

K1538B PYR DISC

PRINCIPLE/DISCUSSION:

Enzymatic hydrolysis of PYR (L-pyroglutamic acid β -naphthylamide, aka pyrrolydonyl- β -naphthylamide) releases free β -naphthylamine which is detected and shown by the color change after adding PEP reagent.

ACTIVE INGREDIENTS/MSDS:

The discs contain L-pyroglutamic acid β -naphthylamide. Naphthylamides have been identified as possible carcinogens however when used as directed the discs present no hazard. PEP reagent contains a small amount of hydrochloric acid, is poisonous, mildly corrosive, and stains clothing and hands. Handle with care. Consult poison control center if ingested.

MATERIAL REQUIRED:

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

- Purified water, pH 6.5-7.5
- Slide
- Loop or sterile stick for harvesting

INSTRUCTIONS:

- (1) Place the disc onto a slide and moisten with a loopful of 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 organism from a fresh, pure 24 hour culture plate or slant from **non-selective media** such as Blood Agar.
- (3) Wait 2-5 minutes (at room temperature) then add 1 drop of PEP reagent. Wait at least 1 but not more than 2 minutes for color to develop.

INTERPRETATION/LIMITATIONS:

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+/indole-) to purple (PYR+/indole+). Green or turquoise (indole+), and yellow (indole-) are both PYR negative. Positive PYR and Indole may be shown by testing with *Citrobacter diversus* and positive indole only can be shown with *E. coli*.) A false negative may occur if discs are too wet. False-negative tests can result if selective media or tube biochemical agars are used to provide inocula. The development of an orange, salmon, or yellow color should be interpreted as a negative reaction.

STORAGE:

Store discs and reagent tightly covered in the dark at 2-8C. Discs may be used cold. Do not freeze reagent.

QUALITY CONTROL:

Each lot should be tested with known positive and negative organisms. The following strains are used routinely by Key Scientific. Dispose of all used material in a manner appropriate for biohazardous material.

Positive:

- *E. faecalis* ATCC 29212
- *S. lugdunensis* ATCC 700328

Other organisms expected to be positive: *S. haemolyticus*, *S. Schleifei*, **Citrobacter*, *Klebsiella*, *Yersinia*, *Enterobacter*, and *Serratia spp.*

Negative:

- *S. aureus* ATCC 25923
- *Strep. agalactiae* ATCC 13813

In some cases, organisms may give a weak positive which is a reaction to the reagent only and **not the result** of the PYR test. This can be cross checked by smearing the organism onto plain filter paper and immediately adding reagent. This may show the pale pink of a negative test which should not be read as positive. *Proteus* or *Providencia spp* and some *S. aureus* strains may give this false reaction which should not be confused with a true positive result. The development of an orange, salmon, or yellow color should be interpreted as a negative reaction.

REFERENCES:

- (1) Manual of Clinical Microbiology, Seventh Edition, Chapter 27, *Enterobacteriaceae*.
- (2) Manual of Clinical Microbiology, Seventh Edition, Chapter 17, *Streptococcus*.
- (3) Manual of Clinical Microbiology, Seventh Edition, Chapter 16, *Staphylococcus* and *Micrococcus*.
- (4) Chagla, Abdul, Alexander A., Borczyk, John E., Aldom, Sergio Dalla Rosa, & Donald D. Cole. July 1993. Evaluation of the L-Pyrrolidonyl- β -Naphthylamide Hydrolysis Test for the Differentiation of Members of the Families *Enterobacteriaceae* and *Vibrionaceae*. Journal of Clinical Microbiology. P. 1946-1948.



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