

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-a d-glucosaminide (NAG). Organisms producing the necessary enzymes hydrolyze this substrate, releasing the yellow nitro-phenol.

2. Enzymes acting on 4-methylumbelliferyl-β-D-glucoside (β-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-β-naphthylamide release free β-naphthylamide which is detected by the addition of aminopeptidase reagent.

MATERIAL SAFETY DATA:

K9140: NGP Wee-Tabs contain: NAG (p-nitrophenol-N acetyl-a-D glucosaminide), β-GLU (4-methylumbelliferyl-β-D-glucoside), PRO (proline-β-naphthylamide), and other inert ingredients necessary for tableting. 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 K51699 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) **β-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.

- 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. stuartii* is positive.
 - h) *Salmonella arizonia* is positive.
 - i) *Ser. rubidea* is usually positive.
 - j) *Yer. enterocolitica* is positive.
 - k) *Yer. enterocolitica* is negative.
 - m) *C. freundii* is negative.
 - n) *E. coli* 0157 is **MUG negative**.



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