Technical Information

Azide Blood Agar Base

Product Code: DM 1158

Application: Azide Blood Agar Base is used for the isolation and cultivation of *Streptococcus* species from clinical and non-clinical specimens.

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
Beef extract	3.000
Sodium chloride	5.000
Sodium azide	0.200
Agar	15.000
Final pH (at 25°C) **Formula adjusted, standardized to suit performance parameters	7.2±0.2

Principle & 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 case of bovine mastitis [(1)]. The original broth medium of Edwards was further modified to a blood agar by Packer who added sodium azide and crystal violet to make media more selective for isolation of Streptococcus from different clinical sample [(2)]. Peptone special and beef extract 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 and preventing the swarming of *Proteus* [(3, 4)]. 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 inhibitory action of sodium azide is pH dependent [(2)]. 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 plate / tube anaerobically. 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 [(5, 6)].

Methodology

Suspend 33.2 grams of powder media in 1000 ml of distilled water. Shake well & heat 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.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Quality Control

Physical 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 Range:- 7.00-7.40

Cultural Response/Characteristics

DM1158: 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
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=50%	alpha/gamma
Escherichia coli ATCC 25922	50-100	none-poor	>=50%	None
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=50%	None
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=50%	beta
Streptococcus pneumonia ATCC 6303	50-100	luxuriant	>=50%	alpha

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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

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