K9140 NGP

PRINCIPLE/DISCUSSION:

It is known that approximately 50% of all clinical isolates and 80% of gram-negative bacilli are from the family *Enterobacteriaceae*(1); the most common of which is *Escherichia coli*. Most *Enterobacteriaceae* can be recognized by their reactions to a few chemical compounds. Though each may be used alone for specific applications, NGP Wee-Tabs provide three tests which, combined with urease and OMPI Wee-Tabs, will identify approximately 95% of such organisms. The tablets detect the presence of enzymes which hydrolyze various chromogenic substrates and employ the following principles:

1. The primary test is p-nitrophenol N-acetyl-α d-glucosaminide (NAG). Organisms producing the necessary enzymes hydrolize this substrate, releasing the yellow nitro-phenol.

2. Enzymes acting on 4-methylumbelliferyl-ß-D-glucoside (ß-GLU) release the fluorescent methyumbelliferone which can be observed under a Wood's lamp.

3. The third test is proline aminopeptidase (PRO). Organisms containing the enzymes necessary for hydrolysis of the Proline-ß-naphthylamide release free ß-naphthylamide which is detected by the addition of amino-peptidase reagent.

MATERIAL SAFETY DATA:

K9140: NGP Wee-Tabs contain: NAG (p-nitrophenol-N acetyl-α-D glucosaminide), ß-GLU (4-methylumbelliferyl-ß-D-glucoside), PRO (proline-ß-naphthylamide), and other inert ingredients necessary for tabletting. None of the substrates are harmful in this form.

MATERIALS REQUIRED:

NGP tablets are sold ready to use, 28 tubes per bottle. Usage requires 24 hour growth on media appropriate for the specimen. Consult a current reference manual for the correct media to use. The following items are also required but not provided:

Microbiological loop or needle

Purified water, pH 6.5 - 7.5

Long-wave fluorescent light KS1699 or equal

K2375 PEP reagent

PROCEDURE:

1) Add 0.3-0.5 ml (about 5-8 drops) of water to the tube.

2) Inoculate the tube with a single colony from a primary agar plate (eg. Blood agar or MacConkey, etc). Mix well.

OPTIONAL: To read at 2 hours or for brighter results, inoculate with 10-15 colonies.

3) Incubate @ 35-37C for 4 hours. Though finished at 4 hours, tests may be held for up to 28 hours. Do not read after more than 28 hours as false positives may occur.

INTERPRETATION:

1) **NAG:** Observe for a yellow color indicating a positive test. Colorless is negative.

2) **ß-GLU**: Observe the tube for fluorescence,

using a long-wave ultra-violet light. A positive 4-MU shows a **bright blue** fluorescence. **IMPORTANT-pale** fluorescence should be considered negative if PYR is positive. Pale fluorescence is only considered as a positive test when the PYR is negative.

3) **PRO:** Add 2 drops of PEP to the tube and reincubate for 15 minutes. A bright pink or red color indicates a positive test. After all tests are completed, refer to the chart.

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NAME	TESTS USING COMBINATION TEST TABLETS OR DISCS								CONFIRMATION	
	ONPG	MUG	INDOLE	IPA	PYR	NAG	BGLU	PRO	UREA	LACTOSE
Cedecea sp.	+	-	-	-	-	+	+	-	-	V-
Citrobacter sp.	+	-	+(m)	-	+	-	+(m)	-	-	V
Edwardsiella sp.	-	-	+	-	-	+	-	+	-	-
Enterobacter sp.	+	-	-	V-	V+	V+	+	-	V-	V+
Escherichia coli	+	+(n)	+	-	-	-	-	-	-	+
Escherichia sp.	+	-	+	-	+	-	V-	-	-	+
Hafnia alvei	-	-	-	-	-	V+	-	+	-	-
Klebsiella sp.	+(a)	-	-(b)	-	+	-(c)	+	-	+(d)	V
Kluyvera sp.	+	-	+	-	-	-	+	-	-	+
Leminorella sp.	-	-	-	-	-	-(e)	-	-	-	-
Morganella morgani	-	-	+	+	-	-	-	-	+	-
Proteus sp.	-	-	-(f)	+	-	-	-	-	+	-
Providencia sp.	-	-	+	+	-	-(g)	-	-	V+	-
Rahnella sp.	+	-	-	+	+	-	+	+	-	+
Salmonella sp.	-(h)	V	-	-	-	-	-	-	-	-
Serratia sp.	+	-	-	-	+	+	+	+	+	-(i)
Shigella sonnei	+	+	-	-	-	-	-	-	-	-
Shigella sp.	-	V	V	-	-	-	-	-	-	-
Yersinia sp.	V	-	V	V-		V_	+(j)	-(k)	-	-

REFERENCES/FOOTNOTES:

1) Manual of Clinical Microbiology, 5th Edition, Chapter 36

a) K. rhinoscleromatis is negative.
b) K. oxytoca and K. ornithinolytica are positive.
c) K. ornithinolytica is positive.
d) K. ozaenae and K. rhinoscleromatis are negative.
e) Lem. grimonti is positive.
f) P. vulgaris may be weakly positive.
g) Only Prov. stuarti is positive.
h) Salmonella arizonia is positive.
i) Ser. rubidea is usually positive.
j) Yer. enterocolitica is negative.
k) Yer enterocolitica is positive.
m) C. freundi is negative.
n) E. coli 0157 is MUG negative.