



Cooked Meat Medium, Modified

M1870

Cooked Meat Medium, Modified is used for the cultivation of *Clostridium perfringens* in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Part A	-
Beef heart, solids	98.000
Proteose peptone	20.000
Dextrose	2.000
Sodium chloride	5.000
Part B	-
Tryptone	10.000
Sodium thioglycollate	1.000
Soluble starch	1.000
Neutral red	0.050
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Part A: Add 1g directly into 20×150mm test tubes

Part B: Suspend 14.05 grams of Part B in 1000ml distilled water.

Complete Medium: Transfer 15ml of Part B medium into the tube containing 1 gram of Part A. Allow to stand so that all the meat particles are rehydrated. Sterilize by autoclaving at 15lbs pressure (121°C) for 15minutes.

Principle And Interpretation

Cooked Meat Medium, Modified is prepared in accordance with FDA BAM, 1998 (1) for the cultivation of *Clostridium* species, particularly *Clostridium perfringens* and *Clostridium botulinum* from food products. *Clostridium* is a large genus of gram-positive spore-bearing anaerobes that are normal inhabitants of the soil and are causative agents of food poisoning. Detection of *C.perfringens* , *C.sporogenes* and *Clostridium botulinum* is of significance because of their ability to produce enterotoxin alpha and botulinum toxins responsible for gas gangrene and botulinism. Cooked Meat Medium was initially developed by Robertson(2) for the cultivation of certain anaerobes isolated from wounds. The medium can be used when the inoculum strength is low and also for maintenance of cultures. This allows the growth of mixed cultures of bacteria without affecting the performance of the other (3).

Cooked Meat Medium contains beef heart, the muscle protein, which provides amino acids and other nutrients. Beef heart also contains glutathione, a reducing substance that permits the growth of obligate anaerobes(4). The sulphhydryl groups, which impart reducing effect, are more available in denatured protein and hence cooked meat is added in the medium. Dextrose allows rapid and heavy growth of anaerobic bacteria in a short time. Sodium Thioglycollate lower the oxidation-reduction potential of the medium. Starch provides the complex carbohydrates that help the growth of anaerobes. Growth in this medium is indicated by turbidity and/or colour change in the medium. *Clostridium perfringens* ferment dextrose, changing the colour of the medium from pink to yellow. Some are late fermenters. Blackening and disintegration of the meat particles indicate proteolysis. It is recommended to use the media in the day of preparation itself; otherwise it should be boiled or steamed for a few minutes and allowed to cool without agitation before inoculation. Inoculation should be made near the bottom of the tube in the meat particles for anaerobic cultures. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.

Representative 25g of food sample is homogenized in 225ml peptone dilution fluids. Appropriate dilutions are prepared and inoculated on TSC Agar for plate count of viable *C. perfringens* . Simultaneously 3or 4 CMM tubes are inoculated with

2ml of homogenates solution as back up for preceding plating procedure. Incubation is carried out at 35°C for 24-48hrs and examined microscopically for morphology appearance as tennis racket shaped *Clostridium* cells.

Quality Control

Appearance

Part A : Brown granules Part B : Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Red coloured slightly opalescent supernatant over insoluble granules

Reaction

Reaction of medium [(6.67% w/v) Part A and (1.41% w/v) Part B] at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours under anaerobic conditions.

Cultural Response

Organism	Inoculum (CFU)	Growth
Cultural Response <i>Clostridium perfringens</i> ATCC 12924	50-100	little-none(no increase in numbers)
<i>Clostridium botulinum</i> ATCC 25763	50-100	luxuriant
<i>Clostridium sporogenes</i> ATCC 11437	50-100	luxuriant

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.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
- 2.Robertson. 1916. J. Pathol. Bacteriol, 20(327).
- 3.Collins, CH., Lyne, PM. and Grange, JM. 1985. Microbiological Methods. .
- 4.MacFaddin, J. F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria vol. 1. Baltimore: Williams and Wilkins.

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