PYR BROTH

INTENDED USE
Remel PYR Broth is a liquid medium recommended for use in qualitative procedures for the rapid, presumptive identification of group A streptococci and enterococci.

SUMMARY AND EXPLANATION
The L-pyroglutamic and acid-β-naphthylamide (PYR) hydrolysis test was first described in 1970 by Mulczyk and Szewczuk.[1] It has proved to be useful in the identification of *Streptococcus pyogenes, Enterococcus* spp., and other gram-positive cocci. In 1981, Godsey et al. incorporated PYR substrate into a Todd Hewitt Broth and tested for enzyme activity of streptococci after overnight incubation.[2] Later, Bosley et al. incorporated PYR substrate into a broth and successfully tested for enzyme activity after 4 hours incubation.[3] The PYR test has been reported to be equally sensitive and more specific than the routine bacitracin test for identification of group A streptococci, while also comparable to the agar salt tolerance test in the differentiation of enterococci and group D streptococci.[4]

PRINCIPLE
Casein peptone supplies essential growth factors and trace elements. Sodium chloride maintains osmotic equilibrium. *S. pyogenes* and *Enterococcus* spp. possess the enzyme L-pyrrolidonyl aminopeptidase which hydrolyzes the substrate L-pyroglutamic acid-β-naphthylamide (PYR) with the formation of a free β-naphthylamine. This amine then combines with the cinnamaldehyde reagent that is added to form a bright red end product.[5]

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	20.0	g	Sodium Chloride	2.0	g
Beef Heart Infusion	3.1	g	Disodium Phosphate	0.4	g
Sodium Carbonate	2.5	g	L-pyroglutamic acid-β-naphthylamide	0.1	g
Dextrose	2.0	g	Demineralized Water	1000.0	ml

pH 7.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Implement appropriate procedures to verify that the test isolate is a streptococci or an enterococci.
2. Inoculate the broth using 3-5 colonies from a pure, 18-24 hour culture.
3. Incubate aerobically at 33-37°C for 4 hours.
4. After incubation, add 1-2 drops of PYR Reagent (REF R21258) to the tube.
5. Observe for a red color development within 1-2 minutes.

INTERPRETATION OF THE TEST
Positive Test - Red color development
Negative Test - Yellow color development

QUALITY CONTROL
All lot numbers of PYR Broth 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
Enterococcus faecalis ATCC® 29212	Aerobic, 4 h @ 33-37°C	Positive
Streptococcus pyogenes ATCC® 19615	Aerobic, 4 h @ 33-37°C	Positive
Streptococcus agalactiae ATCC® 12386	Aerobic, 4 h @ 33-37°C	Negative
Streptococcus gallolyticus ATCC® 9809	Aerobic, 4 h @ 33-37°C	Negative

LIMITATIONS
1. A positive PYR test provides a high probability for presumptive identification of enterococci and *S. pyogenes.* However, other organisms, including gram-positive cocci, may hydrolyze PYR. Further biochemical testing may be necessary for definitive identification. Consult appropriate references as necessary.[5,6]

BIBLIOGRAPHY

1. Mulczyk, M. and A. Szewczuk. 1970. J. Gen. Microbiol. 61:9-13.
2. Godsey, J., R. Schulman, and L. Eriquez. 1981. Abstract #C84. Abstracts of the 81st General Meeting of the American Society for Microbiology. ASM, Washington, D.C.
3. Bosley, G.S., R.R. Facklam, and D. Grossman. 1983. J. Clin. Microbiol. 18:1275-1277.
4. Facklam, R.R., L.G. Thacker, B. Fox, and L. Eriquez. 1982. J. Clin. Microbiol. 15:987-990.
5. Murray, P.R., E.J. Baron, J.H Jorgensen, M.L. Landry, and M.A Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, 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 62084, Revised June 20, 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