DUAL-TEST: NITROPHENOL AND AMINOPEPTIDASE COMBINED TESTS

PRINCIPLE/DISCUSSION:
Some bacteria produce enzymes which hydrolyze various chromogenic substrates. When these substrates are bound to another base, the hydrolysis will produce a color reaction, some apparent immediately and others by the use of special equipment or the addition of color developers. The dual tests make use of these principles and, by combining non-interfering substrates, produce two chromogenic reactions in one product, reducing the cost per test.

1. The primary tests are nitro-phenol bound substrates. Organisms containing the necessary enzymes hydrolyze these substrates, releasing the yellow nitro-phenol which is visible without addition of any reagent. The nitro-phenol test will be the one listed first in the product name and are located on the tablet.
2. The second tests are aminopeptidase tests. Organisms containing the enzymes necessary for hydrolysis of the applicable arylamide release free beta-naphthylamide which is detected by the addition of aminopeptidase reagent. These will be listed last on the labels and are on the disc.

Nitrophenol substrates: (1st test - yellow)

- ONPG (o-nitro-phenol-ß-D galactopyranoside)
- NAG (p-nitro-phenol-N-acetyl-ß-D-glucosaminide)
- AFU (P-nitrophenol-alpha-D-fucopyranoside)
- AGLU (0-nitrophenyl-alpha-D-glucopyranoside)
- BGLU (o-nitrophenyl-beta-D-glucopyranoside)
- AGAL (p-nitrophenyl-alpha-D-galactopyranoside)
- PO4 -(alkaline phosphatase)
- aARA (o-Nitrophenol-a-d-arabinopyranoside)

Discs contain one of the following chemicals:
Naphthylamide substrates: (2nd test - red or purple Aminopeptidase)

- PRO (proline-ß-naphthylamide)
- PYR (pyrrolidonyl-ß-naphthylamide, aka pyroglutamic acid-ß-naphthylamide)
- TRY (Na-Benzoyl-DL-arginine-ß-naphtylamide “trypsin”)
- CHY (N-Glutaryl-Gly-Gly-Phe-ß-naphthylamide-“chymotrypsin”)
- LGY (Leucyl glycine ß naphthylamide)
- SER (Serine ß naphthylamide)
- ARG (Arginine ß naphthylamide)
- PAL (Lphenylalanine αnaphthylamide)
- GLY (Glycine αnaphthylamide)

MATERIALS REQUIRED:
Each type of test is sold separately, ready to use in test tubes, 28 per bottle. Usage requires 24 hour growth. Consult a current reference manual for the correct media to use. The following items are also required but not provided:

- Microbiology loop, needle, or sterile swab for harvesting
- Distilled water, neutral pH

PROCEDURE:

1) Add at least 0.25 ml but not more than 0.5 ml (about 5 drops) of distilled water to the tube provided.
2) Inoculate heavily from fresh 24 hour growth on a plate or slant. The heavier the inoculum the brighter and faster the reaction: use a visible loopful of paste. Mix well by using the loop to move the tablet and disc around in the tube. It is not necessary for the tablet to dissolve.
3) Incubate at 32-37^{O}C for at least 2 hours. Do not read after more than 28 hours as false positives may occur.

INTERPRETATION:
(Read and interpret in the exact sequence listed)

1) **Nitro-phenol tests:** Observe for a yellow color indicating a positive test. Colorless is negative.
2) **Aminopeptidase tests:** Perform the aminopeptidase test last by adding 2 drops of PEP reagent directly to the tube and reincubating for 15 minutes. The water may or may not show the reaction color. If necessary use a loop to pull the disc up the side of the tube to see the reaction color on the disc. Red, blue, or purple is positive. Green, turquoise, or yellow is negative.

REFERENCES:

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

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