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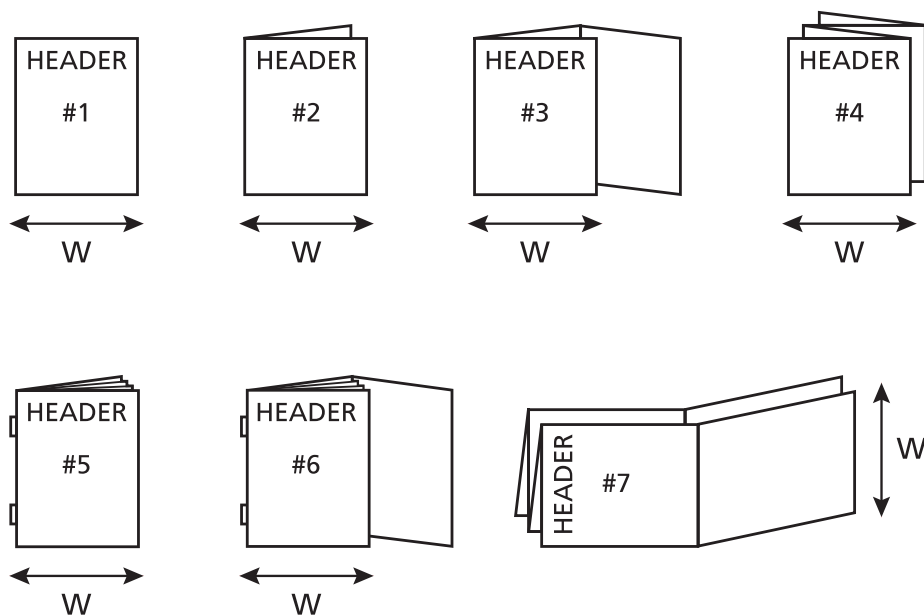
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# BD BBL™ Prepared Tubed Media for Cultivation of Anaerobic Microorganisms

Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub>



8806291JAA(03)  
2018-12

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## INTENDED USE

Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub> is a general-purpose medium used for the cultivation of obligate anaerobes, especially *Clostridium* spp.

## SUMMARY AND EXPLANATION

Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub> is based on the formulation of Robertson.<sup>1</sup> It supports the growth of most sporeforming and nonsporeforming obligate anaerobes and may be used for a variety of purposes.<sup>2</sup> This medium is also useful as an enrichment broth for cultivating anaerobes that may be present in small numbers and as a subculture medium for determination of proteolysis (meat digestion) and spore formation by clostridia. Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub> is also recommended as a subculture medium for anaerobic isolates to be examined by gas-liquid chromatography.<sup>3</sup>

## PRINCIPLES OF THE PROCEDURE

Muscle tissue and animal tissue peptone provide organic nitrogen and other nutrients to support the growth of microorganisms. The muscle tissue also provides reducing substances, particularly glutathione, which permits the growth of strict anaerobes.<sup>4</sup>

Cooked Meat Medium, enriched, is supplemented with added glucose, yeast extract, hemin and vitamin K<sub>1</sub> 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.

## REAGENTS

### Cooked Meat Medium Base

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.

**Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub>** consists of the above ingredients with an additional 3.0 g dextrose, 5.0 g yeast extract, 5.0 mg hemin and 1.0 mg vitamin K<sub>1</sub> added per liter.

### 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 to 25 °C. Avoid freezing and overheating. Tubed media stored as labelled until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation. Do not open until ready to use.

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

## SPECIMEN COLLECTION AND HANDLING

This medium is not suitable for use directly with clinical specimens or other sources containing mixed microbial flora, unless used as an enrichment broth in addition to the primary plating media. Consult appropriate texts for more information.<sup>3,5-9</sup>

## PROCEDURE

**Material Provided:** Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub>

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

**Test Procedure:** Observe aseptic techniques. Liquid media for anaerobic incubation should be reduced by placing the tubes, with caps loosened, under anaerobic conditions for 18 to 24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of a **GasPak™** EZ 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.

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

If the medium is being used as a back-up enrichment medium, in addition to the primary plating medium, tubes should be held at least 1 week before discarding as negative.

### User Quality Control:

1. Examine the tubes for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of tubes with pure cultures of stable control organisms that produce known, desired reactions.

The following cultures are recommended:

| TEST STRAIN                                  | EXPECTED RESULT          |
|----------------------------------------------|--------------------------|
| <i>Clostridium sporogenes</i><br>ATCC® 11437 | Growth.<br>Gas produced. |
| <i>Clostridium perfringens</i><br>ATCC 13124 | Growth.<br>Gas produced. |

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 NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

## RESULTS

Following incubation, growth is indicated by turbidity and, in some cases, by the production of gas. Proteolysis is characteristic of some species and is evidenced by blackening of the meat particles with eventual digestion or dissolution of the meat. A Gram or spore stain should be performed as the location and size of spores is characteristic of *Clostridium* spp.<sup>7</sup>

## LIMITATIONS OF THE PROCEDURE

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers in a specimen. For identification, the organism must be in a pure culture. Biochemical and other identification tests may be performed for complete identification. Appropriate texts should be consulted for further information.<sup>3,5-11</sup>

## PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Cooked Meat Medium with Glucose, Hemin and Vitamin K<sub>1</sub> 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) and *C. sporogenes* (ATCC 11437). The inocula 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 inoculated tubes are incubated in a **GasPak™**, **GasPak™** Plus or **GasPak™** EZ anaerobic system at 35 ± 2 °C and read after 1, 3 and 7 days incubation. Growth and gas production are evident with both *C. perfringens* and *C. sporogenes*.

## AVAILABILITY

| Cat. No. | Description                                                                                                        |
|----------|--------------------------------------------------------------------------------------------------------------------|
| 295982   | <b>BD BBL™</b> Cooked Meat Medium with Glucose, Hemin and Vitamin K <sub>1</sub> ,<br>Pkg of 10 size K tubes, 9 mL |

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JJJJ-MM-TT / JJJJ-MM (MM = Monatsende)  
EEEE-MM-HH / EEEE-MM (MM = τέλος του μήνα)  
AAAA-MM-DD / AAAA-MM (MM = fin del mes)  
AAAA-KK-PP / AAAA-KK (KK = kuu lõpp)  
AAAA-MM-JJ / AAAA-MM (MM = fin du mois)  
GGGG-MM-DD / GGGG-MM (MM = kraj mjeseca)  
EEEE-HH-NN / EEEE-HH (HH = hónap utolsó napja)  
AAAA-MM-GG / AAAA-MM (MM = fine mese)  
ЖЖЖЖ-АА-КК / ЖЖЖЖ-АА / (АА = айдың соңы)  
YYYY-MM-DD/YYYY-MM (MM = 월말)  
MMMM-MM-DD / MMMM-MM (MM = mėnesio pabaiga)  
GGGG-MM-DD/GGGG-MM (MM = mėnesia beigas)  
JJJJ-MM-DD / JJJJ-MM (MM = einde maand)  
AAAA-MM-DD / AAAA-MM (MM = slutten av måneden)  
RRRR-MM-DD / RRRR-MM (MM = koniec miesiąca)  
AAAA-MM-DD / AAAA-MM (MM = fim do mês)  
AAAA-LL-ZZ / AAAA-LL (LL = sfârșitul lunii)  
ГГГГ-ММ-ДД / ГГГГ-ММ (ММ = конец месяца)  
RRRR-MM-DD / RRRR-MM (MM = koniec miesiąca)  
GGGG-MM-DD / GGGG-MM (MM = kraj meseca)  
AAAA-MM-DD / AAAA-MM (MM = slutet av månaden)  
YYYY-AA-GG / YYYY-AA (AA = ayın sonu)  
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