BLOOD AGAR BASE N° 2 ISO FORMULATION

Dehydrated culture medium

BLOOD AGAR SHEEP N° 2 ISO FORMULATION

ready to use plates

Group A β-haemolytic Streptococcus

1 - INTENDED USE

Non-selective medium with strong nutritional properties, to be used with enrichments (e.g. animal blood) for the isolation and cultivation of fastidious and non-fastidious microorganisms and for the determination of bacterial haemolysis.

2 - COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	15.0 g
Liver extract	2.5 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Agar	13.0 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Blood Agar Base N ° 2 ISO Formulation is a general purpose medium with richer nutritive properties than other blood agar base media and with special capacity to promote the pigment production by bacteria; it can be used with the addition of various enrichments such as blood, serum, carbohydrates, for the cultivation of fastidious microorganisms.
Peptone, liver extract and yeast extract are sources of carbon, nitrogen, vitamins and trace elements for microbial growth; sodium chloride contributes to the osmotic balance of the medium.
With the addition of defibrinated animal blood, the medium is used for the isolation and cultivation of fastidious microorganisms and for the highlighting of the haemolytic properties of streptococci, staphylococci, lysteria and other microorganisms. Blood Agar Base N°2 ISO Formulation complies with ISO 11290[1] and ISO 7932[2].

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add 5-7% of sterile defibrinated sheep blood. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Blood agar plates appearance	deep red, opaque
Final pH at 20-25 °C	7.1 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Blood Agar Base N°2 ISO Formulation	Dehydrated medium	401156P2	500 g (12,3 L)
Blood Agar Sheep N°2 ISO Formulation	Ready to use plates	541156P	2 x 10 plates

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Animal blood, autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Blood Agar Base N°2 ISO Formulation supplemented with sheep blood and poured in plates can be directly inoculated with many specimens or with colonies growth on other isolation media. Refer to the quoted literature for specimen types, related to specific applications.[1-4] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.

9- 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% CO_2, and record the results after 18-24, 48 and, if necessary, 72 hours.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milano, Italia
Tel. +39 02 25209.1
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

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.

For the confirmation of *Listeria monocytogenes* according to ISO 112901 proceed as follows.
Inoculate plates of medium supplemented with defibrinated sheep blood to determine the haemolytic reaction of the isolated strain.
Pick an isolated colony and streak it on the surface of the medium. Repeat for each suspected colony.
Incubate at 37°C for 24 h ± 2 h and examine for the presence of areas of haemolysis.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
On Blood Agar Base N°2 ISO Formulation, 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.

Confirmation test for *L. monocytogenes*:
L. monocytogenes shows small zones of clear hemolysis
L. innocua shows no hemolytic activity around the colonies.
L. seeligeri shows mainly a zone of weak hemolysis.
L. ivanovii usually shows large and clearly delineated zones of hemolysis.
Examine the plates with a bright light and compare the results of the test strains with the control strains. The hemolysis reaction is most easily seen by removing the colony growth from the agar surface.

11 - 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
L. monocytogenes ATCC 13932	35-37°C / 18-24H / A o CO_2	good growth, beta haemolysis
L. innocua ATCC 33090	35-37°C / 18-24H / A o CO_2	good growth, no haemolysis
S. pyogenes ATCC 19615	35-37°C / 18-24H / A o CO_2	good growth, beta haemolysis
S. pneumoniae ATCC 6305	35-37°C / 18-24H / A o CO_2	good growth, alpha haemolysis

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Blood Agar Base N°2 ISO Formulation, supplemented with defibrinated sheep blood is tested for productivity and haemolytic pattern by comparing the results with a previously approved Reference Batch.
Productivity on blood sheep supplemented medium is tested by semi-quantitative ecometric technique with the following strains: *S.pyogenes* ATCC 19615*, S. pneumoniae* ATCC 6305*, Group B Streptococcus* ATCC 12389*, S.aureus* ATCC 25923, *E.coli* ATCC 25922, *L.monocytogenes* ATCC 13932, *L.innocua* ATCC 33090, *B.cereus* ATCC 11778. After incubation at 35-37°C for 18-24 hours the type of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth comparable to the Reference Batch, with typical haemolytic or non-haemolytic pattern.

13 - LIMITATIONS OF THE METHOD

- Depending on the specimens analyzed and the microorganisms being tested for, it is recommended for the examination of clinical specimens to use also additional media such us 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 patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[8]; *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 hemolytic reactions of some strains of group D streptococci are influenced by the type of blood used: they are beta-hemolytic with horse, human and rabbit blood and alpha-haemolytic with sheep blood.
- The incubation atmosphere influences the haemolytic reactions of beta-haemolytic streptococci: for optimal performance, incubate the plates in aerobic conditions with 5-10% CO_2 or in anaerobic conditions.
- 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.

14 - PRECAUTIONS AND WARNINGS

- This product is intended for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come in 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.

- Apply Good Manufacturing Practice in the preparation process of plated, tubed, bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium:

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method applied (temperature and packaging).

Ready to use plates:

Upon receipt, store plates in their original pack at +2°C /+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).

16 - REFERENCES

1. ISO 11290:2107. Microbiology of food and animal feeding stuffs. Horizontal Methods for the detection and enumeration of Listeria monocytogenes and of Listeria spp.
2. ISO 7932:2004. Microbiology of food and animal feeding stuffs. Horizontal Methods for the enumeration of presumptive *Bacillus cereus.* Colony count technique at 30°C.
3. 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.
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. 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 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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First release	2025/06

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