

**INSTRUCTIONS FOR USE**

# SCHAEDLER SELECTIVE CNA BLOOD AGAR

## Ready-to-use plates



*Peptostreptococcus anaerobius*  
on Schaedler Selective CNA Blood Agar

### 1 - INTENDED USE

*In vitro* diagnostic device. Moderately selective medium for the isolation of anaerobic Gram-positive 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
Colistin	0.01 g
Nalidixic acid	0.01 g
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<sup>1</sup> of the formulation of basal medium proposed by Schaedler, Dubos and Costello<sup>2</sup>. 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 colistin and nalidixic acid was first described by Ellner *et al*<sup>3</sup> in 1966 for the growth of many Gram-positive organisms and the inhibition of Gram-negative bacteria such as *Proteus*, *Klebsiella* and *Pseudomonas* species. The current formulation of Schaedler Blood Agar supplemented with colistin and nalidixic acid is reported by the reviews of Microbiological Media of Atlas *et al*.<sup>4,5</sup> Schaedler Selective CNA Blood Agar plate, used in combination with a non-selective medium, is recommended for the detection of Gram-positive anaerobic cocci, in clinical specimens.<sup>4,6</sup>

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.<sup>7</sup> Dipotassium hydrogen phosphate and tris buffer are used to prevent the pH decreasing, during glucose fermentation. Colistin and nalidixic acid are inhibitors of facultative anaerobic Gram-negative bacteria, especially *Enterobacteriaceae*.

### 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 CNA Blood Agar	Ready-to-use plates	549907	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 CNA Blood Agar can be directly inoculated with clinical specimens such as tissue biopsy specimens, aspirates (e.g. cerebrospinal fluid, joint fluids and pus), dental root canal exudates and subgingival plaque.<sup>6,8</sup> Mucosal or cutaneous swabs are not recommended. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Specimens must be transported to the laboratory under anaerobic conditions and processed within 24 h.<sup>6</sup>

### 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 under anaerobic conditions at 35-37°C for at least 48 hours and up to 7 days before discard the plates as negative.





### 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.

CONTROL STRAINS		INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>P.anaerobius</i>	ATCC 27337	35-37 °C / 44-48 H / AN	growth
<i>B.fragilis</i>	ATCC 25285	35-37 °C / 44-48 H / AN	growth
<i>P.mirabilis</i>	ATCC 12453	35-37 °C / 44-48 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 CNA 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, *P.anaerobius* ATCC 27337, *C.perfringens* ATCC 13124 and incubating at 35-37°C for 44-48°C in anaerobic atmosphere. All target Gram-positive strains show a good growth.

Selectivity is evaluated with modified Miles-Misra surface drop method, inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *P.mirabilis* ATCC 12453. After incubation at 35-37°C for 44-48 hours in anaerobic conditions, the growth of the non-target strain is totally inhibited.

### 12 - LIMITATIONS OF THE METHOD

- It is recommended to inoculate together with Schaedler Selective CNA Blood Agar other non-selective and selective media: Columbia Blood Agar incubated in aerobic atmosphere with 5-10% CO<sub>2</sub>, on which only the facultative anaerobes will grow, Schaedler Selective Blood Agar (with kanamycin and vancomycin) incubated in anaerobic conditions, on which Gram-negative obligate bacteria 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.<sup>8</sup>
- Plates should not be exposed to air during the first 48 hours of incubation to avoid loss of the more oxygen-sensitive species.<sup>8</sup>
- Growth on Schaedler Selective CNA 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 these products do 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](http://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](http://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. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. *Am. J. Clin. Path* 1966; 45: 502-504
4. Atlas R, Snyder J. Reagent stains and media: bacteriology. In Carrol KC, Pfaller MA et al. editors. *Manual of clinical microbiology*, 12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Atlas R, Parks LC. *Handbook of Microbiological Media*. 2nd edition. CRC Press, 1997
6. Butler-Wu SM, She RC. Actinomyces, Lactobacillus, Cutibacterium and other non-spore-forming Gram-positive 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. 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.

**TABLE OF APPLICABLE SYMBOLS**

 <b>REF</b> or <b>REF</b> Catalogue number	 <b>LOT</b> Batch code	 <b>IVD</b> <i>In vitro</i> 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.

