B. Group D *Enterococcus*

In a comparison study, one hundred and twenty-eight (128) alpha or gamma hemolytic suspect *Streptococcus* or *Enterococcus* culture isolates were tested comparing bile esculin and salt tolerance with Pulse PYR Disc Test results.

1. Comparison of test results revealed a 98.4% agreement (126 isolates) and 1.6% disagreement (2 isolates).
2. Based on these test results, together with alpha or gamma hemolytic activity of a *Streptococcus*, the Pulse PYR Disc Test demonstrates a sensitivity of 100.0% and a specificity of 93.3%
3. This data demonstrates the value of the Pulse PYR Disc Test and alpha or gamma hemolytic activity of a *Streptococcus* as differential parameters to aid in the presumptive identification of Group D *Enterococcus*.
4. Detailed information on this comparative study is available upon request.

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PYR Disc Test

INTENDED USE

The Pulse PYR Disc Test is used to detect the hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR). The hydrolysis of PYR provides a valuable differential parameter to aid in the presumptive identification of Group A *Streptococcus* and Group D *Enterococcus* and provide differentiation from other *Streptococcus* and Group D Non-*Enterococcus* from pure culture.

SUMMARY & PRINCIPLES

The identification of *Streptococcus* and *Enterococcus* is necessary to diagnose clinical infections, initiate appropriate antimicrobial therapy, provide insight into the origin of the infection, and provide valuable information concerning the prognosis of the disease process.

Traditionally, the identification of clinically significant *Streptococcus* and *Enterococcus* has involved parameters such as hemolysis; resistance to such selective agents as bacitracin, optochin, trimethoprim / sulfamethoxazole and bile salts; hydrolysis of esculin and sodium hippurate; the CAMP reaction; and salt tolerance (3,5).

Utilizing "PYR" reaction, the Pulse PYR Disc Test provides a valuable differential parameter to aid in the presumptive identification of Group A *Streptococcus* and Group D *Enterococcus*. Investigators have reported that a positive PYR test provides excellent presumptive identification of Group A *Streptococcus* and Group D *Enterococcus*. The remaining *Streptococcus* groups demonstrate a negative PYR test (1,2,4)

The by-products of metabolized PYR (L-pyrrolidonyl-β-naphthylamide) react with Indole (Sutter's) Reagent (N,N-dimethylaminocinnaldehyde) to form an immediate bright-red complex. A negative reaction demonstrates no color reaction or a slow dull, pink complex; A positive reaction usually results in a formation of pink color within 30 seconds.

The Pulse PYR Disc Test uses a paper disc which is impregnated with L-pyrrolidonyl-β-naphthylamide. The ability and inability of a test isolate to metabolize PYR is determined after addition of the Indole (Sutter's) Reagent. Utilizing this paper-disc technology provides a standardized and stable test parameter, thus eliminating the need for bacitracin sensitivity, bile esculin, and 6.5% salt tolerance parameter.

MATERIALS SUPPLIED

Cat. Number	0715B	0715S25	0715	0715A
# of PYR Paper Disc	50	25	25	50
Indole Reagent	2.5 mL	1.3 mL	-	-

Additional Items Required:

Method I: Distilled water (pH 7.0 – 7.2), Slide, Sterile loop or stick, Indole (Sutter's) Reagent (if not provided).

Method II: Tryptic Soy Agar or Tryptic Soy Agar with 5 % Defibrinated Sheep Cells; inoculating loop; sterile swabs; 35 Degree C incubator; Indole (Sutter's) Reagent (if not provided).

STORAGE & STABILITY

The components should be stored at 2–8 Degree C in their original, tightly sealed containers. The PYR Disc Test should not be used if there is evidence:

a) that hydration has occurred as indicated by the desiccant turning from blue to pink;
b) of contamination;
c) of improper storage;
d) that the expiration date has passed; and
e) that product specifications and product performance cannot be met.

PRECAUTIONS

This product is for in-vitro diagnostic use by trained, qualified personnel. Established laboratory procedures must be followed in the handling and disposal of all infectious bacteria. This product is only one integral part of the overall plan for the collection, transportation, isolation, identification, and antimicrobial agent susceptibility testing of disease-producing microorganisms. Consult appropriate references for additional methods and procedures.

The specification, performance, and intended use of these products have been set forth in the literature. The references cited include several of these application (3, 5).

PROCEDURE

Method I: (Rapid Method)

1. Place the disc onto a slide and moisten with a loopful of distilled water. Do not use excessive water. The disc should be only wet enough to hold it in place.
2. Smear with a paste of the suspect organism from a fresh pure 24-hour culture plate or slant.
3. Wait 2 – 5 minutes (at room temperature). Then add one drop of Indole (Sutter's) Reagent directly to the paper disc.
4. Wait for one minute. DO NOT read result after two minutes. Observe and record results.

Method II:

1. Select a suspect, well-isolated *Streptococcus* or *Enterococcus* colony on primary agar medium (i.e. aerobic, Gram-positive, catalase-negative coccus).
2. From the center of the representative colony, pick a portion of the colony and inoculate approximately one quarter of a petri dish containing Tryptic Soy Agar or Tryptic Soy Agar with 5 % Defibrinated Sheep Cells.
3. Place a PYR Disc into the center of the inoculated area and gently tap the disc into firm contact with the medium.
4. Incubate the agar plate at 35 Degree C, under normal atmospheric conditions for 18-24 hours.
5. After incubation, and when a lawn of growth is present in the area of the inoculated medium, add one drop of Indole (Sutter's) Reagent directly to the paper disc.
6. Wait for 30 seconds. Observe and record results.

INTERPRETATION OF RESULTS

COLOR STATUS IN THE PAPER DISC	RESULT INTERPRETATION
Formation of a pink color within specified time	PYR - Positive
No formation of a pink color within specified time	PYR - Negative

Notes: It is essential that the reaction be read within the prescribed period. Delayed reading may result in an apparent false-positive reaction. The Indole (Sutter's) Reagent can break down the hemoglobin in the red blood cells resulting in the formation of a brown-red hemin complex. Also, prolonged exposure to the Indole (Sutter's) Reagent will begin to break down the PYR substrate resulting in a weak and delayed positive reaction.

Beta-Hemolytic *Streptococcus* – GROUP A Alpha or Gamma-Hemolytic *Enterococcus* – GROUP D	Beta-Hemolytic *Streptococcus* – NOT GROUP A Alpha or Gamma-Hemolytic *Enterococcus* – NOT GROUP D
• PYR-positive	• PYR-negative

A gamma-hemolytic or alpha-hemolytic *Streptococcus / Enterococcus* and PYR-positive test parameters compare favorably with a gamma-hemolytic or alpha-hemolytic *Streptococcus / Enterococcus* AND Bile Esculin-positive / Salt Tolerance-positive test parameters to aid in the presumptive identification of Group D *Enterococcus.*

QUALITY CONTROL

Follow the same procedure as outlined above using the following microorganisms as control:

PYR Positive

- *Streptococcus pyogenes* (Group A *Streptococcus*), ATCC # 19615
- *Enterococcus faecalis* (Group D *Enterococcus*), ATCC # 29212

PYR Negative

- *Streptococcus agalactiae* (Group B *Streptococcus*), ATCC # 12386 or ATCC # 13813
- *Streptococcus bovis* (Group D Non-*Enterococcus*), ATCC # 9809

Note: The microorganisms suggested are examples only. Selection of microorganism strains for quality control testing is the responsibility of each individual laboratory.

LIMITATIONS

1. The Pulse PYR Disc Test must ONLY be used as a parameter to aid in the presumptive identification of Group D *Enterococcus* IF the isolate demonstrates gamma or alpha hemolysis.
2. The Pulse PYR Disc Test must ONLY be used as a parameter to aid in the presumptive identification of Group A *Streptococcus* IF the isolate demonstrates beta hemolysis.
3. Situations may arise when more definitive biochemical and / or serological identification of a *Streptococcus* or *Enterococcus i*solate may be required. Examples might include systemic infections (i.e. blood cultures) and when definitive isolate identification is required for the interpretation of antibiotic susceptibility test results.

PERFORMANCE CHARACTERISTICS

A. Group A *Streptococcus*

In a comparison study, one hundred and twenty-five (125) beta hemolytic *Streptococcus* culture isolates were tested comparing bacitracin differential disc and the Pulse PYR Disc Test results.

1. Comparison of test results revealed a 98.4% agreement (123 isolates) and 1.6% disagreement (2 isolates).
2. Based on these test results, together with beta-hemolytic activity of a *Streptococcus*, the Pulse PYR Disc Test demonstrates a sensitivity of 100.0% and a specificity of 91.3%
3. This data demonstrates the value of the Pulse PYR Disc Test and beta hemolytic activity of a *Streptococcus* as differential parameters to aid in the presumptive identification of Group A *Streptococcus*.
4. Detailed information on this comparative study is available upon request.