Azide Blood Agar Base

Intended Use

Azide Blood Agar Base is used for isolating streptococci and staphylococci and, supplemented with blood, for determining hemolytic reactions.

Summary and Explanation

In 1933, Edwards[1] used a liquid medium containing crystal violet and sodium azide as a selective broth in the isolation of mastitis streptococci. Snyder and Lichstein[2,3] reported that 0.01% sodium azide in blood agar prevented the swarming of *Proteus* species, and permitted the isolation of streptococci from mixed bacterial populations. Packer[4] modified Edwards' medium and prepared Infusion Blood Agar containing 1:15,000 sodium azide and 1:500,000 crystal violet for the study of bovine mastitis. Mallmann, Botwright and Churchill[5] reported that sodium azide exerted a bacteriostatic effect on gram-negative bacteria. The Azide Blood Agar Base formulation was based on the work of these researchers.

Azide Blood Agar Base is used in the isolation of gram-positive organisms from clinical and nonclinical specimens. Azide Blood Agar Base can be supplemented with 5-10% sheep, rabbit or horse blood for isolating, cultivating and determining hemolytic reactions of fastidious pathogens.

Principles of the Procedure

Peptones and beef extract provide nitrogen, vitamins, carbon and amino acids. Sodium chloride maintains osmotic balance. Sodium azide is the selective agent, suppressing the growth of gram-negative bacteria. Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms, and is used to determine hemolytic patterns of bacteria.

Formula

Difco™ Azide Blood Agar Base

Approximate Formula* Per Liter		
Proteose Peptone No. 3	4.0	g
Pancreatic Digest of Casein	5.8	g
Beef Extract	3.0	g
Sodium Chloride	5.0	g
Sodium Azide	0.2	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 33 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium when cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Azide Blood Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slight to slightly opalescent.
Prepared Appearance:	Plain–Light to medium amber, very slightly opalescent. With 5% blood–Cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Azide Blood Agar Base

Prepare the medium per label directions, without and with 5% sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Enterococcus faecalis	19433	10^2-10^3	Good	Alpha/gamma
Escherichia coli	25922	10^3-2×10^3	Inhibition	–
Staphylococcus aureus	25923	10^2-10^3	Good	Beta
Staphylococcus epidermidis	12228	10^2-10^3	Good	Gamma
Streptococcus pneumoniae	6305	10^2-10^3	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Good	Beta

Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions demonstrating both oxygen-stable and oxygen-labile streptolysins.[6]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 in accordance with established laboratory procedures.

Expected Results

Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of hemolysis on blood agar media can be described:[7]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.

d. Alpha-prime (α)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Azide Blood Agar Base is intended for selective use and should be inoculated in parallel with nonselective media.
2. Hemolytic patterns of streptococci grown on Azide Blood Agar Base are somewhat different than those observed on ordinary blood agar. Sodium azide enhances hemolysis. Alpha and beta zones may be extended.[4]
3. Hemolytic patterns may vary with the source of animal blood or base medium used.[6]

References

1. Edwards. 1933. J. Comp. Pathol. Therap. *46*:211.
2. Snyder and Lichstein. 1940. J. Infect. Dis. *67*:113.
3. Lichstein and Snyder. 1941. J. Bacteriol. *42*:653.
4. Packer. 1943. J. Infect. Dis. *67*:113.
5. Mallmann, Botwright and Churchill. 1943. J. Bacteriol. *46*:343.
6. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Azide Blood Agar Base

Cat. No. 240920 Dehydrated – 500 g