PRAS GELATIN MEDIUM

INTENDED USE

Remel PRAS Gelatin Medium is a liquid medium recommended for use in qualitative procedures for determination of gelatin liquefaction by anaerobic bacteria.

SUMMARY AND EXPLANATION

Dr. R.E. Hungate pioneered the development of Pre-Reduced Anaerobically Sterilized (PRAS) media, which are processed in a reduced condition and remain reduced up to and after inoculation.[1] The PRAS biochemical system is considered the standard method for Level III identification of anaerobic bacteria.[2] Definitive identification of anaerobic organisms should be obtained for all blood isolates and visceral-space isolates, when the patient is gravely ill and not responding to treatment, and when a prolonged and expensive treatment is indicated.[3,4] It may also be indicated in unusual case presentations, when a nosocomial infection is suspected, and in teaching-hospital settings.

PRINCIPLE

PRAS Gelatin Medium is prepared and processed in an atmosphere of hydrogen and nitrogen and maintained in an oxygen-free environment. Cysteine, a reducing agent, contains sulfhydryl groups which bind hydrogen ions and produce anaerobic conditions. Resazurin, an Eh indicator, turns from colorless to pink upon exposure to oxygen indicating loss of anaerobic conditions. Peptone, yeast extract, salt solutions, hemin, and vitamin K supply growth factors required by many anaerobic bacteria. Gelatin serves as the substrate to detect an organism's ability to produce gelatinase, a proteolytic enzyme which hydrolyzes gelatin and renders the medium liquid.

REAGENTS (CLASSICAL FORMULA)*

Gelatin	120.0	g	
Yeast Extract	10.0	g	
Peptone	5.0	g	
Dextrose	1.0	g	
L-Cysteine Hydrochloride	0.5	g	
Sodium Bicarbonate	0.4	g	
Sodium Chloride	80.0	mg	
Monopotassium Phosphate	40.0	mg	
Dipotassium Phosphate	40.0	mg	
Calcium Chloride	8.0	mg	
Magnesium Sulfate	8.0	mg	
Hemin	5.0	mg	
Resazurin	1.0	mg	
Vitamin K	1.0	mg	
Demineralized Water	1000.0	ml	

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

Inoculation 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 using a needle and syringe. Prior to inoculation, decontaminate the diaphragm of the rubber stopper with alcohol. Allow the alcohol to evaporate. **Note:** Do not use media that are pink, indicating contamination with oxygen (oxidized).

1. Implement appropriate procedures to verify that the test isolate is an anaerobe.
2. Inoculate PRAS Gelatin Medium with 4 drops from a young, actively growing broth culture. Prior to inoculation, mix the broth culture well by inverting the tube. (**Note**: Use of older cultures may yield unreliable results due to predominance of non-viable organisms.)
3. To verify purity and viability of the test isolate, inoculate one drop of the broth culture onto a nonselective anaerobic blood agar plate. Incubate the plate at 33-37°C under anaerobic conditions.
4. Incubate PRAS Gelatin Medium aerobically at 33-37°C for 48 hours or until 2+ turbidity is obtained.
5. Following incubation, refrigerate the tube along with an uninoculated PRAS Gelatin Medium tube (negative control) for at least one hour.
6. Remove tubes to room temperature and invert immediately. Observe the test and control tubes for liquefaction every 5 minutes.

INTERPRETATION OF THE TEST

Positive Test -	Gelatin fails to solidify at 2-8°C (i.e., drops to the top of the inverted tube immediately).
Weak Positive Test -	Gelatin solidifies at 2-8°C and becomes liquid when it reaches room temperature (<30 minutes).
Negative Test -	Gelatin fails to liquefy when it reaches room temperature (>30 minutes).

QUALITY CONTROL

All lot numbers of PRAS Gelatin Medium 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
Clostridium perfringens ATCC® 13124	Aerobic, 48 h @ 33-37°C	Gelatin liquid after refrigeration
Clostridium innocuum ATCC® 14501	Aerobic, 48 h @ 33-37°C	Gelatin solid after refrigeration

(Continued on back)

LIMITATIONS

1. This test is only part of the overall scheme for identification of anaerobic bacteria. Additional biochemical characterization may be required for definitive identification. Consult appropriate references for further instructions.[1-4]
2. The test isolate must be in pure culture before inoculation of PRAS Gelatin Medium. Do not read the reactions of mixed cultures.

BIBLIOGRAPHY

1. Holdeman, L.V., E.P. Cato, and W.E.C. Moore. 1977. Anaerobe Laboratory Manual. 4th ed. Virginia Polytechnic Institute, Blacksburg, VA.
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4. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Lippincott Williams and Wilkins, Philadelphia, PA.

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.

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