PRAS PY (PEPTONE YEAST EXTRACT) MEDIA w/ and w/o ADDITIVES

INTENDED USE

Remel PRAS PY Media w/ and w/o Additives are tubed media recommended for use in qualitative procedures for differentiation of anaerobic bacteria based on biochemical utilization.

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] Identification may also be indicated in unusual case presentations, when a nosocomial infection is suspected, and in teaching-hospital settings.

PRINCIPLE

This medium is autoclaved in a reduced state 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. Peptones and yeast extract supply basic nutrients for growth. The medium is only lightly buffered so that acid production from carbohydrate utilization may be detected by changes in the pH. Vitamin K and hemin are included to satisfy the growth requirements for various anaerobic organisms.

REAGENTS (CLASSICAL FORMULA)*

PY Basal Medium:

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

pH 7.1 ± 0.3 @ 25°C

*Adjusted as required to meet performance standards.

The following optional ingredients are available per liter of PY basal medium:

		Fructose	10.0 g	Lactate	9.0 ml	Melibiose	5.0 g	Starch	10.0 g
Arabinose	5.0 g	Galactose	10.0 g	Lactose	10.0 g	Raffinose	10.0 g	Sucrose	10.0 g
Arginine	3.0 g	Glucose	10.0 g	Maltose	10.0 g	Rhamnose	10.0 g	Threonine	3.0 g
Cellobiose	10.0 g	Glycerol	8.0 ml	Mannitol	10.0 g	Ribose	5.0 ml	Trehalose	5.0 g
Erythritol	5.0 g	Glycogen	5.0 g	Mannose	10.0 g	Salicin	10.0 g	Xylan	10.0 g
Esculin	5.0 g	Inositol	10.0 g	Melezitose	5.0 g	Sorbitol	10.0 g	Xylose	10.0 g

Glucose Agar Slant (PYG):		**PYG w/ Bile:**		**PYG w/ Formate, Fumarate:**		**PYG w/ Polysorbate 80:**	
Glucose	10.0 g	Oxgall	20.0 g	Formate	1.8 g	Polysorbate 80	1.0 g
Agar	20.0 g			Fumarate	1.8 g		

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 (Hungate cap) using a needle and syringe. The diaphragm of the Hungate cap should be decontaminated with alcohol and allowed to dry prior to injection. **Note:** Do not use media if they are pink (oxidized), indicating oxygen contamination.

1. The inoculum must be from a young, actively growing broth culture (without carbohydrate) of at least 2+ turbidity. Mix the broth culture well by inverting the tube and inoculate 5 drops into selected PRAS biochemical tubes.
2. Include a PRAS Base Control or a PYG tube, as appropriate, with each test isolate.
3. Inoculate one drop of the broth culture onto a nonselective anaerobic blood agar plate to verify purity and viability. Incubate the plate at 35-37°C under anaerobic conditions.
4. Inoculate one drop of the broth culture onto a blood agar or chocolate agar plate and incubate at 35-37°C in 5% CO_2.
5. Incubate the tubes at 35-37°C until good growth is achieved (greater than 2+). If good growth is not apparent after five days of incubation, supplement the PRAS tube(s) following established laboratory procedures and reincubate for 48-72 additional hours. Consult appropriate references as necessary.[1,2]
    a. Carbohydrate fermentation results are determined by measuring acid production using a pH meter. Invert each PRAS tube to resuspend settled organisms and record the turbidity as 1+ to 4+. At least 2+ growth should be observed for reliable interpretation.
    b. Catalase reaction is determined by adding 2-3 drops of 3% hydrogen peroxide to growth in a PYG tube after exposure to air.
    c. Esculin hydrolysis is determined by adding 2-3 drops of 1% ferric ammonium citrate solution (R21215) to PY Esculin tube.
    d. Lactate and threonine utilization are confirmed by detection of propionic acid using gas-liquid chromatography (GLC). Consult appropriate references as necessary.[1,2]
    e. Starch hydrolysis is determined by adding 2-3 drops of Gram iodine (R40056) to PY Starch tube.

(Continued on back)

INTERPRETATION

Carbohydrate Fermentation: Lower electrode into PRAS tube and gently swirl until reading is stabilized. Read pH to the nearest tenth.
Positive - pH $\leq$ 5.5 = acid
pH 5.5-6.0 = weak acid
Negative - pH > 6.0

Note: The pH of cultures in arabinose, ribose, or xylose should be below 5.7 to interpret results as positive (acid).[1]

Bile Tolerance:
Sensitive - Less growth in PYG w/ Bile tube than in control tube (PYG)
Resistant - Equal or greater growth in PYG w/ Bile tube when compared to PYG

Catalase: Expose PYG growth to air for at least 30 minutes. Add 3% hydrogen peroxide and observe for production of bubbles.[1]
Positive - Bubbles
Negative - No bubbles

Esculin Hydrolysis: Add 2-3 drops of 1% ferric ammonium citrate solution to PY Esculin tube and observe for color development.
Positive - Black or dark brown color development and no fluorescence under ultraviolet light
Negative - No color development or white-blue fluorescence under ultraviolet light

Polysorbate 80 Growth Enhancement Test:
Positive - Growth substantially better in PYG w/ Polysorbate 80 tube than in control tube (PYG)
Negative - Little or no increase in the magnitude of growth in PYG w/ Polysorbate 80 tube as compared to PYG

Note: Polysorbate 80 enhances the growth of gram-positive organisms.

Growth Stimulation Test: (Arginine; Formate, Fumarate)
Positive - Growth substantially better in the supplemented tube than in the PY Base Control
Negative - Little or no increase in the magnitude of growth in the supplemented tube as compared to the PY Base Control

Note: Arginine is required by *E. lentum*. Formate, Fumarate is required by the *B. ureolyticus* group.

Lactate & Threonine: Chromatograph the ether extracts of both the supplemented tube(s) and the control tube(s), each acidified to pH 2.
Positive - More propionate produced in the supplemented tube than in the PY Base Control.
Negative - Equal amount or less propionate produced in the supplemented tube as compared to the PY Base Control

Starch: Add 2-3 drops of Gram iodine to PY Starch tube and observe for clearing of the agar.
Positive - No color (hydrolysis of starch)
Negative - Blue-black color (no hydrolysis or incomplete hydrolysis of starch)

QUALITY CONTROL

All lot numbers of PRAS PY Media w/ and w/o Additives have been tested for performance and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Ideally, control organisms should be selected that demonstrate a positive and negative reaction for each biochemical tested. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. These tests are only part of the overall identification scheme Further biochemical testing may be necessary for definitive identification. Consult appropriate references as necessary.[1-4]
2. The anaerobe tested must be in pure culture and viable in PRAS tubes. Do not read reactions of mixed cultures.[2]
3. Use of older cultures for testing may yield unreliable results due to predominance of non-viable organisms.[2]
4. If the isolate fails to grow on the purity and viability plate, but appears turbid in the biochemical tubes, Gram stain media to verify turbidity is not merely inoculum or a contaminant.[2]
5. Do not incubate non-turbid tubes for more than 5 days, as growth rarely occurs beyond that time.[2]
6. Inconsistencies in the magnitude of growth within a series of tubes should be investigated by either a Gram stain or subculture of the tube(s) to rule out contamination.[2]
7. Measurement of pH cannot be used to determine lactate utilization.[1]
8. Do not use nichrome or plastic loops because they may oxidize the media.[1]
9. Serum (1%) can be used to stimulate the growth of gram-positive and gram-negative anaerobes.[2]

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. Summanen, P., E.J. Baron, D.M. Citron, C.A. Strong, H.M. Wexler, and S.M. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual. 5th ed. Star Publishing, Belmont, CA.
3. Dowell, B.R. and T.M. Hawkins. 1974. Laboratory Methods in Anaerobic Bacteriology CDC Laboratory Manual. U.S. of Dept. H.H.S., Atlanta, GA.
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.

IFU 5161, Revised June 13, 2012 Printed in U.S.A.

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