

**INSTRUCTIONS FOR USE****BLOOD AGAR BASE N° 2**

Dehydrated culture medium



Group A  $\beta$ -haemolytic Streptococcus on Blood Agar Base n° 2 supplemented with sheep blood.

**1 - INTENDED USE**

*In vitro* diagnostic. Improved blood agar base to be used with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of their haemolytic properties.

**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 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 serum or other enrichments, Blood Agar Base n° 2 becomes suitable for the cultivation of fastidious microorganisms such as streptococci, pneumococci, meningococci, *Haemophilus*.

Supplemented with sterile defibrinated sheep or horse blood, the medium is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of haemolytic properties of streptococci, staphylococci and other microorganisms. With the addition of a suitable selective supplement, the growth supplement and 5-7% of defibrinated sheep or horse blood, Blood Agar Base N° 2 is apt for isolation of *Campylobacter* spp.

Blood Agar Base n° 2 conforms to the formulation reported by FDA-BAM.<sup>1</sup> The formulations according to ISO standard 7932<sup>2</sup> and 11290<sup>3</sup> differ for their final pH values (ISO 7032:  $7.0 \pm 0.2$ ; ISO 11290:  $7.2 \pm 0.2$ ).

**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 or horse 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	$7.4 \pm 0.2$

**6 - MATERIALS PROVIDED - PACKAGING**

Product	Type	REF	Pack
Blood Agar Base N° 2	Dehydrated medium	4011562	500 g (12,3 L) CND: W0104010101; EDMA:14.01.01.01; RDM: 1852951/R
		4011564	5 kg (123 L) CND: W0104010101; EDMA:14.01.01.01; RDM: 1852953/R

**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 supplemented with sheep blood and poured in 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 specimen types, related to specific infections.<sup>4-6</sup> Blood Agar plates are 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.<sup>4</sup>

### 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<sub>2</sub>, 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.

### 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies. By cultivation on sheep blood agar plates prepared with Blood Agar Base N°2, 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 colonies characteristics of some microorganisms which can be isolated on blood agar sheep plates.<sup>7</sup>

- 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 CO<sub>2</sub> incubators, are surrounded by a fairly large zone of α-haemolysis.
- The 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 plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

### 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, it is responsibility of the end-user to perform Quality Control testing 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>S. pyogenes</i> ATCC 19615	35-37°C / 18-24H / A or CO <sub>2</sub>	good growth, beta haemolysis
<i>S. pneumoniae</i> ATCC 6305	35-37°C / 18-24H / A or CO <sub>2</sub>	good growth, alpha haemolysis
<i>S. aureus</i> ATCC 25923	35-37°C / 18-24H / A or CO <sub>2</sub>	good growth
<i>E. coli</i> ATCC 25922	35-37°C / 18-24H / A or CO <sub>2</sub>	good growth

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, 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, *S. agalactiae* ATCC 12386, *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 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 patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood<sup>8</sup>; *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<sub>2</sub> 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.
- 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.

**14 - 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.
- 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 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.
- 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](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.








**15 - STORAGE CONDITIONS AND SHELF LIFE**

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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

**16 - REFERENCES**

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), M22 Blood Agar Base #2
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. ISO 11290:2107. Microbiology of food and animal feeding stuffs. Horizontal Methods for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp
4. 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.
5. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneva: World Health Organization.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
7. 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.
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 chocolate 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**

<b>REF</b> or REF 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	 Keep away from direct light	 Store in a dry place

**REVISION HISTORY**

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/06

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

