PURPOSE: The PYR test is used, in conjunction with others, for the identification of catalase negative, gram positive cocci, including *Enterococci* and Group A *Streptococcus;* to differentiate *Escherichia coli* from other indole-positive, lactose positive Gram-negative rods and to differentiate coagulase-negative *Staphylococci*.

SAMPLE INFORMATION:

Type	Few, well isolated colonies

REAGENTS and/or MEDIA:

Type	remel PYR DISK with REAGENT
Stability and Storage Requirements	• Store in the dark at 2°C to 8°C. • Do not freeze or overheat. • Allow to come to room temperature before use. • Do not incubate prior to use. • Protect disks from moisture by removing from the vial only those disks necessary for testing. • Promptly replace the cap and return the vial to 2°C to 8°C.

SUPPLIES:

- Forceps
- Glass microscope slides
- Sterile saline
- Sterile pipettes
- Wooden sticks
- Disposable loops

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed weekly:
  - Positive: *Enterococcus faecalis* ATCC 29212
  - Negative: *Streptococcus agalactiae* ATCC 12386
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the PYR test	
1	Using forceps, place a disk on a clean microscope slide or on the surface of the Blood agar plate.
2	If the disk was placed on a slide, moisten with sterile saline. Do not oversaturate the disk.
3	With a wooden stick or disposable loop, remove a heavy visible paste (5 to 10 colonies) of the test isolate and rub it into the disk.
4	Incubate at room temperature for 2 minutes.
5	Add one drop of Colour Developer to the disk.
6	Allow up to one minute for colour change to occur.

INTERPRETATION OF RESULTS:

IF	THEN
Pink to red colour	**PYR = Positive**
Cream, yellow or no colour change	**PYR = Negative**

LIMITATIONS/PRECAUTIONS:

1. This test is only part of the overall scheme for identification.
2. *Aerococcus viridans*, *Lactococcus garvieae*, certain *Staphylococci* and most *Corynebacterium haemolyticum* strains are PYR positive, as are some Enterobacteriaceae and other gram-negative bacilli. Consult appropriate references for differential tests when necessary.
3. Some *Streptococcus pyogenes* may give negative PYR reactions.
4. Some *Enterococci* do not possess detectable Group D antigen although they give a positive reaction in the PYR test. The identity of such organisms should be confirmed using the Vitek 2 instrument.
5. Some strains of *Enterococci* are beta-hemolytic; therefore, colony morphology must be carefully evaluated to differentiate between *Enterococci* and *Streptococcus pyogenes*.
6. False negative reactions may occur if inadequate inoculum is used or if inoculum is removed from selective media or if disk is too moist.
7. The product should not be used if the color of the disk has changed from white, the expiration date has passed or there are other signs of deterioration.
8. PYR reagent is toxic and may cause harm to the environment; harmful by inhalation, contact with skin or eyes, or if swallowed.

REFERENCES:

- remel PYR Disk package insert, December 18, 2008
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	5 APR 19	Initial Release	L. Steven