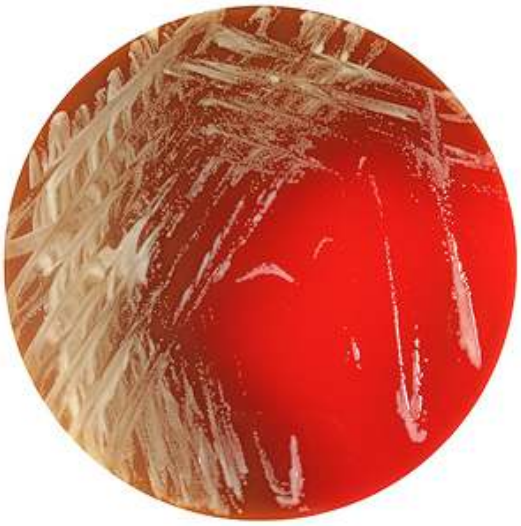
INSTRUCTIONS FOR USE

SCHAEDLER SELECTIVE BLOOD AGAR

Ready-to-use plates



Bacteroides fragilis on Schaedler Selective Blood Agar

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of anaerobic Gram-negative bacteria from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.7 g
Enzymatic digest of soya bean	1.0 g
Sodium chloride	1.7 g
Dipotassium hydrogen phosphate	0.8 g
Special peptone	5.0 g
Yeast extract	5.0 g
Glucose	5.8 g
Cysteine HCl	0.4 g
Haemin	0.01 g
Tris Buffer	0.75 g
Agar	13.5 g
Vitamin K1	10 mg
Kanamycin	100 mg
Vancomycin	7.5 mg
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Schaedler Selective Blood Agar is based on the modification by Mata, Carillo and Villatoro[1] of the formulation of basal medium proposed by Schaedler, Dubos and Costello[2]. The modifications, evaluated in their studies on anaerobic human faecal microflora, consisted in the substitution of pancreatic digest of casein with 1% Tryptic Soy Broth.
The use of the aminoglycoside paromomycin 100 mg/L together with vancomycin 7.5 mg/L was first described by Finegold *et al* [3] in 1965 and reported in the NCDC Laboratory Methods in Anaerobic Bacteriology[4]. The current formulation of Schaedler Selective Blood Agar contains kanamycin in substitution of paromomycin.[5]
The kanamycin-vancomycin blood agar plate, used in combination with a non-selective medium, is recommended for the detection of Gram-negative anaerobic bacilli, especially *Bacteroides* and *Prevotella* species, in clinical specimens.[6]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride supplies essential electrolytes and maintains the osmotic balance. Yeast extract, haemin, vitamin K1 and sheep blood enable the growth of the most fastidious obligate and facultative anaerobes. Dextrose provides an energy source and is a reducing agent; cysteine is a reducing agent too and is inhibitory for *E.coli* growth.[7] Dipotassium hydrogen phosphate and tris buffer are used to prevent the pH decreasing, during glucose fermentation. Kanamycin suppresses the growth of aerobic and facultative anaerobic Gram-negative bacteria while vancomycin is active against Gram-positive bacteria.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.6 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Schaedler Selective Blood Agar	Ready-to-use plates	549990	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, anaerobic atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Schaedler Selective Blood Agar can be directly inoculated with clinical specimens such as specimens such as tissue biopsy specimens, aspirates (e.g. cerebrospinal fluid, joint fluids, and pus), dental root canal exudates and subgingival plaque.[6,] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[8] Specimens must be transported to the laboratory under anaerobic conditions and processed within 24 h.[6]

8- TEST PROCEDURE

Allow plates to come to room temperature. Inoculate the specimen as soon as possible after collection. Streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the 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. Incubate in anaerobic condition at 35-37°C for at least 40-48 h or longer (up to 7 days) depending on type of culture being studied or suspected microorganism(s). An incubation period of 48 h will reveal the presence of rapidly growing strains, such as *Bacteroides* spp.,

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but re-incubation for 5 to 7 days is recommended since some species such as *Bilophila, Desulfovibrio* and *Porphyromonas*, may not be detected with shorter incubation times and require at least 4 to 5 days for growth.[6]

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic haemolytic characteristics of the colonies. Different anaerobic bacteria grow with different colony morphologies. Confirmatory evidence is required.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
Bacteroides fragilis	ATCC 25285	35-37 °C / 24-48 H / AN	growth
P.mirabilis	ATCC 12453	35-37 °C / 24-48 H / AN	inhibited
E.faecalis	ATCC 29212	35-37 °C / 24-48 H / AN	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use plates of Schaedler Selective Blood Agar is tested for productivity and selectivity.
Productivity is tested by semi-quantitative ecometric technique, by inoculating the plates with the target-strains *B.fragilis* ATCC 25285 and *F.nucleatum* ATCC 25586 and incubating at 35-37°C for 44-48 hours in anaerobic atmosphere. Both Gram-negative target strains show a good growth.
Selectivity is evaluated with modified Miles-Misra surface drop method and by semi-quantitative ecometric technique, inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *C.perfringens* ATCC 13124, *P.anaerobius* ATCC 27337, *E.faecalis* ATCC 29212, *P.mirabilis* ATCC 12453. After incubation at 35-37°C for 44-48 hours in anaerobic conditions, the growth of non-target strains is observed and recorded: *E.faecalis*, *P.mirabilis* and *P.anaerobius* are totally inhibited, while *C.perfringens* is partially or totally inhibited.

12 - LIMITATIONS OF THE METHOD

- It is recommended to inoculate together with Schaedler Selective Blood Agar other non-selective and selective media: Columbia Blood Agar incubated in aerobic atmosphere with 5-10% CO_2, on which only the facultative anaerobes will grow, Schaedler Selective CNA Blood Agar incubated in anaerobic conditions, on which Gram positive obligate anaerobic cocci will grow and Schaedler Blood Agar, on which all anaerobic bacteria will grow. The comparison of the growths on the four media can help to orient the detection of the isolates.
- The use of solid selective medium together with non-selective medium increases the yield and saves time in term of recognition and isolation of colonies.[6]
- The presence of vancomycin 7.5 mg/L may be inhibitory for some strains of *Porphyromonas* and *Fusobacterium*.[10]
- Plates should not be exposed to air during the first 48 hours of incubation to avoid loss of the more oxygen-sensitive species.[6]
- In many chronic infections, if not in all, several uncultivable anaerobic Gram-negative phylotypes can be present.[6]
- Growth on Schaedler Selective Blood Agar depends on the metabolic requirements of each individual microorganism; some target strains, with specific requirements may not grow on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product does not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Mata LJ, Carrillo C, Villatoro EF. Fecal microflora in healthy persons in a preindustrial region. Appl Microbiol 1969;17: 596-599
2. Schaedler RW, Dubos R, Castello R. The development of bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965;122: 59-66.
3. Finegold SM, AB Miller, Posnick DJ. Further studies on selective media for *Bacteroides* and other anaerobes. Ernährungsforschung 1965; 10:517-528.
4. Dowell, VR, Hawkins TM. Laboratory Methods in Anaerobic bacteriology, NCDC Laboratory Manual. Public Health Service Publication n° 1803, June 1968.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Conrads G, Nagy E, Kononen E. Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic Gram negative rods. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
7. Kari C, Nagy Z, Kovacs P and Hernadi F. Mechanism of the growth inhibitory effect of cysteine on Escherichia coli. J Gen Microbiol 1971; 68:349-356.
8. McElvania E, Singh K. Specimen Collection, Transport and Processing:Bacteriology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
9. Australian Society for Microbiology: Guidelines for assuring quality of medical microbiological culture media. 2nd Ed, July 2012.
10. Jousimies-Somer HR. et al. Bacteroides, Porphyromonas, Prevotella,Fusobacterium, and other anaerobic Gram-negative bacteria. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors). Manual of clinical microbiology, 8th ed. Washington, DC: American Society for Microbiology; 2003.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/11
Revision 2	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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