IFU

Instructions for Use

COOKED MEAT MEDIUM

Cat. no. K19	Cooked Meat Medium, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K219	Cooked Meat Medium, 20x125mm Tube, 15ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Cooked Meat Medium is recommended for the cultivation of aerobic, microaerophilic, and anaerobic microorganisms, especially *Clostridium* species.

SUMMARY

The use of animal tissue for culturing anaerobic organisms was first employed by Theobald Smith in 1890.[4] Von Hibler later used brain tissue for cultivating and classifying anaerobic bacilli.[5] Robertson replaced brain tissue with beef heart and used this medium to differentiate putrefactive and saccharolytic species.[8]

The formulation presently used is a modified version of Robertson's formulation. This medium is also referred to as Chopped Meat Medium.[2]

Nutritional requirements needed by most bacteria are provided by beef heart, peptone and dextrose. Dextrose, yeast extract, hemin and vitamin K are added to enhance the growth of anaerobic microorganisms. Amino acids and other nutrients are supplied by the muscle protein in the heart tissue granules. Reducing substances, which permit the growth of strict anaerobes, are supplied by the muscle tissue and the iron filings.[9] It is thought that the meat particles act as a reducing and detoxifying substance, thereby disabling harmful by products that may be produced by the replicating organism.[11] Because reducing substances are more available in denatured protein, the meat particles are cooked before use in the medium.

Growth of spore-forming and non-spore-forming obligate anaerobes is supported by this medium. Cooked Meat Medium is also useful as an enrichment broth for cultivating organisms from a very small inoculum.[2,3,7,9,10] Additionally, researchers have found that Cooked Meat Medium preserves viability of organisms over a long period of time and is useful in maintaining anaerobic stock organisms.[13] The Food and Drug Administration recommends its use in the enumeration and identification of *Clostridium perfringens* from food.[14]

FORMULA

Ingredients per liter of deionized water:*

Peptic Digest of Animal Tissue	17.5gm
Dextrose	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm

Cooked Meat Medium	250.0gm
Iron Filings	10.0gm
Hemin	10.0ml
Vitamin K	10.0ml

Final pH 6.8 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-3,6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of Use: Consult the listed references for the appropriate cultivation techniques using this medium.[1-3,6] It is recommended that liquid media for anaerobic incubation should be reduced prior to inoculation by placing tubes (with loosened caps) under anaerobic conditions for 18-24 hours. Alternatively, the media may be reduced by bringing the media up to 100ºC. in a boiling waterbath. Loosen screw caps slightly before heating, and tighten during cooling to room temperature. The boiling serves to reduce media intended for the culture of anaerobic organisms.

1. The medium can be inoculated with a pure culture of an isolated colony, macerated tissue or liquid from a clinical specimen.

2. Heavily inoculate in the area of meat particles.

3. Incubate the tubes with caps tightened at 35 +/- 2.0ºC. for up to 7 days.

4. Growth or turbidity should be confirmed by gram stain and subcultured onto an appropriate plated growth medium.

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth and other identification tests to identify growth of organism in this medium.[1-3,6]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Meat particles in the medium may cause turbidity, which could be misinterpreted as positive growth.

Meat particles blacken only in the presence of alkali, which is a result of ammonia production by proteolytic enzymes.

The reactions observed in the medium are useful for characterization, not speciation, of the organism.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacteroides fragilis ATCC® 25285	A	24-48hr	35°C	Aerobic	Growth
Streptococcus pyogenes ATCC® 19615	A	24-48hr	35°C	Aerobic	Growth
Clostridium perfringens ATCC® 13124	A	24-48hr	35°C	Aerobic	Growth

* Refer to the document "Inoculation Procedures for Media QC" for more information.

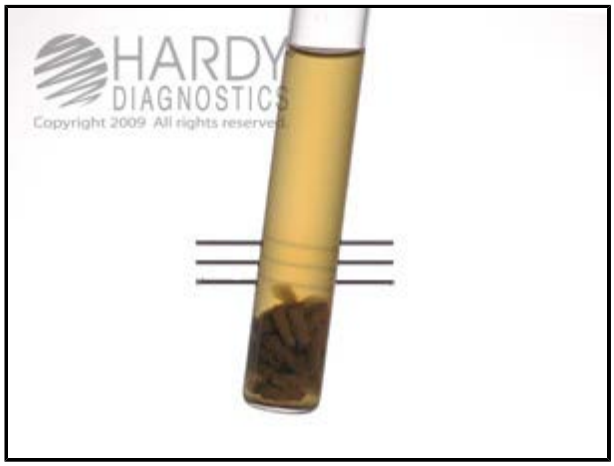
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends

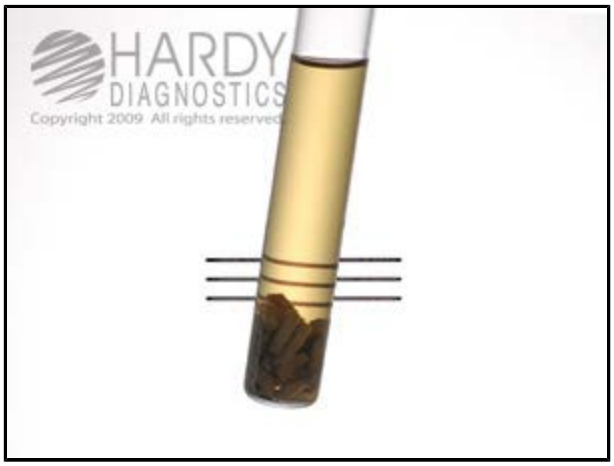
end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Cooked Meat Medium should appear amber in color, with approximately one inch of chopped meat on the bottom. Black iron filings should also be present on the bottom of the medium.



Bacteroides fragilis (ATCC® 25285) growing in Cooked Meat Medium (Cat. no. K19). Incubated aerobically (with cap screwed down tightly) for 24 hours at 35ºC.



Uninoculated tube of Cooked Meat Medium (Cat. no. K19).

REFERENCES

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]