



Azide Blood Agar Base

M158

Intended use

Azide Blood Agar Base is used for the selective isolation and cultivation of *Staphylococcus* and *Streptococcus* species from mixed bacterial flora.

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
HM Peptone B#	3.000
Sodium chloride	5.000
Sodium azide	0.200
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 33.2 grams in 1000 ml of purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. For preparing Blood Agar plates, 5% v/v sterile defibrinated blood is added aseptically. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Azide Blood Agar Base is recommended for the isolation and cultivation of *Streptococcus* species from clinical and non-clinical specimens. It is a modification of the broth medium originally formulated by Edwards for the detection of Streptococci from bovine mastitis cases (1). The original broth medium of Edwards was modified to a blood agar by Packer containing sodium azide and crystal violet (5).

Peptone special and HM Peptone B are the sources of carbon, nitrogen and essential growth factors. Sodium azide acts as a selective agent by suppressing the growth of gram-negative bacteria. It also prevents the swarming of *Proteus* (3,6). Sodium chloride helps to maintain the osmotic balance of the medium. The media can be supplemented with sterile defibrinated blood to prepare blood agar. Blood serves as an additional source of growth factors and it also helps to visualize the haemolytic pattern. The pH of the medium influences the inhibitory action of sodium azide (5). At pH 7.2, sodium azide does not interfere with the haemolytic reactions of Streptococci; however, haemolytic pattern of Streptococci is different on Azide Blood Agar as compared on nonselective blood agar.

For best results, use light inoculum and incubate anaerobically for enhancement in haemolytic reaction. Different types of haemolysis can be visualized on blood agar plates (2).

Type of specimen

Clinical samples - Blood

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The degree of haemolysis or the haemolytic pattern obtained differs with the type of blood used for preparation of blood agar, and also the composition of blood agar used (4).
2. Sodium azide effects haemolysis, hence the haemolytic pattern may be different in comparison with non- selective Blood Agar.
3. Haemolytic pattern is affected by type of blood used (e.g. sheep blood, horse blood)

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium : Yellow coloured, clear to slightly opalescent gel. After addition of 5%w/v sterile defibrinated blood : Cherry red coloured, opaque gel forms in Petri plates, which darkens on standing

Reaction

Reaction of 3.32% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M158: Cultural characteristics observed with added 5%w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
<i>Enterococcus faecalis</i> ATCC 50-100 29212 (00087*)		luxuriant	≥50%	alpha/gamma
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	≤10%	none
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	luxuriant	≥50%	none
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	good-luxuriant	≥50%	beta
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	luxuriant	≥50%	alpha

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,4).

Reference

1. Edwards, 1933, J. Comp. Pathol. Therap., 46:211.
2. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I., American Society for Microbiology, Washington, D.C.
3. Lichstein and Snyder, 1941, J. Bacteriol., 42:653.
4. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover F. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
5. Packer, 1943, J. Bacteriol., 1943, 46:343
6. Snyder and Lichstein, 1940, J. Infect. Dis., 67:113.

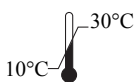
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