

Azide Blood Agar Base

For the isolation of streptococci and staphylococci. With blood, for researching hemolytic reactions

Practical information

Applications	Categories
Selective isolation	Staphylococcus
Selective isolation	Streptococcus

Industry: Water / Clinical / Food



Principles and uses

Azide Blood Agar Base contains Sodium azide which has been proved to have a bacteriostatic effect on Gram-negative bacteria, thus, this medium is used for the isolation of streptococci and staphylococci in clinical specimens, water, foods, etc.

Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. Sodium chloride supplies essential electrolytes for transport and osmotic balance. 0.02% Sodium Azide in blood agar was reported to prevent the swarming of Proteus and allows the selective isolation from mixed bacterial populations. Gram-negative organisms are inhibited by Sodium azide.

The medium can be supplemented with 5% sheep blood that allows for the investigation of hemolytic reactions of fastidious pathogens. Hemolytic patterns may vary with the type of blood or base medium used. For instance, defibrinated sheep blood gives best results for Group A streptococci.

Formula in g/L

Bacteriological agar	15	Beef extract	3
Peptone mixture	10	Sodium azide	0,2
Sodium chloride	5		

Preparation

Suspend 33,2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. To prepare blood agar, cool to 45-50 °C and aseptically add 5% sterile defibrinated blood. Homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.

Instructions for use

- » For clinical diagnosis, the type of sample is secretions of the respiratory tract, sputum.
 - Inoculate sample onto the surface of the medium.
 - For isolation, streak with an inoculating loop.
 - Incubate plates aerobically, anaerobically or under CO₂ (5-10%) in accordance to standard procedures at 35±2 °C.
 - Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours.

- » For other uses not covered by the CE marking:
Isolation of streptococci and staphylococci in foods:
 - Prepare the initial suspension and the appropriate dilutions of the sample.
 - Streak 0,1 ml aliquots of the dilutions onto the surface of the prepared medium.
 - Incubate the plates at 35±2 °C under appropriate atmospheric conditions and observe after 18-24 and 40-48 hours.

*Results:

1. Alpha-hemolysis: greenish discoloration of medium.
2. Beta-hemolysis: clear zone surrounding colony.
3. Gamma-hemolysis: no change.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Amber, opalescent / With blood: Cherry red.	7,2±0,2

Microbiological test

Incubation conditions: (35±2 °C /18-24 h).

Microrganisms	Specification	Characteristic reaction
Staphylococcus epidermidis ATCC 12228	Good growth	Hemolysis gamma
Enterococcus faecalis ATCC 19433	Good growth	Hemolysis alpha/gamma
Streptococcus pyogenes ATCC 19615	Good growth	Hemolysis beta
Escherichia coli ATCC 25922	Inhibited growth	
Streptococcus pneumoniae ATCC 6305	Good growth	Hemolysis alpha

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

Edwards, S. J. 1933 The diagnosis of Streptococcus mastitis by cultural methods. J. Comp. Pathol. Ther. 46:211.
Lichstein, H. C., and M.L. Snyder. 1941. The inhibition of the spreading growth of Proteus and other bacteria to permit the isolation of associated streptococci. J. Bacteriol. 42:653