



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Cooked Meat Medium is a medium for the cultivation of anaerobes.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Using a 0.01 mL calibrated loop, inoculate the *Clostridium* species using 18- to 72-h Cooked Meat Medium cultures (or colonies from 48-h CDC Anaerobe 5% Sheep Blood Agar plate cultures that have been transferred to pre-reduced tubes of Fluid Thioglycollate Medium and adjusted to a 1.0 McFarland standard) and the *Bacteroides* species using 18- to 48-h Thioglycollate Medium, Enriched with Vitamin K₁ and Hemin.
 - b. Incubate tubes at 35 ± 2 °C in an anaerobic atmosphere (**GasPak™** EZ Anaerobic System or equivalent).
2. Examine tubes for up to 7 days for growth, digestion of meat, blackening and gas production. Blackening may be delayed or absent if the medium remains acid. Blackening of meat particles occurs only in the presence of alkali; the reaction is a combination of the action on iron and an alkaline environment.¹
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Clostridium perfringens</i>	13124	Growth with heavy gas production
* <i>Clostridium sporogenes</i>	11437	Growth with heavy gas production and digestion, may show blackening
<i>Clostridium septicum</i>	6008	Growth with gas production, may show blackening
<i>Bacteroides fragilis</i>	25285	Growth with or without gas production, may show blackening
<i>Bacteroides vulgatus</i>	8482	Growth with or without gas production, may show blackening

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Cooked Meat Medium is used in the cultivation of anaerobes, especially pathogenic clostridia.

V 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 used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes. 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. This medium is also useful as an enrichment broth as backup to plated media² or 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.

VI 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. 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.

In the **BBL** formula, the content of beef heart is expressed as weight of dry heart tissue granules.

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.

VII REAGENTS

Cooked Meat Medium

Approximate Formula* Per Liter Purified Water

Heart Tissue Granules	98.0 g
Peptic Digest of Animal Tissue	20.0 g
Dextrose	2.0 g
Sodium Chloride	5.0 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 – 25 °C. Avoid freezing and overheating. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Minimize exposure to light.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{2,3} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Cooked Meat Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18 – 24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **GasPak EZ** anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling for 10 min with caps loosened and cooling 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.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X 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.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.²⁻⁴

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Cooked Meat Medium are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are inoculated with cultures of *Clostridium perfringens* (ATCC 13124), *C. septicum* (ATCC 6008), *C. sporogenes* (ATCC 11437), *Bacteroides fragilis* (ATCC 25285) and *B. vulgatus* (ATCC 8482). The inocula for the clostridia are taken from either Cooked Meat Medium or from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and subsequently diluted to a 1.0 McFarland Standard in Fluid Thioglycollate Medium. The inocula for *Bacteroides* are taken from Thioglycollate Medium Enriched with Vitamin K₁ and Hemin. The inoculated tubes are incubated in a **GasPak Plus** or **GasPak EZ** anaerobic system at 35 ± 2 °C and read after 1, 3 and 7 days incubation. Growth is moderate to heavy for all organisms within 7 days. Gas is produced with *C. perfringens*, *C. septicum* and *C. sporogenes*. Digestion is evident with *C. sporogenes* and may or may not be evident with *C. septicum*. Blackening may or may not be observed with the clostridia. *B. fragilis* and *B. vulgatus* may or may not produce gas and may show a slight amount of blackening.


XIII AVAILABILITY

Cat. No.	Description
221507	BD BBL™ Cooked Meat Medium, 8 mL, Pkg. of 10 size K tubes
221508	BD BBL™ Cooked Meat Medium, 8 mL, Ctn. of 100 size K tubes

XIV REFERENCES

- Robertson, M. 1916. Notes upon certain anaerobes isolated from wounds. J. Pathol. Bacteriol. 20:327-349
- Forbes, B.A., and P.A. Granato. 1995. Processing specimens for bacteria, p. 265-281. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
- Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
- Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual. of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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