 100 g

BBL™ Schaedler Broth with Vitamin K_1

Cat. No. 221541 Prepared Tubes – Pkg. of 10*
221542 Prepared Tubes – Ctn. of 100*

**Store at 2-8°C.*