Technical Information

Tryptose Blood Agar Base

Product Code: DM 1097

Application: Tryptose Blood Agar Base is recommended for the isolation of fastidious organisms and determining the haemolytic reactions.

Composition**

Ingredients	Gms / Litre
Tryptose	10.000
Beef extract	3.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Tryptose Blood Agar Base is a tryptose based medium used for the cultivation of fastidious organisms, on supplementation with blood [1, 2]. This medium is without dextrose and therefore useful in determining the haemolytic reactions more clearly.

Tryptose Blood Agar Base is recommended both by FDA and APHA [3, 4]. Tryptose Blood Agar Base can be used as a general-purpose medium without supplementation of blood. These media when supplemented with blood can be used to determine the heamolytic reactions of fastidious organisms. The four different types of haemolysis observed are as follows:

a) Alpha haemolysis: partial lysis of the erythrocytes surrounding colony, causing a grey green or brownish discolouration in the media.

b) Beta haemolysis: complete lysis of the red blood cells surrounding a colony, causing a clearing of blood from the medium.

c) Gamma haemolysis: no haemolysis and consequently, no colour change of the medium surrounding a colony. Organisms showing no haemolysis are generally termed non-hemolytic rather than gamma haemolytic.

d) Alpha-prime or wide zone alpha: a small zone of intact erythrocytes immediately adjacent to the colony, with a zone of complete red cell haemolysis surrounding the zone of intact erythrocytes. This type of haemolysis may be confused with beta haemolysis [6].

Tryptose and beef extract provide nitrogenous and carbonaceous compounds, sulphur, vitamin B complex and trace elements essential for bacterial metabolism. Blood provides additional nutrients and serves as a base to study haemolytic reactions. This medium not only keeps the blood cells in a good state but also help in forming distinct haemolysis. Perform biochemical test for further identification [5] which has added advantage in the confirmation of micro organism.

Methodology

Suspend 33 grams of powder media in 950 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the autoclaved medium to 45 - 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix thoroughly, avoiding air bubbles and pour into sterile Petri plates.

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 forms in Petri plates. After addition of 5% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 3.3% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range:- 7.00-7.40

Cultural Response/Characteristics

DM 1097: Cultural characteristics observed with added 5% v/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth w/ blood	Haemolysis	Recovery w/ blood
Neisseria meningitides ATCC 13090	50-100	good-luxuriant	>=70%	luxuriant	none	>=70%
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%	luxuriant	beta/gamma	>=70%
Staphylococcus epidermidis ATCC 12228	50-100	good-luxuriant	>=70 %	luxuriant	gamma	>=70%
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant	40-50%	good	alpha	50-70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	40-50%	good	beta	50-70%

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. Casman E. P., 1942, J. Bacteriol., 43:33.
2. Casman E. P., 1947, Am. J. Clin. Pathol., 17: 281.
3. FDA Bacteriological Analytical Manual, 8th Ed., 1995, AOAC International, Gaithersburg, Md.
4. American Public Health Association, 1970, Diagnostic Procedures and Reagents, 5th Ed., APHA Inc., New York.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
6. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.

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