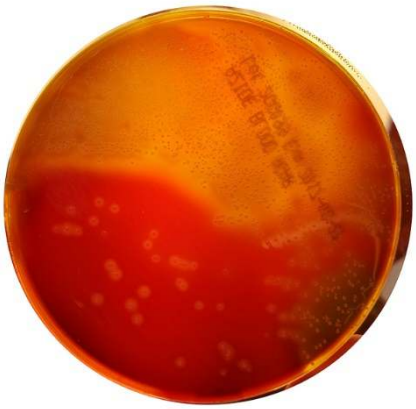
AZIDE BLOOD AGAR BASE

Powdered culture medium



Azide Blood Agar:
group A beta haemolytic Streptococcus colonies

1 - INTENDED USE

Selective culture medium, for use with defibrinated sheep blood, for the isolation of Gram-positive cocci from clinical specimens and other materials.

2 - COMPOSITION

TYPICAL FORMULA PER LITRE, AFTER DISSOLVING IN WATER *

Tryptose	10 g
Meat extract	3 g
Sodium chloride	5 g
Sodium azide	0.2 g
Agar	15 g

* The ground can be compensated and/or corrected to match its performance to specifications.

3 - DESCRIPTION AND PRINCIPLE OF THE METHOD

Azide Blood Agar Base, fortified with defibrinated mutton blood, is recommended for the selective isolation of Gram-positive cocci, especially streptococci and staphylococci, from pharyngeal swabs, faeces, water, food and other samples grossly contaminated with Gram-negative flora.
The review by Hartman and co-workers[1] lists more than forty types of selective media for streptococci based on sodium azide: this substance has a bacteriostatic effect on numerous bacterial species, in particular Gram-negative (by blocking metalloporphyrin enzyme systems catalase, cytochrome C oxidase), inhibits the swarming of proteus colonies, does not interfere with the phenomenon of haemolysis and allows the development of some Gram-positive species, in particular streptococci, staphylococci and some anaerobes.

4 - PREPARATION

Suspend 33.2 g of powder in 1000 mL of cold purified water. Bring to the boil under stirring, and autoclave at 121°C for 15 minutes.
Cool in a thermoregulated water bath to approximately 47-50°C and add, with asepsis precautions, 5% sterile defibrinated sheep blood.
Mix well and distribute in sterile Petri dishes.

5 - TERRAIN CHARACTERISTICS

Appearance of powderfine	homogeneous grain size, beige
Appearance of the soil in solution	clear
Appearance of	opaque blood-red soil
Final pH at 25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Packaging
Azide Blood Agar Base	Powdered culture medium	4011002	500 g (15 L)

7 - MATERIALS NEEDED AND NOT PROVIDED

Autoclave, water bath, thermostat and other calibrated and controlled laboratory instrumentation, defibrinated sheep blood, microbiology loops, reagents and accessory culture media, materials for generating the controlled incubation atmosphere.

8 - SAMPLES

All types of clinical or non-clinical samples can be used; they must be sown on the surface of the plate medium. Apply good laboratory practice standards for specimen collection, storage and transport to the laboratory.

9 - ANALYSIS PROCEDURE

Bring the plates to room temperature. Rotate the swab with which the sample was collected over a small area of the plate, then swab four quadrants of the plate with a loop to disperse the inoculum and obtain isolated colonies.
Incubate the inverted plates at 37 °C for 24-48 hours in aerobiosis and/or anaerobiosis. Since streptolysin O is inactivated by oxygen, and since some streptococcus strains grow better at reduced oxygen tension, several authors recommend incubating in anaerobiosis, or to set up a double series of seeding and incubate one in aerobiosis and one in anaerobiosis.

10 - READING AND INTERPRETATION OF RESULTS

After incubation, observe bacterial growth and the presence of any haemolytic zones around the colonies. The appearance of haemolysis in Azide Blood Agar is typical:

- alpha-haemolysis: greenish-brown halo, sometimes surrounded by a clear halo, on microscopic observation the corpuscles appear discoloured but intact;
- beta-haemolysis: transparent red halo, on microscopic observation the red blood cells appear broken.

The complete identification of the micro-organisms grown on the medium must be carried out by biochemical, immunological, molecular or mass spectrometric techniques, after purification of the colonies by subculture on appropriate medium.

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

11 - QUALITY CONTROL

Each batch of the product described herein is released for sale after it has undergone quality control to verify its conformity to specifications. It is up to the user to carry out his own quality control in accordance with the relevant regulations and according to his own laboratory experience. The following table shows some useful strains for quality control.

CONTROL STRAINS		INCUBATION (T°/ t / ATM)	EXPECTED RESULTS
S.pyogenes	ATCC 19615	37°C / 24h / A	good growth, beta haemolysis
S.pneumoniae	ATCC 6305	37°C / 24h / A	good growth, alpha haemolysis
S.aureus	ATCC 25923	37°C / 24h / A	good growth
P.mirabilis	ATCC 12453	37°C / 24h / A	growth inhibited

A: incubation in aerobiosis; ATCC is a registered trademark of American Type Culture Collection

13 - LIMITS OF THE METHOD

- The medium described here is selective for Gram-positive cocci; to isolate and recognise the pathogens in the sample, sow the test material also on appropriate non-selective media.
- *Proteus* spp. can grow on the soil but swarming is inhibited. *E. coli* is completely or partially inhibited.
- The growth and type of haemolysis on the medium described here depends on the metabolic requirements of each micro-organism; it is possible that some strains are unable to grow on the medium and/or show different haemolytic patterns than expected.
- Haemolysis in media containing sodium azide sometimes appears different to that obtained in common blood media: *S.agalactiae* is normally gamma haemolytic but in media with sodium azide exhibits beta haemolysis; the alpha and beta haemolysis in media with sodium azide are usually wider.
- The medium described here is intended as an aid to the diagnosis of infections sustained by Gram-positive cocci. Interpretation of the results must be made considering the patient's medical history, the origin of the sample and the results of microscopic and/or other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The medium described here is intended for microbiological control, is for professional use and must be used in the laboratory by appropriately trained operators, with approved methods of asepsis and safety against pathogens.
- Powdered soils must be handled with appropriate protection. Consult the safety data sheet before use.
- Apply good manufacturing practice in the process of preparing culture media.
- The culture medium described here contains materials of animal origin. *Ante-mortem and post-mortem* controls of the animals and those during the production and distribution cycle of the raw materials cannot absolutely guarantee that this product does not contain any transmissible pathogens; for these reasons it is recommended to handle the product with the specific safety precautions for potentially infectious materials (do not ingest, do not inhale, avoid contact with skin, eyes, mucous membranes). Download from the website www.biolifeitaliana.it the TSE Statement document, with the measures implemented by Biolife Italiana S.r.l. to contain the risk of transmissible animal diseases.
- Treat samples as potentially infectious.
- The laboratory environment must be controlled to avoid contaminants such as culture media or microbial agents.
- Sterilise all biohazardous waste before disposal. Dispose of unused soil and soil inoculated with samples or microbial strains and sterilised in accordance with relevant legislation.
- Do not use the product as an active ingredient for pharmaceutical preparations or as a material for production for human or animal consumption.
- The Certificates of Analysis and Safety Data Sheet for the product are available at www.biolifeitaliana.it.
- The information contained in this document has been established to the best of our knowledge and ability and represents a guideline for the correct use of the product, but without commitment or liability. The end user must, in all cases, comply with local laws, regulations and standard procedures for the examination of samples collected from the various human and animal organic districts, environmental samples and products intended for human or animal consumption. Our information does not relieve the end-user of his responsibility to check the suitability of our products for their intended purpose.

15 - STORAGE AND VALIDITY

Store at +10°C /+30°C protected from light and moisture. Under these conditions the product remains valid until the expiry date indicated on the label. Do not use after this date. Avoid opening the bottle in humid environments. Once opened, store the product by keeping the cap of the container tightly closed. Dispose of the product if the container and/or cap are damaged, if the containers are not tightly closed or in the event of obvious deterioration of the powder (colour changes, hardening, presence of large lumps).
The user is responsible for the production and control process of the media prepared in the laboratory and for defining their shelf life, depending on the type (test tubes/flasks/plates) and storage conditions.

16 - BIBLIOGRAPHY

1. Hartman. P.A. Beinbold, G.W. & Saraswat D.S. (1966) - Adv. Appl. Micr. 8, 253-289.
2. Mac Faddin, J.F. (1985) Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Baltimore: The Williams & Wilkins Company.
3. Moody, M.D. (1972) - Old and new techniques for rapid identification of group A streptococci. In: 'Streptococci and streptococcal diseases', ed. Wannamaker, S.W. & Matsen J.M., London & New York Academic Press.

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	LOT Lot number	Use within	Manufacturer	
Temperature limits	Sufficient content for <n> essays	Consult the Instructions for Use	Protect from light	Protect against moisture

REVISION HISTORY

Version	Description of Changes	Date
Revision 3	Updating content and layout	11/2023

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

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