



Sheep Blood Agar Base

M1301

Sheep Blood Agar Base with added sheep blood gives improved haemolytic reactions of organisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	14.000
Peptic digest of animal tissue	4.500
Yeast extract	4.500
Sodium chloride	5.000
Agar	12.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.5 grams in 1000 ml 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 and aseptically add 7% sterile sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Haemolysins are exotoxins produced by bacteria that lyse red blood cells. The haemolytic reaction can be visualized on blood agar plates. On blood agar plates colonies of haemolytic bacteria may be surrounded by clear, colourless zone where the red blood cells have been lysed and the haemoglobin destroyed to a colourless compound. This is beta haemolysis. Other types of bacteria can reduce haemoglobin to methaemoglobin which produces a greenish zone around the colonies and is called alpha haemolysis (1). Gamma haemolysis is no haemolysis where no change in the medium is observed (2). Blood Agar Base No. 2 (M834), supplemented with sheep blood is used to study haemolytic reactions (patterns) of organisms. But this gave mixed haemolytic (a and b) reactions due to the physiological differences between sheep blood and horse blood (3).

Sheep Blood Agar Base with added sheep blood was developed to allow maximum recovery of organisms without interfering with their haemolytic reactions. Sheep Blood Agar Base was formulated to be compatible with sheep blood and give improved haemolytic reactions of organisms.

Casein enzymic hydrolysate and yeast extract provide nitrogen, carbon, amino acids and vitamins. Peptic digest of animal tissue is the nitrogen source. Sodium chloride maintains the osmotic balance.

Sheep Blood Agar Base showed considerable improvement and the expected beta haemolytic reactions with *S. pyogenes* in comparison to other blood agar bases supplemented with blood.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.25% Agar gel.

Colour and Clarity of prepared medium

Basal medium yields light yellow coloured clear gel without any precipitate. On addition of 7% sheep blood, cherry red coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.05% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

M1301: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added 7% v/v sterile sheep blood.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	luxuriant	>=70%	alpha
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	>=70%	beta

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, New Delhi.
2. 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. Lippincott Company.
3. Spector W. S., (Ed.), 1961, Handbook of Biological Data, W. B. Saunder Company, Philadelphia and London.

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