anaeroGRO™
Pre-Reduced Anaerobic Culture Media

CHOPPED MEAT MEDIA

Cat. no. AG19H	Chopped Meat Glucose Broth, 16x125mm Tube with Hungate Septum Cap, 9ml	20 tubes/box
Cat. no. AG20H	Chopped Meat Carbohydrate Broth, 16x125mm Tube with Hungate Septum Cap, 9ml	20 tubes/box
Cat. no. AG21H	Chopped Meat Broth, 16x125mm Tube with Hungate Septum Cap, 9ml	20 tubes/box

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Chopped Meat Media is recommended for the cultivation of microaerophilic, facultative and obligate 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) Growth of spore-forming and non-spore-forming obligate anaerobes is supported by this medium. Chopped 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 Chopped Meat Medium preserves viability of organisms over a long period of time and is useful in maintaining anaerobic stock organisms.(16) The Food and Drug Administration recommends its use in the enumeration and identification of *Clostridium perfringens* from food.(14)

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 obligate 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. Various formulations are available containing different carbohydrates, Cat. no. AG19H contains glucose while Cat. no. AG20H contains glucose, cellobiose, maltose, and

starch. These two media with additional components are better suited to enhance the productions of toxin by anaerobes such as: *Clostridium* spp. compared to the unsupplemented medium (Cat. no. AG21H). Chopped meat carbohydrate medium is recommended for the use with gas-liquid chromatography for analysis of anaerobic metabolic products.[16]

FORMULA

Ingredients per liter of deionized water:*

Chopped Meat Broth (Cat. no. AG21H):	
Peptic Digest of Animal Tissue	17.5gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm
Cooked Meat	250.0gm
Iron Filings	10.0gm
Hemin	10.0ml
Vitamin K	10.0ml

In addition, AnaeroGRO™ Chopped Meat Glucose Broth (Cat. no. AG19H) contains:

Glucose	3.0gm

In addition, AnaeroGRO™ Chopped Meat Carbohydrate Broth (Cat. no. AG20H) contains:

Glucose	4.0gm
Maltose	1.0gm
Cellobiose	1.0gm
Starch	1.0gm

Final pH 7.1 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

The expiration dating 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 quality control incubation times.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

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

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-3,6,11,15,16] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat, cold, and oxygen exposure. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium (Cat. no. S120D) and refrigerated until inoculation.

Method of Use: Consult the listed references for the appropriate cultivation techniques using this medium.[1-3,6,11,15,16]

1. The medium can be inoculated with a pure culture of an isolated colony, macerated tissue or liquid from a clinical specimen.To avoid oxygen exposure liquid specimens may be injected directly through the rubber septum of the hungate screw cap with a needle and syringe.

2. Heavily inoculate in the area of meat particles.

3. Incubate the tubes with caps tightened at 35ºC. for up to seven days in an ambient air incubator.

4. Growth or turbidity should be confirmed by Gram stain and subcultured onto an appropriate plated growth medium, such as Brucella Agar with H and K (Cat. no. AG301).

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth and other identification tests to identify growth of organism in this medium.[1,2,3,6,11,15,16]

LIMITATIONS

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

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, swabs, applicator sticks, other culture media,

transports (Cat. no. S120D) 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 Certificates of Analysis (CofA). The following organisms 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.

** Tubes are incubated in an aerobic incubator with the caps screwed down tightly to create an atmosphere of low oxygen tension within the tube.

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

AnaeroGRO™ Chopped Meat Media 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 Chopped Meat Glucose Broth (Cat. no. AG19H). Incubated aerobically (with cap screwed down tightly) for 24 hours at 35ºC.

Uninoculated tube of Chopped Meat Glucose Broth (Cat. no. AG19H).

REFERENCES

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15. Summanen, P., and E.J. Baron. 2002. *Wadsworth Anaerobic Bacteriology Manual,* 6th ed. Star Publishing Company, Belmont, CA.

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

HDQA 2207A [C]