

PYR

Rapid Identification of Group A Streptococci And Enterococci by the Identicult-AE

I. PRINCIPLE

The Identicult-AE is intended for use as a rapid test for identifying enterococci and group A streptococci from clinical isolates. A chromogenic substrate (L-pyrrolidonyl- β -naphthylamide or PYR) which when hydrolyzed by PYR-ase (L-pyrroglutamyl-peptide hydrolase) produces a red color upon the addition of a specific reagent. PYR is a substrate that is hydrolyzed by 100% of the enterococci and group A streptococci, but not by any other streptococcal strains.

II. MATERIALS

- A. Demineralized water.
- B. Reagent #2, Color developer (15ml). Store at 2-8°C until stated expiration date.
- C. Identicult -AE test strips. Store at 2-8°C until stated expiration date.
- D. Wooden applicator sticks or inoculating needles

III. SAFETY

- A. Avoid contact of Reagent #2 with skin and eyes. Wash affected areas with water upon exposure.

IV. QUALITY CONTROL

- A. Quality control is performed when each new lot or shipment. The control organisms and their PYR reactions are as follows:
 - 1. *E. faecalis* ATCC 29212 = Positive
 - 2. *S. agalactiae* ATCC 12386 = Negative
- B. If controls do not display expected results, quality control must be repeated. Notify the supervisor

V. PROCEDURE

- A. Using microbiological techniques, determine that you are working with pure colonies of streptococci. Note hemolytic reaction from BAP.
- B. Moisten strip slightly with DI water.
- C. Pick up 3-5 colonies of the streptococci and rub onto the test strip. Be sure not to use a mixed culture.
- D. Incubate the inoculated strip at room temperature for 2 min.
- E. Apply 1 drop of Reagent #2 to the test strip.

VI. INTERPRETATION

- A. An intense red color develops immediately around the colonies in the presence of hydrolyzed PYR.
- B. The PYR test is negative if no color, an orange color, or a weak pink-color develops. Questionable results should be confirmed with bile esculin and 6.5% NaCl testing.
- C. PYR positive streptococci can be identified as *Enterococcus* or *S. pyogenes* (group A streptococci).

VII. Notes

- A. *S. pyogenes* is frequently isolated from throat cultures but may also be isolated from blood cultures, wound cultures, CSF, and other sites.
- B. Enterococci are normal inhabitants of the human gastrointestinal tract. They may cause bacteremia, urinary tract infections, wound infections and endocarditis. The medically important enterococci include *E. faecalis*, *E. faecium*, and *E. durans*.

VIII. LIMITATIONS

You must be sure that you are working with a pure culture of streptococci. Other organisms, such as staphylococci, may cause a positive PYR reaction.

VIII. REFERENCES

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