PRAS COOKED MEAT BROTH w/ and w/o ADDITIVES

INTENDED USE
Remel PRAS Cooked Meat Broth w/ and w/o Additives is a liquid medium recommended for use in qualitative procedures for the cultivation of anaerobic microorganisms.

SUMMARY AND EXPLANATION
Pre-Reduced Anaerobically Sterilized (PRAS) media are processed in a reduced condition and remain reduced up to and after inoculation.[1] R.E. Hungate developed the basis for these media, referred to as the "roll tube" technique. The use of a steam sterilized emulsion of brain tissue in water to support the growth of anaerobic bacilli was first described by von Hibler.[2] Robertson observed comparable results in cooked meat medium, in which she substituted beef heart for the brain tissue.[3] Henry used the cooked meat medium for cultivating anaerobes and to study their saccharolytic and proteolytic properties.[4] Cooked Meat Medium is recommended by the Food and Drug Administration (FDA) for use in the enumeration and identification of *Clostridium perfringens* from food and by the Association of Official Analytical Chemists (AOAC) for the detection of *Clostridium botulinum*.[5] This medium is also referred to as chopped meat medium.

PRINCIPLE
PRAS products are prepared and processed in a hydrogen and nitrogen atmosphere. Cooked meat medium contains minimal salts, beef heart, peptone, and dextrose which provide reducing substances, amino acids, and other nutrients. Solid meat particles provide favorable growth conditions for anaerobes due to the reducing action of sulfhydryl groups (-SH) from muscle protein. Cysteine also contains sulfhydryl groups which act as reducing agents to bind hydrogen ions and produce anaerobic conditions. Sulfhydryl groups are more accessible in denatured protein, therefore the meat is cooked. Vitamin K and hemin are added to stimulate the growth of certain anaerobes, specifically *Prevotella melaninogenica*. A mixed carbohydrate-starch source was developed to provide a carbon source for all anaerobes including those which do not use glucose. When glucose or other carbohydrates are incorporated, a growth medium results which is useful in obtaining rapid growth from single colonies for chromatographic analyses, susceptibility testing, or inoculation of different media. Cooked Meat Broth has the capacity to initiate growth of bacteria from minute inocula and maintain viability of cultures over extended periods of time.

REAGENTS (CLASSICAL FORMULAE)*

Beef Heart Infusion	454.0 g	Dextrose	2.0 g
Proteose Peptone	20.0 g	L-Cysteine Hydrochloride	0.5 g
Sodium Chloride	5.0 g	Hemin	5.0mg
Yeast Extract	5.0 g	Vitamin K	0.1mg
		Demineralized Water	1000.0 ml

pH 7.2 +/- 0.2 @ 25°C

The following optional ingredients are available per liter of medium:

Cooked Meat Glucose:

Glucose	3.0 g

Cooked Meat Carbohydrates:

Glucose	2.0 g	Maltose	1.0 g
Cellobiose	1.0 g	Starch	1.0 g

*Adjusted as required to meet performance standards.

PROCEDURE
Note: Inoculation of clinical material (specimen) or test isolate should be performed under anaerobic conditions using an anaerobic chamber, a specialized apparatus which supplies continuous gas flow into the tube during manipulation, or through a rubber stopper (Hungate cap) using a needle and syringe. Consult appropriate references for further guidelines.[1,6]

1. Inoculate specimens for anaerobic culture as soon as possible after receipt in the laboratory.
2. Test isolates for subculture should be removed from an 18-24 hour old pure culture.
3. If using the closed or Hungate method, use a syringe and needle to inoculate through the rubber stopper. The diaphragm of the Hungate cap should be decontaminated with alcohol and allowed to dry prior to injection.
4. If using the open method, remove the cap and insert a cannula that has oxygen-free gas flowing from the tip. While the tube is open, inoculate using a Pasteur pipette. If inoculation occurs in an anaerobic chamber, the use of a cannula and oxygen-free gas is not required.
5. Inoculate heavily in the area of meat particles.
6. Incubate the tubes aerobically at 35-37°C for up to 7 days.
7. Examine daily for growth.

NOTE: When cultivating clostridia, saccharolytic organisms produce acid and gas but no digestion of the meat. Proteolytic organisms usually produce blackening and decomposition of the meat particles. However, some saccharolytic strains also produce H_2S which causes blackening but to a lesser degree than with proteolytic organisms.

(Continued on back)

QUALITY CONTROL

All lot numbers of PRAS Cooked Meat Broth w/ and w/o Additives have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Bacteroides fragilis ATCC® 25285	Anaerobic, 48 h @ 35-37°C	Good growth
Clostridium perfringens ATCC® 13124	Anaerobic, 48 h @ 35-37°C	Good growth
Peptostreptococcus anaerobius ATCC® 27337	Anaerobic, 48 h @ 35-37°C	Good growth
Prevotella melaninogenica ATCC® 25845	Anaerobic, 48 h @ 35-37°C	Good growth

BIBLIOGRAPHY

1. Holdeman, L.V., E.P. Cato, and W.E.C. Moore. 1977. Virginia Polytechnic Institute Anaerobic Manual. 4th ed. VPI, Blacksburg, VA.
2. von Hibler, E. 1899. Centr Bakteriol. 25:513.
3. Robertson, M. 1916. J. Pathol. Bacteriol. 20:327-349.
4. Henry, H. 1917. J. Pathol. Bacteriol. 21:358-359, 380-382.
5. Food and Drug Administration. 1995. Bacteriological Analytical Manual. 8th ed. AOAC International, Gaithersburg, MD.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology. 11th ed. Mosby, Inc., St. Louis, MO.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

IFU 5030, Revised February 12, 2007 Printed in U.S.A.

remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Technical Service: (800) 447-3641 Order Entry: (800) 447-3635
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128
Website: www.remel.com Email: remel@remel.com