

Cooked M-Medium

LQ080

Intended Use:

Recommended for cultivation of aerobes and anaerobes, especially pathogenic *Clostridia* and also for the maintenance of stock cultures.

Composition**

Ingredients	g/ L
HMH Peptone B #	98.000
Proteose peptone	20.000
Dextrose (Glucose)	2.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

#- Equivalent to Beef heart, solids

Directions

Label the ready to use LQ080 bottle. Unscrew the cap and inoculate the sample. Incubate at specified temperature and time.

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. 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 M- Medium was originally developed by Robertson (1) for the cultivation of certain anaerobes isolated from wounds. The present formulation is a modification, also called as Chopped M 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 M-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 (2,3).

FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods (4). Cooked M-Medium contains HMH peptone B, which provides nitrogenous and carbonaceous compounds, long chain amino acids and other nutrients. The sulphhydryl groups, which impart reducing effect, are more available in denatured protein and hence this medium is used. 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. 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 (LQ089) 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 Plate (MP579). Incubate duplicate plates aerobically and anaerobically to distinguish between clostridia and other organisms. Add some of the suspension to two tubes of Cooked M-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).

Type of specimen

Clinical samples - faeces, wound exudate/discharge, skin and tissue swab around wound, Food and dairy samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). .

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further isolation and biochemical tests must be carried out for confirmation.

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

Sterile Clear Cooked M-Medium in glass bottles.

Colour

Medium amber coloured supernatant over insoluble granules.

Quantity of medium

10 ml of medium in bottles.

pH

7.00- 7.40

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed after incubation at 35-37°C for 40-48 hours.

Organism	Growth (under anaerobic conditions)	Growth (under aerobic conditions)
<i>Clostridium botulinum</i> ATCC 25763	Luxuriant	-
<i>Clostridium perfringens</i> ATCC 12924	Luxuriant	-
<i>Clostridium sporogenes</i> ATCC 11437	Luxuriant	-
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	-	Luxuriant
<i>Streptococcus pneumoniae</i> ATCC 6303	-	Luxuriant

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label. 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 (8,9).

