INSTRUCTIONS FOR USE

BLOOD AGAR HORSE

Ready-to-use plates

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

1 - INTENDED USE

In vitro diagnostic device. General purpose medium with defibrinated horse 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	13.5 g
Growth factors	1.5 g
Defibrinated horse 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 agar[1]; in their 1903 Manual of Bacteriology, Muir and Ritchie[2] 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 Horse is prepared from Tryptic Soy Blood Agar Base with 5% defibrinated horse blood.
Blood Agar Horse is a general purpose, enriched medium used to grow fastidious and non-fastidious organisms and to differentiate bacteria based on their haemolytic properties.
Horse blood provides X (hemin) and V (NAD) factors required for the growth of some fastidious microorganisms including *Haemophilus influenzae*. Selected casein and soy peptones improve the bacterial haemolytic reactions and provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The inclusion of a mixture of growth factors enhances the growth of fastidious organisms. The presence of horse blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.

4 - PHYSICAL CHARACTERISTICS

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

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Blood Agar Horse	Ready-to-use plates	541180	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, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Blood Agar Horse plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens types, related to specific infections.[3-5] Blood Agar Horse 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 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% CO_2, 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.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.

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By cultivation on Blood Agar Horse, 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.

Haemolytic properties referred to blood agar with sheep blood might be different with Blood Agar Horse plates.
Blood Agar Horse provides clearer β-haemolysis of streptococci than Blood Agar Sheep.
- Group A streptococci: colonies surrounded by a well-defined zone of complete haemolysis
- Group B and C haemolytic streptococci: larger colonies (2-4 mm) surrounded by a zone of transparency (β-haemolysis)

H.haemolyticus colonies produce β-haemolysis and mimic *Streptococcus pyogenes*.
Enterococci produce β-haemolysis on horse blood and are not normally haemolytic with sheep blood.
S.aureus which is usually β-haemolytic on sheep blood, will often be non-haemolytic on horse blood.

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
S.pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, beta haemolysis
S.pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, alpha haemolysis
S.aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth
E.coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth
H.influenzae	ATCC	10211	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 Horse is tested for productivity and haemolytic pattern. Productivity is tested by semi-quantitative ecometric technique with the following strains: *S.pyogenes* ATCC 19615, *S.pneumoniae* ATCC 6305, *H.influenzae* ATCC 10211, *S.aureus* ATCC 25923, *E.coli* ATCC 25922. After incubation at 35-37°C for 18-24 hours in aerobic conditions with 5-10% CO_2, the types of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth with typical haemolytic pattern.

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 us selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected. *Neisseria*, *Mycobacterium*, *Bordetella, Legionella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies present on the plates are differentiated on the basis of their morphological, chromatic and 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, 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. 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. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019

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	2020/08
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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