# Cooked Meat Medium (R.C. Medium)

**M149**

Cooked Meat Medium (R.C. Medium) is used for cultivation of aerobes and anaerobes, especially pathogenic Clostridia. This can also be used as a maintenance medium for stock cultures.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Beef heart, solids | 98.000 |
| Proteose peptone | 20.000 |
| Dextrose | 2.000 |
| Sodium chloride | 5.000 |
| Final pH ( at 25°C) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 12.5 grams in 100 ml distilled water (or suspend 1.25 grams in 10 ml distilled water in test tubes). Mix thoroughly and allow to stand for 15 minutes until all the particles are thoroughly wetted. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

## Principle And Interpretation

*Clostridium* is a large genus of gram-positive spore-bearing anaerobes. They are normally present in soil, some are responsible for human and animal diseases and others are associated with food spoilage. They may be saccharolytic, decomposing sugars to form butyric and acetic acids and alcohols. The meat in Robertsons Medium is reddened and gas is produced. Other proteolytic species attack the amino acids. Meat in Robertsons medium is blackened and decomposed by *Clostridium* species, giving the culture a foul odour. The mesophilic spore-forming anaerobes are of primary importance in the spoilage of low acid foods packed in sealed containers, because of their high heat resistance, their ability to grow in the absence of oxygen and a growth range which covers the temperature of normal storage of canned and other processed foods including the refrigerated storage of cured meats.

Cooked Meat Medium was originally developed by Robertson (3) for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped Meat Medium (2), which supports the growth of many spore forming and non-spore forming strict anaerobes. It has the ability to initiate growth of bacteria from very small inocula and to maintain the viability of cultures over long period. Mixed cultures of bacteria survive in Cooked Meat Medium without displacing the slower-growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic organisms. It is used for cultivation and maintenance of Clostridia and for determining proteolytic activity of anaerobes (1, 2). FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods (4).

Cooked Meat Medium contains beef heart, the muscle protein, which provide amino acids and other nutrients. Beef heart also contains glutathione, a reducing substance that permits the growth of obligate anaerobes. The sulphydryl groups, which impart reducing effect, are more available in denatured protein and hence cooked meat is added in the medium. The addition of dextrose allows rapid and heavy growth of anaerobic bacteria in a short time and leads to more rapid identification of important anaerobes. Growth in this medium is indicated by turbidity or bubble formation by some organisms. Blackening and disintegration of the meat particles indicate proteolysis. For best results, medium should be used on the day it is prepared, otherwise it should be boiled or steamed for a few minutes and allowed to cool without agitation and then inoculated.

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. For the isolation of *Clostridium* from food, use a stomacher to prepare 10% suspension of the food in Peptone Water (M028) diluent. Make dilutions and plate, both suspensions and dilutions on Willis and Hobbs Medium Base (M1375), Tryptose Sulphite Cycloserine (T.C.S.) Agar Base (M837). Place a

metronidazole disc on the inoculum. Incubate anaerobically at 37°C overnight. To count the clostridia, pour the plates with the dilutions on Perfringens Agar Base (O.P.S.P.) (M579). Incubate duplicate plates aerobically and anaerobically to distinguish between clostridia and other organisms. Add some of the suspension to two tubes of Cooked Medium. Heat one tube for 10 min at 80°C and incubate as above. Growth of clostridia is visualized as turbidity or gas bubbles. This medium can be further tested for presence of *Clostridium* (5).

## Quality Control

**Appearance**
Brown coloured granules

**Colour and Clarity of prepared medium**
Medium amber coloured, clear to slightly opalescent supernatant over insoluble granules.

**Reaction**
Reaction of 12.5% w/v aqueous suspension at 25°C. pH : 7.2±0.2

**pH**
7.00-7.40

**Cultural Response**
M149: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

| Organism | Inoculum (CFU) | Growth |
|---|---|---|
| *Clostridium botulinum ATCC 25763* | 50-100 | luxuriant |
| *Clostridium perfringens ATCC 12924* | 50-100 | luxuriant |
| *Clostridium sporogenes ATCC 11437* | 50-100 | luxuriant |
| *Enterococcus faecalis ATCC 29212* | 50-100 | luxuriant |
| *Streptococcus pneumoniae ATCC 6303* | 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. MacFaddin J. F., 1985, Media for Isolation - Cultivation - Identification - Maintenance of Medical bacteria, Vol. I, Williams & Wilkins, Baltimore.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Robertson, 1916, J. Pathol. Bacteriol., 20:327.
4. U. S. Food and Drug Administration, 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va.
5. Collins C. H., Lyne P. M., Grange J. M., 1985, 7th Ed., Microbiological Methods.

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