GLYCOSIDASE 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 glycosidase and naphthylamide tablets provide an easy to setup, inexpensive way to detect these enzymes.

Glycosidase: When bound to nitrophenol, the hydrolysis of the colorless aryl-substituted glycoside or phospoester releases the nitrophenol base with its yellow color. The most common test using this principle is o-Nitrophenol-ß-d-galactopyranoside (ONPG) used for enterics and *Neisseria* species.

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:

p-Nitrophenol-N-acetyl ß-d-glucosaminide (NAG)
o-Nitrophenol-α-d-arabinopyranoside (αARA)
p-Nitrophenol-α-l-fucopyranoside (Aφυ)
p-Nitrophenol-ß-d-fucopyranoside (ßFU)
o-Nitrophenol-α-d-galactopyranoside (αGAL)
o-Nitrophenol-ß-d-galactopyranoside (ONPG)
p-Nitrophenol-α-d-glucopyranoside (αGLU)
p-Nitrophenol-ß-d-glucopyranoside (ßGLU)
o-Nitrophenol-α-d-mannopyranoside (αMAN)
p-Nitrophenol phosphate (alkaline phosphatase) (PO4)
o-Nitrophenol-ß-d-xylopyranoside (ßXYL)

MATERIAL SAFETY DATA:

None of the nitrophenol bases are known at this time to be hazardous. 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 water, pH 7.0-7.2
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 separately in 10 ml bottles and in Droppits.

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 34-37C for 2 hours. . Tests may be incubated up to 24 hours..

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

GLYCOSIDASE: No change at 2 hours is negative. The appearance of a yellow color at any time during the 2 hours is a positive glycosidase test. At 24 hours only very bright yellow is positive. PO4 tablets should only be considered positive at any time if very deep yellow. SEE **TIPS.**L TEST After reading the glycosidase results or after incubation of single naphthylamide test, 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)**

STORAGE:

Consult individual package label for storage instructions.

QUALITY CONTROL:

Each lot of tablets should be tested with known positive and negative organisms. Some suggested strains are listed. Dispose of all used material in a manner appropriate for biohazardous material.

Organism codes

01. *Arcanobacterium spp.*
02. *Bacteroides fragilis*
03. *Bacteroides ureolyticus*
04. *Corynebacterium spp.*
05. *Escherichia coli*
06. *Klebsiella pneumoniae*
07. *Bacteroides cacae*
08. *Prevotella denticola*
09. *Streptococcus intermedius*
10 Streptococcus constellatus

GLYCOSIDASE:	POSITIVE	NEGATIVE
K1046 αARA	07.	08.
K1235 αFUC	02.	03.
K1230 ßFUC	09.	10.
K1270 αGLU	02.	03.
K1275 ßGLU	02.	03.
K1442 αMAN	01.	04.
K1463 NAG	02.	03.
K1490 ONPG	02.	03.
K1045 PO4	02.	03.
K1692 ßXYL	06.	05.

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.

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