NAPHTHYLAMIDE DISCS

PRINCIPLE/DISCUSSION:

Some bacteria produce enzymes which hydrolyze various chromogenic substrates. Because the organism produces these enzymes during the growth process and the presence of the enzymes can be detected rapidly, more substrates can be used in a wide variety of applications which would normally require special growth conditions. KEY naphthylamide discs provide an easy to setup, inexpensive way to detect these enzymes. Enzymatic hydrolysis of the arylamide in the disc releases free-naphthylamine which is detected and shown by the color change after adding reagent. Any naphthylamide disc may be used as a single test or added to any nitrophenol based WEE-TAB for dual setup (ADD-A-TEST).

ACTIVE INGREDIENTS:

The discs contain approximately 0.05 mg. of one of the following substrates (see Quality control section for catalog #) dissolved in an inorganic solvent:

- L-pyroglutamic acid ß-naphthylamide (PYR)
- L-arginine ß-naphthylamide (ARG)
- L-phenylalanine ß-naphthylamide (PAL)
- L-proline ß-naphthylamide (PRO)
- L-leucine ß-naphthylamide (LAP)
- L-cystine DI-ß-naphthylamide (CYST)
- L-alanyl-L-alanyl-ß-naphthylamide (ALN)
- L-serine ß-naphthylamide (SER)
- Glycine ß$^{\gamma}$naphthylamide (GLY)
- Leucyl-Glycine ß-naphthylamide(LGY)
- Na-Benzoyl-DL Arginine ß-naphthylamine (trypsin) (TRY)
- N-glutaryll-gly-gly-phe-ß naphthylamide (chymotrypsin) (CHY)
- Glutamic acid -(ß naphthylamide (GLU-AMP)
- PEP reagent contains p-dimethyl-amino-cinnamaldehyde in hydrochloric acid.

MATERIAL SAFETY DATA:

Naphthylamides have been identified as possible carcinogens and should be handled accordingly. Avoid excessive handling. When used only as directed there is no hazard involved. PEP reagent is poisonous, mildly corrosive, and stains clothing and hands. Handle with care. Consult poison control center if ingested.

MATERIAL REQUIRED:

All tests require fresh 24 hour growth on solid media. Broth media are not acceptable. Consult a suitable manual for recommended media for the specimen. PEP Reagent is provided. The following items are required but not provided:

- Inoculating loop
- Distilled water, pH 7.0-7.2
- Droppers
- Small test tubes or glass slides

PROCEDURE FOR DIRECT TEST:

(1) Place the disc onto a slide or the lid of a plate and moisten with a loopful of distilled 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.
(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.

PROCEDURE FOR ADD-A-TEST:

1) Add one disc to the tube containing the WEE-TAB.
2) Follow the set up and incubation instructions for the Wee-tab.
3) After evaluating results of the Wee-tab, add 2 drops of PEP reagent. Incubate 5 minutes for color development. It is recommended that you pull the disc up the side of the tube to observe the color of the disc.

INTERPRETATION OF RESULTS:

Positive tests will be red while negative tests are yellow to a very light peach color. The color will be brightest where the smear is, and spread from there. In a test tube, the disc will turn red, but not necessarily the water. **Note**: Indole reactions of organisms grown on any media containing tryptophane may interfere with the naphthylamide reaction. From such media, positive naphthylamide reactions may be red (naphth+/indol-) or very dark blue to purple (naphth+/indole+). Green or turquoise (indole+), and yellow (indol-) are naphthylamide negative.

STORAGE:

Consult individual package label for storage instructions.

QUALITY CONTROL:

Each lot of discs should be tested with known positive and negative organisms. Some suggested strains are listed but any strain of known reactivity may be used. Dispose of all used material in a manner appropriate for bio-hazardous material.

Organism codes

01. *Bacteroides fragilis* ATCC 25285
02. *Clostridium difficile* ATCC 9689
03. *Clostridum perfringens* ATCC 13124
04. *Corynebacterium diphtheriae* ATCC 13812
05. *Corynebacterium xerosis* ATCC 373
06. *Fusobacterium nucleatum* ATCC 25586
07. *Porphyromonas canoris* ATCC 122835
08. *Porphyromonas gingivalis* ATCC 33277
09. *Streptococcus pyogenes* ATCC 19615
10. *Aerococcus viridans* ATCC 70040
11. *Escherichia coli* ATCC 25922
12. *Lactobacillus acidophilus* ATCC 4356
13. *Clostridium septicum* ATCC 12464
14. *Serratia Marcesans* ATCC 8100

NAPHTHYLAMIDE:	POSITIVE	NEGATIVE
K9145 ALN	01.	06.
K9083B ARG	03	13
K9161B CHY	07.	08.
K9595B CYST	04.	05.
K9265B GLU-AMP	14.	11.
K9305B GLY	03.	01.
K1378B LAP	09.	10.
K9376B LGY	01.	03.
K9525B PAL	01.	08.
K1532B PRO	02.	03.
K1538B PYR	09.	11.
K9575B SER	12.	02.
K9162B TRY	08.	07.

REFERENCES:

(1) Manual of Clinical Microbiology, Fifth Edition, Chapter 36, Enterobacteriaceae
(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.

KEY SCIENTIFIC PRODUCTS, INC
1113 EAST REYNOLDS STREET
STAMFORD, TEXAS 79553
VOICE 800-843-1539
FAX 888-440-4208
WWW.KEYSCIENTIFIC.COM