K9162B TRY & K9161B CHY

PRINCIPLE:
K9162B discs detect the trypsin enzyme in bacterial cells. Hydrolysis of Na-Benzoyl- DL-arginine-ß-naphtylamide releases pure naphthylamide which is red after adding PEP (cinnamaldehyde) reagent. K9161B discs detect the chymotrypsin enzyme. In this test the substrate hydrolyzed is N-Glutaryl-Gly-Gly-Phe-ß- naphthylamide, which is also red after addition of PEP reagent. These tests are useful in the identification of anaerobes, specifically of value for *Porphyromonas* species.(1) TRY is also useful in the speciation of non-fermentative aerobic gram-negative bacilli and when used with other chromogenic tests produced by KEY can expedite identification.

MSDS:
Discs should be used only by trained individuals. Unbound naphthylamide in considered hazardous: the disc contains the bound form and unbound naphthylamide is present only in a positive test. Do not handle the used test- discard in a manner appropriate for biohazardous materials. PEP reagent is a 0.1% solution of p-dimethylaminocinnamaldehyde in weak hydrochloric acid. Hydrochloric acid can cause irritation or burns-in case of contact flush with water. PEP reagent will stain skin and clothing.

STORAGE:
Store discs at 2-8 degrees C. Do not use beyond the expiration date. Do not freeze reagent.

MATERIALS REQUIRED:
Key TRY and CHY discs are in packs of 50 discs with PEP reagent provided. The tests require fresh growth on media appropriate for the specimen, however, chocolate based media can cause a false positive reaction (see LIMITATIONS). A sterile loop or stick for harvesting, a slide, and distilled water are required but not provided.

PROCEDURE:
The discs are for in vitro diagnostic use only. Observe aseptic techniques when working with clinical specimens and microbiological cultures. The discs should be white to cream colored. If discs have changed colors do not use them. For best results use fresh cultures less than 48 hours old.

1. Place a disc onto a clean slide and moisten slightly.
2. Using a sterile stick or loop, smear the disc with a visible paste of the suspected isolate. False negatives may result from insufficient inoculum.
3. Incubate at room temperature for 5 minutes.
4. Add 1 drop of PEP reagent and wait 2 minutes to observe color.

INTERPRETATION:
After adding reagent, a positive test will be a deep red to deep purple while a negative test will be colorless, yellow, or blue-green: the blue green color indicates a negative enzyme test and positive indole. Disregard pale pink reactions (see LIMITATIONS).

QUALITY CONTROL:
Each lot of discs should be tested with organisms of known reactivity prior to use. We recommend: *Porphyromonas gingivalis*, ATCC 33277 (TRY +, CHY -) and *Porphyromonas levii*, ATCC 29147, (TRY -, CHY +). We also recommend smearing a plain paper disc as a negative control if the growth media contains vitamins (see LIMITATIONS).

LIMITATIONS:
Some organisms may react with the reagent to produce a pale pink reaction which is not related to the substrate being tested. This can also happen from colonies grown on chocolate agar or other vitamin enriched agar. This should not be read as positive. If in doubt, smear the organism onto a piece of plain filter paper and immediately add reagent. If the organism turns pale pink on the plain paper, it is negative for the substrate. *Enterobacter aerogenes* may be used as an example of this reaction.

REFERENCES:

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