PRODUCT SPECIFICATION SHEET

Cooked Meat Medium / R.C. Medium (DM248)

Intended Use
Cooked Meat Medium/R.C. Medium (DM248) is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation
In 1890, Smith used fresh unheated animal tissue for cultivating anaerobic organisms.[1] Tarozzi confirmed Smith's findings and discovered meat broth could be heated to 104 – 105°C for 15 minutes without destroying nutrients.[2] A steam sterilized emulsion of brain tissue in water was employed by von Hibler.[3,4] Von Hibler found organisms in cooked brain broth were less susceptible to harmful effects of toxic metabolic products than in carbohydrate serum media.[3,4] Robertson substituted beef heart for brain tissue and Cooked Meat Medium is prepared according to this formula.[5] Cooked Meat Medium initiates growth from a small inoculum, important for clinical specimens. Cooked Meat Medium is recommended in standard methods for food testing.[6,7] Cooked Meat Medium provides an effective maintenance medium. This medium can be used to differentiate saccharolytic from proteolytic *Clostridium* spp.[8] Saccharolytic species decompose sugars to form butyric and acetic acids and alcohols. The meat in Robertsons Medium is reddened and gas is produced without digesting meat. Proteolytic species break down meat to amino acids. Meat in Robertsons medium is blackened and decomposed to form sulfur compounds leading to blackening and a putrid smell.
The mesophilic spore-forming anaerobes are of primary importance in the spoilage of low acid foods packed in sealed containers, due to 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. FDA has recommended this medium for enumeration and identification of *Clostridium perfringens* from foods.[6,10]

Principles of the Procedure
The nitrogen, vitamin, and carbon sources are provided by Enzymatic digest of animal tissue and Beef Heart. The low concentration of Dextrose is sufficient as the energy source, but not high enough to accumulate toxic metabolites. Sodium Chloride maintains the osmotic balance. Solid meat particles provide favorable growth conditions for anaerobes due to reducing action of -SH (sulfhydryl) groups of muscle protein.[2-4] Sulfhydryl groups are more accessible in denatured proteins, therefore use of cooked meat particles is preferred.[8] 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.

Formula / Liter

Ingredients	Gms / Litre
Beef Heart	454.00
Enzymatic Digest of Animal Tissue	20.00
Dextrose	2.00
Sodium Chloride	5.00
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Place 1.25 g of meat granules into a test tube and add 10 mL of purified/distilled water. (Or add 12.5g in 100mlD/W)
2. Mix thoroughly and allow to stand for 15 minutes until all the particles are thoroughly wetted.

3. Autoclave at 121°C for 15 minutes.

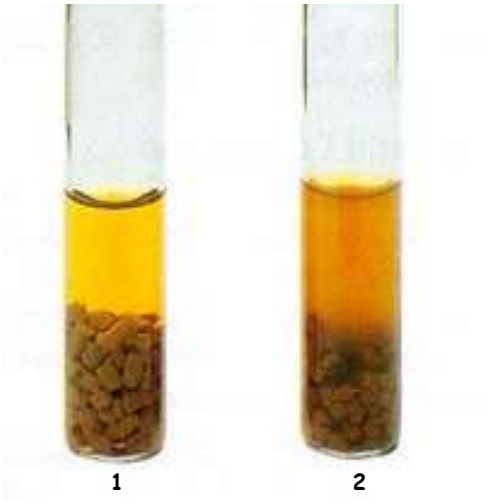
Quality Control Specifications

Dehydrated Appearance	Brown granules or particles
Solution	12.5% Solution in Distilled or deionized water is medium amber colored, and clear with insoluble granules.
Prepared Medium	Clear medium amber supernatant over insoluble granules at the base of the test tube
Reaction of 12.5% Solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural response in Cooked Meat Medium at 35-37°C after 48 - 72 hours incubation.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Clostridium botulium* ATCC 25763	50-100	luxuriant
2.	*Clostridium perfringens* ATCC 12924	50-100	luxuriant
3.	*Clostridium sporogenes* ATCC 11437	50-100	luxuriant
4.	*Streptococcus pneumonia* ATCC 6303	50-100	luxuriant
5.	*Entercoccus faecalis* ATCC 29212	50-100	luxuriant

The organisms listed are the minimum that should be used for quality control testing.



Cooked Meat Medium (DM248)
1. Control, uninoculated tube
2. *Clostridium sporogenes* ATCC 11437

Test Procedure

1. For anaerobic cultures, inoculate specimen deep into meat particles (bottom of the tube). Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.
2. For the isolation of *Clostridium* from food, tissue specimens should be ground prior to inoculation or use a stomacher to prepare 10% suspension of the food in Peptone Water (DM192) diluent.
3. Make dilutions and plate, both suspensions and dilutions on Willis and Hobbs Medium Base (DM1455), Tryptose Sulphite Cycloserine (T.C.S.) Agar Base (DM566). Place a metronidazole disc on the inoculum.
4. Incubate anaerobically at 37°C overnight. To count the clostridia, pour the plates with the dilutions on Perfringens Agar Base (O.P.S.P.) (DM623).
5. Incubate duplicate plates aerobically and anaerobically to distinguish between clostridia and other organisms.

6. 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).
7. For a complete discussion on the isolation and identification of aerobic and anaerobic bacteria, refer to appropriate procedures.

Results
In the cultivation of clostridia, saccharolytic organisms usually produce acid and gas. Growth of proteolytic organisms is generally characterized by blackening and dissolution of the meat particles.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging
Product Name : Cooked Meat Medium
Product Code : DM248
Available Pack sizes : 100gm / 500gm

References

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11. Collins C. H., Lyne P. M., Grange J. M., 1985, 7th Ed., Microbiological Methods.

Further Information

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