RML # 21-258 1/2

remel

PYR REAGENT
TI No. 21258

INTENDED USE
REMEL's PYR Reagent is recommended for use in qualitative procedures for the rapid presumptive identification of group A streptococci and enterococci.

SUMMARY AND EXPLANATION
In 1981, Godsey, Schulman, and Eriquez described a test to differentiate group A streptococci and enterococci from other streptococci based on their ability to cleave L-pyrrolidonyl-B-naphthylamide (PYR).[1] In 1982, Facklam, Thacker, Fox, and Eriquez used the PYR test in conjunction with the CAMP and bile esculin tests to presumptively identify streptococci.[2] Facklam et al. incorporated PYR substrate into agar and tested for enzyme activity after overnight incubation. Bosely et al. incorporated PYR substrate into a broth and tested for enzyme hydrolysis after 4 hours incubation.[3] Ellner et al. described a colorimetric method for the PYR test using filter paper strips containing PYR substrate.[4] A positive test was visible within 15-20 minutes. The ease of performance and interpretation of the PYR test, coupled with the evidence cited, suggests that the PYR test is more specific than the routine bacitracin test for group A streptococci and is at least as specific as the routine 6.5% NaCl test for enterococci.[2,5]

PRINCIPLE
L-pyrrolidonyl-B-naphthylamide (PYR) serves as the substrate for the detection of pyrrolidonyl peptidase. Following hydrolysis of the substrate by the peptidase enzyme, the resulting beta naphthylamine produces a red color upon the addition of N,N-Dimethylaminocinnamaldehyde (PYR Reagent).

REAGENTS (CLASSICAL FORMULA)*

Glacial Acetic Acid (CAS 64-19-7)	25.0	ml
2-Methoxyethanol (CAS 109-86-4)	50.0	ml
N,N-Dimethylaminocinnamaldehyde (CAS 6203-18-5)	0.15	g
Sodium Lauryl Sulfate (CAS 151-21-3)	25.0	g
Formamide (CAS 75-12-7)	20.0	ml
Deionized Water (CAS 7732-18-5)	905.0	ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
CAUTION! May cause irritation to skin, eyes and respiratory tract. Avoid breathing vapor and eye/skin contact. This product is *For In Vitro Diagnostic Use* and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after their use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE
This product is ready for use and no further preparation is necessary. The product should be stored in its original container at 2-8°C until used. Allow product to come to room temperature before use. Do not incubate prior to use. Protect product from light.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION
Specimens should be collected and handled following recommended guidelines.[6]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) PYR Broth (REMEL #06-2084), PYR/Esculin Disk (REMEL #21-138), Strep ID Triplate (REMEL #02-382), (7) Applicator stick.

PROCEDURE
PYR Broth:
1. Inoculate the PYR Broth using 3-5 colonies from a pure, 18-24 hour culture.
2. Incubate the tube aerobically at 35-37°C for 4 hours.
3. After incubation, add a drop of PYR Reagent to the tube.
4. Observe for a red color development within 1-2 minutes.

PYR/Esculin Disk:
1. Place the disk on the agar surface of a primary isolation plate of colonies to be tested. Isolated colonies from an 18-24 hour culture should be selected for testing.
2. Inoculate a small area of the disk surface with a sterile loop or wooden applicator stick and replace the lid.
3. Incubate the plate aerobically at 35-37°C for 15 minutes.
4. After interpreting the esculin reaction, dispense 1-2 drops of PYR Reagent onto the disk.
5. Observe for a red color development within 1-2 minutes.

Strep ID Triplate:
1. Select 3-4 well isolated colonies from a pure culture of streptococci and streak the colonies across the surface of Section I on the triplate.
2. Incubate aerobically at 35-37°C for 18-24 hours.
3. Following incubation, add 1-2 drops of the PYR Reagent to the agar surface of the PYR section of the triplate.
4. Observe for the development of a red color within 1-2 minutes.

INTERPRETATION OF THE TEST
Positive Test - a red color development
Negative Test - a yellow color development or no color change

QUALITY CONTROL
All lot numbers of the PYR Reagent have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control 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
Streptococcus pyogenes ATCC® 19615	Aerobic, 24h @ 35°C	Positive
Streptococcus agalactiae ATCC® 12386	Aerobic, 24h @ 35°C	Negative

BIBLIOGRAPHY

1. Godsey, J., R. Schulman, and L.A. Eriquez. 1981. The Hydrolysis of L-pyrrolidine-Beta-naphthylamide as an Aid in the Rapid Identification of *Streptococcus pyogenes*, *S. avium*, and group D streptococci with a New Test System. Abstracts Am. Soc. Microbio. C84.
2. Facklam, R.R., L.G. Thacker, B. Fox, and L. Eriquez. 1982. J. Clin. Microbiol. 15:987-990.
3. Bosely, G.S., R.R. Facklam, and D. Grossman. 1983. J. Clin. Microbiol. 18:1275-1277.
4. Ellner, P.D., D.A. Williams, M.E. Hosmer, and M.A. Cohenford. 1985. J. Clin. Microbiol. 22:880-881.
5. Morgan, J.W. 1987. Laboratory Medicine. 18:682-683.
6. Balows, A., W.J. Hausler, Jr., K.L. Herrmann, H. Isenberg, and H.J. Shadomy. 1991. Manual of Clinical Microbiology. 5th ed. ASM, Washington, D.C.
7. Baron, E.J., L. R. Peterson, and S.M. Finegold. 1994. Bailey and Scott's Diagnostic Microbiology. 9th ed. Mosby, St. Louis, MO.

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12076 Santa Fe Drive, Lenexa, KS 66215-3594 800-447-3641 (Technical Service) 800-447-3635 (Order Entry)

MATERIAL SAFETY DATA SHEET

SECTION I-IDENTIFICATION

Product Name: PYR Reagent
Product No.: 21-258
Formula: Mixture

SECTION II-HAZARDOUS INGREDIENTS

Material:	Acetic Acid	Formamide	2-Methoxyethanol
CAS #:	64-19-7	75-12-7	109-86-4
% Weight:	2.5	2.0	5.0
NFPA Rating:	Health - 2 Flammability - 2 Reactivity - 1	Health - 2 Flammability - 1 Reactivity - 0	Health - 2 Flammability - 2 Reactivity - 0

SECTION III-PHYSICAL DATA

	Acetic Acid	Formamide	2-Methoxyethanol
Boiling Point:	244.4°F (118°C)	410°F (210°C)	255°F (123.8°C)
Vapor Pressure:	11	0.08	6
Vapor Density (air=1):	2.1	1.55	2.6
Water Solubility:	Complete.	Complete.	Miscible.
Appearance and Odor:	Colorless, pungent.	Colorless liquid.	Clear liquid, pleasant odor.
Melting Point:	62.1°F (16.7°C)	35.6-37.4°F (2-3°C)	-121°F (-85°C)
Specific Gravity:	1.05	1.134	1.0
% Volatile by Volume:	100	Data not available.	Data not available.
Evaporation Rate: (ether = 1)	0.97	Data not available.	0.6

SECTION IV-FIRE AND EXPLOSION DATA

	Acetic Acid	Formamide	2-Methoxyethanol
Flash Point:	106°F (41.1°C)	310°F (154°C)	102°F (38.9°C)
Explosive Limits:	LEL 5.4 UEL 16.0	Data not available.	LEL 2.3 @ 200°F (93.2°C) UEL 24.5 @ 200°F (93.2°C)

Flash point (mixture:) >210°F (>98.8°C) (Pensky-Martens Closed Tester, ASTM D 93).
Extinguishing Media: Carbon dioxide, dry chemical or alcohol foam.
Special Fire-fighting Procedures: Wear self-contained breathing apparatus and protective equipment.
Unusual Fire and Explosive Data: Emits toxic fumes when heated to decomposition.

SECTION V-HEALTH HAZARD AND EMERGENCY

Exposure Limits:	Acetic Acid	Formamide	2-Methoxyethanol
OSHA TWA:	10ppm, 25mg/m^3	20ppm, 30mg/m^3	Data not available.
OSHA STEL:	Data not available.	30ppm, 45mg/m^3	Data not available.
ACGIH (TLV) TWA:	10ppm, 25mg/m^3	10ppm, 18mg/m^3 (Skin)	5ppm, 16mg/m^3 (Skin)
ACGIH STEL:	15ppm, 37mg/m^3	Data not available.	Data not available.
ORAL RAT LD_{50}:	3310 mg/kg	5570 mg/kg	2460 mg/kg

Acute Health Effects: Caution! May cause irritation to skin, eyes and respiratory tract. Avoid breathing vapor and eye/skin contact.
Primary Routes of Entry/First Aid:
Inhalation: Not applicable under normal conditions of use. Seek medical assistance if necessary.
Eye/Skin Contact: Flush immediately with copious amounts of water for at least 15 minutes. Remove contaminated clothing. Call physician.
Ingestion: Do not induce vomiting. If victim is conscious, flush out mouth with water; give water, milk, or milk of magnesia. Call physician.
Carcinogen Potential: None of the components specified are regulated by the IARC, NTP or OSHA as a carcinogen or potential carcinogen.
Target Organ Effects: Formamide - may cause congenital malformation in the fetus (target organ-blood). 2-Methoxyethanol - central nervous system depressant (nephrotoxin), may also affect the hematopoietic system and liver.
Medical Conditions Aggravated by Exposure: Persons with pre-existing eye, skin or respiratory disorders may be more susceptible to the effects of this substance.

SECTION VI-REACTIVITY

Stability: Stable.
Incompatibility: Can react with strong oxidizers, alkalies and amines.
Hazardous Polymerization: Will not occur.
Hazardous Decomposition Products: Fumes of sodium oxide and oxides of carbon.
Conditions to Avoid: Data not available.

SECTION VII-SPILL AND DISPOSAL

Spill Procedures: Wear protective clothing and equipment as specified in Section VIII. Wear self-contained breathing apparatus or respirator with approved NIOSH/MSHA cartridges if necessary. Contain spill, neutralize with soda ash and absorb with inert material. Scoop up and place in an approved hazardous waste container.
Disposal Procedures: Ensure compliance with all local, state and federal regulations or contact an approved and licensed disposal agency.

SECTION VIII-PROTECTION DATA

Eyes: Chemical safety goggles.
Gloves: Chemical-resistant gloves.
Respiratory: Not required under normal conditions of use.
Ventilation: Mechanical exhaust, fume hood.
Other: Protective apron or gown as required.

SECTION IX-HANDLING AND STORAGE

Store at 36-46°F (2-8°C). Keep container tightly closed. Separate from oxidizing materials.

SECTION X-MISCELLANEOUS DATA

This information is believed to be correct, however no warranties are made with respect to this information and REMEL assumes no liability resulting from use. Make independent determinations of the suitability and completeness of information from all sources, to assure proper use and disposal of this product and personnel safety and health. Data for each component is specified where applicable as mixtures have not been tested.