K1532B PRO DISC

PRINCIPLE/DISCUSSION:
Enzymatic hydrolysis of L-proline-ß-naphthylamide releases free-ß naphthylamine which is shown by the color change after adding PEP reagent. This test is especially useful in screening for *Clostridium difficile.* A negative test indicates the organism cannot be *C. difficile.* The test is also of value when used with other tests in the speciation of many anaerobes and other organisms such as enterics. Comprehensive charts for these organisms are available on request.

ACTIVE INGREDIENTS/ MSDS:
The discs contain L-proline ß-naphthylamide. Naphthylamides have been identified as possible carcinogens however when used as directed the discs present no hazard. PEP reagent contains para-dimethyl amino-cinnamaldehyle in a weak hydrochloric acid solution. Pep reagent is mildly corrosive and stains clothing and hands.

MATERIAL REQUIRED:
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:

- Slide
- Inoculating loop or stick
- purified water (pH. 6.5-7.5)

INSTRUCTIONS:
(1) Place a disc onto a slide and moisten slightly. (Do not over saturate.)
(2) Smear the disc with a paste of the organism from a fresh pure 24 hour culture plate or slant.
(3) It is not necessary to incubate. Wait 2 to 5 minutes (not longer) then add 1 drop of PEP reagent. Wait at least 1 but not more than 2 minutes for color to develop.

INTERPRETATION:
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 PRO reactions will range from dark pink or red (PRO+/indole-) to purple (PRO+/indole+). Green or turquoise (indole+), and yellow (indole-) are both PRO negative.

STORAGE:
Store discs and reagent tightly covered with desiccant at 2-8 C. Discs may be used while cold. Do not freeze reagent.

QUALITY CONTROL:
Each lot should be tested with known positive and negative organisms. Some suggested strains are *C. difficile* ATCC 9689 (positive) and *C. perfringens* ATCC 13124 (negative). Positive PRO and Indole may be shown by testing with *C. sordelli.* Dispose of all used material in a manner appropriate for hazardous material.

REFERENCES:
(1) Manual of Clinical Microbiology, Fifth Edition, ASM, Washington,D.C.
(2) Wadsworth Anaerobic Bacteriology Manual, 5th Edition, 1993,Glucosidase tests, page 152.

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