

ESC/PYR WEE-TAB AND SPOT TEST

PRINCIPLE/DISCUSSION:

The ability to hydrolyse both esculin and PYR are valuable tests to identify an assortment of different organisms, in particular *Streptococci* and *Enterococci*. Key Scientific has combined these two tests into one easy to use, inexpensive tablet.

ESC: A positive esculin is apparent by hydrolysis of p-nitrophenol-β-D-glucopyranoside which releases yellow para-nitrophenol. PYR: Enzymatic hydrolysis of L-pyrroglutamic acid-β-naphthylamide (PYR) (aka pyrrolidonyl-β-naphthylamide) releases free β-naphthylamine which is detected and shown by the color change after adding PEP reagent.

ACTIVE INGREDIENTS/ MSDS

The tablets contain approximately 0.05 mg. each of L-pyrroglutamic acid β-naphthylamide and p-nitrophenol-β-D-glucopyranoside in a Sodium Chloride and Dicalcium phosphate base: The naphthylamides have been identified as possible carcinogens and should be handled accordingly. Avoid excessive contact with the tablets. When used only as directed there is no hazard involved.

MATERIAL REQUIRED:

ESC/PYR (K1137) tablets are sold in tubes ready-to-use, 28 per pack.

The tests require fresh 24 hour growth. Consult a suitable manual for recommended media for the specimen. The following items are required but not provided:

- Inoculating loop
- Distilled water, pH 7.0-7.2
- Disposable pipette
- Filter paper (Optional - for smear method)
- PEP reagent (K 2375 or K982375.)

INSTRUCTIONS:

If using differential media it is best to use the direct test method. (see INTERPRETATION NOTES for comments).

DIRECT TEST

- (1) Add 3-5 drops of distilled water to the test tube.
- (2) Inoculate heavily with a loopful of organism from a fresh pure 24 hour culture plate or slant. Mix with the loop until the organism is in suspension.
- (3) Incubate aerobically, uncovered, at 35-37C for 2 hours.

4) After incubation, observe for yellow color indicating positive esculin reaction. After recording reaction, add 2 drops of **PEP** reagent. Incubate 15 more minutes for PYR color development. Positive PYR tests will be red while negative tests are yellow to a very light peach color (or green. See INTERPRETATION NOTES). If reaction is peach colored, vortex to confirm negative. (SEE TIPS)

SMEAR METHOD (PYR only)

- 1) Rehydrate the tablet by adding 5 drops of water to the tube. Mix thoroughly with a pipette.
- 2) Moisten a piece of filter paper with 1 drop of the rehydrated substrate. Disregard any powder which may form on the surface of the paper. This is from the tableting process and will not interfere with the test being performed.
- 3) Smear several colonies onto the moist spot and incubate covered for 5-20 minutes (not longer).

4) If desired, inoculate remaining liquid for esculin reaction.

5) After incubation, add 1 drop of PEP reagent to the smeared colonies and allow to sit 1-2 minutes. In a positive test, the smear will turn dark pink, red, or purple while negative tests will remain unchanged or turn green (see INTERPRETATION NOTES).

INTERPRETATION NOTES / TIPS

Esculin: A yellow color at any time during the incubation period indicates a positive esculin reaction.

PYR: The appearance of a dark pink to red color is positive. Indole reactions of organisms grown on any media containing tryptophane (e.g. blood agar) may interfere but will still produce predictable reactions. From such media, positive PYR reactions will range from dark pink or red (PYR+/indol-) to very dark blue or purple (PYR+/indole+). Green or turquoise (indole+), and yellow (indol-) are both PYR negative.

When doing the direct test, vortexing or shaking the test vigorously will enhance color development of the PYR. A denser suspension will also produce brighter and faster reactions for both tests. PYR tests may be brighter if done by dipping a swab to the bottom of the tube then adding 1 drop of reagent to the wet swab.

If doing the smear method, avoid using media which create pigmentation (e.g. MacConkey, EMB, etc.) as this will interfere, often making it impossible to read the results. Pigmentation of the colony from differential media does not interfere with the direct tube method.

When reading a smear, use only the color of the smear itself and disregard any discoloration around the perimeter of the circle created by the reagent. One drop of rehydrated substrate produces a large enough circle to do both a positive and negative control on the paper at the same time a test is being performed.

STORAGE:

Store tightly covered with desiccant at less than 0C.

QUALITY CONTROL:

Each lot of tablets should be tested with known positive and negative organisms. Some suggested strains are listed. Dispose of all used material in a manner appropriate for biohazardous material.

	PYR	ESC
<i>E. faecalis</i> ATCC29212	+	+
<i>S. agalactiae</i> ATCC13813	-	-

(Positive PYR and Indole may be shown by testing with *Citrobacter diversus*)

REFERENCES

- (1) Manual of Clinical Microbiology, Fifth Edition, Chapter 36, Enterobacteriaceae
- (2) Kilian, M and Bulow, P. 1976. Rapid Diagnosis of Enterobacteriaceae, Acta path. microbiol. Scan, Sect B, 84:245-251
- (3) Wadsworth Anaerobic Bacteriology Manual, 5th Edition, 1993, Glucosidase tests, page 152.



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