Cooked Meat Medium • Cooked Meat Medium with Glucose, Hemin and Vitamin K_1

Intended Use

Cooked Meat Medium and the enriched medium are used for the cultivation of anaerobes, especially pathogenic clostridia.

Summary and Explanation

In 1916, Robertson developed a cooked meat medium for use in the cultivation of certain anaerobes isolated from wounds.[1] The present formulation for Cooked Meat Medium is a modification of Robertson's original formula.

Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes. For example, the medium is recommended for use in the enumeration and identification of *Clostridium perfringens* from food.[2] It supports the growth of most sporeforming and nonsporeforming obligate anaerobes and may be used for a variety of purposes including the maintenance of stock cultures. The medium is also useful as an enrichment broth for cultivating anaerobes that may be present in small numbers in a population and as a subculture medium for determination of proteolysis (meat digestion) and spore formation by *Clostridium* species.

Cooked Meat Medium with Glucose, Hemin and Vitamin K_1, is also recommended as a subculture medium for anaerobic isolates to be examined by gas liquid chromatography.[3]

Principles of the Procedure

Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue granules is a source of amino acids and other nutrients. The muscle tissue also provides reducing substances, particularly glutathione, which permits the growth of strict anaerobes.[4] The sulfhydryl groups, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium.

Cooked Meat Medium with Glucose, Hemin and Vitamin K_1 is supplemented with added glucose, yeast extract, hemin and vitamin K_1 to enhance the growth of anaerobic microorganisms.

Growth is indicated by turbidity and, with some organisms, by the presence of gas bubbles in the medium. Disintegration and blackening of the meat particles indicates proteolysis. Gram stains or spore stains should be made to determine the shape and location of spores.

User Quality Control

Identity Specifications

Difco™ Cooked Meat Medium

Dehydrated Appearance:	Brown, homogeneous pellets.
Solution:	12.5% solution, partially insoluble in purified water. Solution is medium amber, clear to very slightly opalescent supernatant fluid over insoluble pellets.
Prepared Appearance:	Medium amber, clear to very slightly opalescent supernatant fluid over insoluble pellets.
Reaction of 12.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Cooked Meat Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides vulgatus	8482	10^2-10^3	Good
Clostridium novyi	7659	10^2-10^3	Good
Clostridium perfringens	12924	10^2-10^3	Good
Clostridium sporogenes	11437	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Uninoculated Tube

Clostridium sporogenes ATCC™11437

Formula

Difco™ Cooked Meat Medium

Approximate Formula* Per Liter

Beef Heart (from 454 g)	98.0	g
Proteose Peptone	20.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 12.5 g of the particles in 100 mL purified water (1.25 g/10 mL).
2. Let stand until all particles are thoroughly wetted and form an even suspension.
3. Autoclave at 121°C for 15 minutes. Reduce pressure slowly and cool without agitation.
4. If not used within 24 hours, reheat (100°C) prior to use to drive off absorbed oxygen.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **GasPak**™ EZ anaerobic system or an alternative anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation. Organisms to be cultivated must first be isolated in pure culture in an appropriate medium.

Using a sterile inoculating loop or needle, transfer growth from a fresh subculture medium, inoculating heavily in the area of meat particles. Incubate the tubes at 35 ± 2°C under anaerobic conditions for up to 7 days. It is recommended that an indicator of anaerobiosis be used.

Expected 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.

References

1. Robertson. 1916. J. Pathol. Bacteriol. *20*:327.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC, International, Gaithersburg, Md.
3. Holdeman, Cato and Moore. 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnical Institute and State University, Blacksburg, Va.
4. Willis. 1977. Anaerobic bacteriology: clinical and laboratory practice, 3rd ed. Butterworths, London, England.

Availability

Difco™ Cooked Meat Medium

AOAC BAM CCAM COMPF

Cat. No. 226730 Dehydrated – 500 g

BBL™ Cooked Meat Medium

AOAC BAM CCAM COMPF

Cat. No. 221507 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10
221508 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100

BBL™ Cooked Meat Medium with Glucose, Hemin and Vitamin K_1

BS12 CMPH2 MCM9

Cat. No. 297809 Prepared Tubes, 10 mL (C Tubes) – Ctn. of 100
295982 Prepared Tubes, 9 mL (K Tubes) – Pkg. of 10
299455 Prepared Tubes, 9 mL (K Tubes) – Ctn. of 100