NAPHTHYLAMIDE WEE-TABS

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 tablets provide an easy to setup, inexpensive way to detect these enzymes. Enzymatic hydrolysis of the arylamide in the tablet releases free a-naphthylamine which is detected and shown by the color change after adding reagent. Any naphthylamide tablet may be used as a single test or added to any nitrophenol based tablet for dual setup.

ACTIVE INGREDIENTS

The tablets contain aproximately 0.05 mg. of one or more of the following substrates (see catalog #) in a Sodium Chloride and Dicalcium phosphate base:
L-pyroglutamic acid a-naphthylamide (PYR)
L-arginine a-naphthylamide (ARG)
L-phenylalanine a-naphthylamide (PAL)
L-proline a-naphthylamide (PRO)
L-serine a-naphthylamide (SER)
Glycine a-naphthylamide (GLY)
Leucyl-Glycine a-naphthylamide(LGY)
Na-Benzoyl-DL Arginine ß-naphthylamine (trypsin) (TRY)
N-glutaryll-gly-gly-phe-ß naphthylamide (chymotrypsin) (CHY)
PEP reagent contains p-dimethyl-amino-cinnamaldehyde in 10% hydrochloric acid.

MATERIAL SAFETY DATA:

The 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 not available from KEY. Broth media are not acceptable. Consult a suitable manual for recommended media for the specimen. The following items are required but not provided:
Inoculating loop
Distilled or purified water
Droppers
Each product is sold ready-to-use, 28 tubes per pack. The catalog numbers of the tablets are listed in the QUALITY CONTROL section. K2375 PEP is sold in 10 ml bottles and in dropITS.

SINGLE TEST SETUP

(1) Add 3-5 drops of distilled water to the test tube.
(2) Inoculate heavily with a loopful of organism from a fresh pure 24 hour culture plate or slant. Mix with the loop until the organism is in suspension. Go to step 3 below.

MULTIPLE TEST SETUP

1) Harvest sufficient colonies into 1-2 ml of distilled water to make a suspension equal to #5 McFarland. This suspension may be stored aerobically for later use. (up to 5 days).
2) Add 3-5 drops of this solution to the tube containing the tablet, shaking to disintegrate the tablet.
3) Incubate all tests aerobically, uncovered, at 32-37C for 2-3 hours. Tests may be incubated up to 24 hours but reactions should be read slightly different.

TIPS:

Vortexing or shaking the test vigorously will enhance color development. A denser suspension will also produce brighter and faster reactions. Naphthylamide tests are brighter if done by dipping a swab to the bottom of the tube then adding 1 drop of reagent to the wet swab.

INTERPRETATION OF RESULTS

After incubation, add 2 drops of **PEP** reagent. Incubate 15 minutes for color development. Positive tests will be red while negative tests are yellow to a very light peach color. If reaction is peach colored, vortex to confirm negative. (SEE **TIPS)**
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 tablets should be tested with known positive and negative organisms such as those listed below.. Dispose of all used material in a manner appropriate for biohazardous material.
Organism codes

01. *Bacteroides fragilis*	02. *Bacteroides ureolyticus*
03. *Clostridium difficile*	04. *Clostridum perfringens*
05. *Peptostreptococcus hydrogenalis*	06. *Prevotella buccalis*
07. *Bacteroides cacae*	08. *Prevotella denticola*
09. *Streptococcus intermedius*	10. *Porphyromonas canoris*
11. Porphyromonas gingivalis	

	POSITIVE	NEGATIVE
NAPHTHYLAMIDE:		
K1083 ARG	01.	07.
K1086 CHY	11	10
K1305 GLY	06	08.
K1376 LGY	01.	02.
K1525 PAL	09.	05.
K1535 PRO	03.	04.
K1536 PYR	05.	09.
K1575 SER	09.	05
K1084 TRY	10	11

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

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