



BLOOD AGAR SHEEP

Ready-to-use plates



Blood Agar Sheep:
Group A β -haemolytic *Streptococcus*

1 - INTENDED USE

In vitro diagnostic device. Non selective, general-purpose medium with defibrinated sheep blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for determination of haemolytic properties.

2 - COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	15.0 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	15 g
Defibrinated sheep blood	50.0 mL
Purified water	1000.0 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

The history of blood agar is uncertain. The inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar¹; in their 1903 Manual of Bacteriology, Muir and Ritchie² list its inclusion before they discuss "agar-agar" as a replacement for gelatine as a solidifying agent.²

The term "blood agar", as we know it today, generally refers to an enriched base medium to which defibrinated mammalian blood has been added. Biolife Blood Agar Sheep is prepared from Tryptic Soy Agar with 5% defibrinated sheep blood.

Blood Agar Sheep is a general purpose, enriched medium used to grow fastidious and non-fastidious organisms and to differentiate bacteria based on their haemolytic properties.

Blood Agar Sheep is prepared with selected casein and soy peptones for improving the haemolytic reactions: they provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The presence of sheep blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.

Blood Agar Sheep is useful for performing the CAMP (Christie Atkins Munch-Petersen) test for presumptive identification of *Streptococcus agalactiae* and for use with optochin and bacitracin discs for presumptive identification of group A streptococci.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 \pm 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Blood Agar Sheep	Ready-to-use plates	541151	2 x 10 plates \varnothing 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, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Blood Agar Sheep plates can be directly inoculated with many clinical specimens (e.g. sputum, nose, throat, wounds, urine etc.). Refer to the quoted literature for specimens' types, related to specific infections.³⁻⁵ Blood Agar Sheep is not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.³

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and 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 at 35-37°C in aerobic conditions with or without 5 -10% CO₂, and record the results after 18-24, 48 and if necessary, 72 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

CAMP test: a known haemolytic strain of *S. aureus* (ATCC 33862) is streaked in a straight line across the centre of the plate. Test inoculum is streaked in a straight line (2-3 cm in length) perpendicular to *S. aureus* streak but without touching it. A known Group B *Streptococcus* may also be streaked similarly as a positive control. Four-five test organisms may be tested per plate. The plate is incubated at 35-37°C for 18-24 hours.





9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies. By cultivation on Blood Agar Sheep, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α -haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β -haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α -prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Here below are summarized the colony characteristics of some microorganisms which can be isolated on Blood Agar Sheep plates.⁶

- The colonies of Group A streptococci are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of Group B streptococci are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or subsurface β -haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of Group A colonies to be of any value in identification.
- Group D streptococcal colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO₂ incubators, are surrounded by a fairly large zone of α -haemolysis.
- *Viridans* streptococcal colonies may be surrounded by a small zone of α -haemolysis or have no zone of haemolysis; rarely they show an α -prime haemolysis.
- Staphylococci colonies are yellow or white with or without the β -haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small β -haemolytic zone.

Once colonies have grown on Blood Agar Sheep plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

CAMP: a positive test for CAMP factor appears as "arrowhead" haemolysis between the junction of growth of *S. aureus* and Group B *Streptococcus*. There is no enhanced or "arrowhead" haemolysis if the test isolate is not Group B *Streptococcus*.

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.⁷

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>S. pyogenes</i> ATCC 19615	35-37°C / 18-24H / A or CO ₂	good growth, β -haemolysis
<i>S. pneumoniae</i> ATCC 6305	35-37°C / 18-24H / A or CO ₂	good growth, α -haemolysis
<i>S. aureus</i> ATCC 25923	35-37°C / 18-24H / A or CO ₂	good growth, diffuse β -haemolysis
<i>E. coli</i> ATCC 25922	35-37°C / 18-24H / A or CO ₂	good growth

A: aerobic 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 Blood Agar Sheep and of the raw material used for the production of prepared plates (dehydrated Tryptic Soy Agar REF 402150) are tested for productivity and haemolytic model by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative echometric technique with the following strains: *S. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 6305, *S. agalactiae* ATCC 12386, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. After aerobic incubation at 35-37°C for 18-24 hours the types of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth with typical haemolytic model.

CAMP test is performed with *S. aureus* ATCC 33862 and *S. agalactiae* ATCC 12386. After incubation at 35-37°C for 18-24 hours an arrowhead haemolysis of the test strain *S. agalactiae* is observed.

Accuracy was assessed by reviewing the Quality Control data. The results of 70 batches produced from 1/1/2019 to 22/6/2020 were evaluated. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity and differential properties with target strains.

12 - LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional media such as selective media and Chocolate Agar.
- The growth and type of haemolysis depend on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected. *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium⁸; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- The device is not intended to diagnose infections or to guide the antimicrobial therapy. It is used in a diagnostic set of investigations to provide microbial colonies isolated from clinical samples of patients with suspected bacterial infection. Appropriate tests are required for complete identification and epidemiological typing of colonies; if necessary, perform antimicrobial susceptibility tests using recommended methods.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic device intended for professional use only, is not automated and is not a companion diagnostic tool. It must be used by adequately trained and qualified laboratory personnel, observing 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. 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 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 and dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains, in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify the Manufacturer (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The Manufacturer may not be held responsible for any loss or damage in any way resulting from or related to use of the product in manners not compliant with the instructions provided.

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. Buxton T. Blood agar plates and hemolysis protocols. ASM Science, 2005
2. Robert M, Ritchie J. 1903. Manual of Bacteriology. The MacMillan Company, London, 1903.
3. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
4. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
5. The Royal College of Pathologists. Bacteriology. <https://www.rcpath.org/profession/publications/standards-for-microbiology-investigations/bacteriology.html>
6. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
8. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

TABLE OF APPLICABLE SYMBOLS

REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> diagnostic medical device	Manufacturer	This way up	For single use only	CE European conformity mark
Temperature limitations	Contents sufficient for <n> tests	Consult electronic instructions for use	Use by	Keep away from sunlight	Fragile, handle with care	UDI Unique device identifier

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03
Revision 3	Specimens, performances characteristics, limitation of the method, precautions and warnings, table of applicable symbols.	2025/10

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

