PYR Test

PYR (Pyrrolidonyl Aminopeptidase) Test is used for the detection of pyrolidonyl arylamidase (also called pyrrolidonyl aminopeptidase) activity in *Streptococcus pyogenes* (group A strep), *Enterococcus* spp., some coagulase-negative staphylococci, and some *Enterobacteriaceae*. It is also known as PYR (L-pyrrolidonyl-β-naphthylamide) which serve as a substrate for the detection of pyrrolidonyl peptidase.

Principle of PYR Test

PYR is a rapid method for presumptive identification of bacteria based on the pyrrolidonyl arylamidase enzyme. The enzyme *L*-pyrrolidonyl arylamidase hydrolyzes the L-pyrrolidonyl- β-naphthylamide substrate to produce a β-naphthylamine. The β-naphthylamine can be detected in the presence of N,N-methylaminocinnamaldehyde reagent by the production of a bright red precipitate.

Following hydrolysis of the substrate by the peptidase, the resulting b-naphthylamide produces a red color upon the addition of 0.01% cinnamaldehyde reagent. When a visible inoculum of microorganism is rubbed onto a small area of a disk impregnated with the substrate, the hydrolysis occurs within 2 min, at which time the cinnamaldehyde reagent is added to detect the reaction by a color change to purple.

Procedure of PYR Test

Broth Method

1. Inoculate PYR broth with 3-5 colonies from 18-24 hours pure culture.
2. Incubate the tube aerobically at 35-37°C for 4 hours.
3. Add 2-3 drop of PYR reagent and observe for color change.
4. Observe for the red color development within 1-2 minutes.

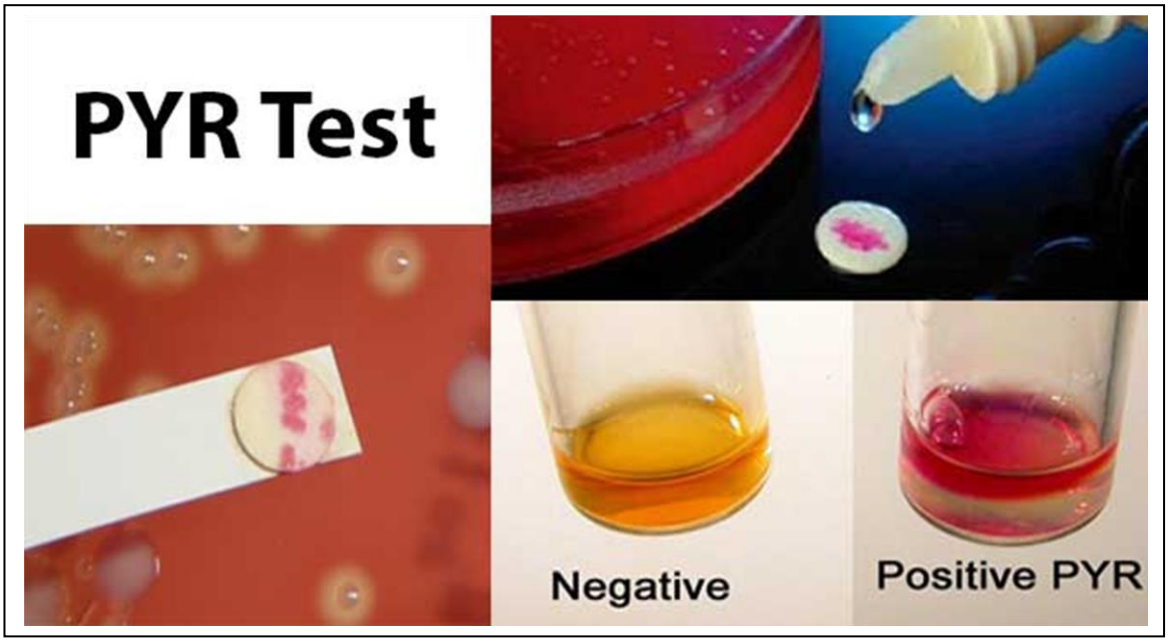
Disk Method (Rapid)

1. Wet the PYR test disc on the strip with 10 µl sterile distilled water or deionized water.

Note: Do not flood the disk.

2. Put 5-10 colonies of the tested strain from 18-24 hours culture on the surface of the disc with a loop and smear them lightly on it.
3. Incubate the disc for 1-2 minutes at room temperature.
4. After incubation, add 1 drop of N, N-dimethylaminocinnamaldehyde.
5. Observe for red color development within 1-2 minutes

Result Interpretation of PYR Test

Positive: Bright pink or cherry-red color within 1-2 minutes.

Negative: No color change.