Sheep Blood Agar Base, Modified

M1956

Used for cultivation and studying haemolytic reactions of *Bacillus cereus* in accordance with ISO 21871:2006.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of casein	15.000
Enzymatic digest of soya	5.000
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 37.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% w/v 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 ofbacteria 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).

Bacillus cereus is Gram -positive aerobic or facultatively anaerobic, motile, spore forming, rod shaped bacterium that is widely distributed environmentally. *B.cereus* is associated mainly with food poisoning it is increasingly reported to be cause of serious and fatal non- gastointestinal-tract infections Sheep Blood Agar Base, Modified with added sheep blood was developed to allow maximum recovery of *B.cereus* without interfering with their haemolytic reactions. This medium is formulated in accordance with ISO(3). It was formulated to be compatible with sheep blood and give improved haemolytic reactions of organisms.

Enzymatic digest of casein and soya peptone provide nitrogen, carbon, amino acids and vitamins. Sodium chloride maintains the osmotic balance.

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 : Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinated blood<(>,<)> after an incubation at 35-37°C for 18-48 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)	Recovery	Haemolysis
Cultural Response				
Bacillus cereus ATCC 10876	luxuriant	50-100	>=70%	beta

Storage and Shelf Life

Reference

1.Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, NewDelhi.
2.Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook ofDiagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.
3.International Organization for Standardization (ISO),Draft ISO 21871:2006 Microbiology of Food & Animal feeding stuffs. Horizontal method for the determination of low numbers of presumptive Bacillus cereus-Most probable number technique and detection methods.

Revision : 1 / 2011