Sheep Blood Agar Base, Modified

M1956

Intended Use:

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

Composition**

Ingredients	Gms / Litre
Tryptone ##	15.000
Soya peptone#	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
#- Equivalent to Enzymatic digest of soya
##- Equivalent to Enzymatic digest of casein

Directions

Suspend 37.5 grams in 1000 ml purified / 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 of bacteria can reduce haemoglobin to methaemoglobin which produces a greenish zone around the colonies and is called alpha haemolysis (5). Gamma haemolysis is no haemolysis where no change in the medium is observed (4).

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 (1). It was formulated to be compatible with sheep blood and give improved haemolytic reactions of organisms.

Tryptone and Soya peptone provide nitrogen, carbon, amino acids and vitamins. Sodium chloride maintains the osmotic balance.

Type of specimen

Food and animal feeding stuff samples.

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.This medium is general purpose medium and so biochemical testing is required for further identification of the species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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.

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. 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.
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3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. 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.
5. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, NewDelhi.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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