K9101 MOT DISCS

PRINCIPLES/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. M.O.T. discs provide 3 tests (ONPG, Indole, and MUG) needed to identify many such organisms. The others require further testing.

- **ONPG:** Fermentation of lactose depends on the presence of two enzymes: permease, which allows the lactose to enter the bacterial cell, and ß-galactosidase, which splits lactose into glucose and galactose, which are subsequently metabolized. Organisms which ferment lactose slowly are deficient in permease. The demonstration of beta-galactosidase is accomplished by the hydrolysis of o-nitrophenol-ß-D-galactopyranoside liberating o-nitrophenol with its characteristic yellow color.
- **MUG:** Most *Escherichia coli* have the enzyme ß-glucuronidase. This enzyme reacts with 4-methylumbelliferyl-ß-D- glucuronide and releases 4-methylumbelliferone which is fluorescent under long-wave ultraviolet light. Rare strains of *Salmonella*, *Shigella*, and *Yersinia* may also possess the enzyme but these organisms are easily differentiated by their inability to ferment lactose and their indole reactions.
- **Indole:** The oxidation of tryptophane forms indole which is indicated by the formation of a red ring after addition of Kovac's reagent.

MATERIALS REQUIRED:

M.O.T. discs are sold 50 per bottle with 1 vial of Kovac's reagent provided. Usage requires 24 hour growth on Blood agar or MacConkey plates. The following items are also required but not provided:

- Non-fluorescent test tubes
- Microbiology loop or needle
- Sterile distilled or purified water
- Long-wave fluorescent light KS1699 or equal

PROCEDURE:

1) Place one M.O.T. disc into a non-fluorescing tube and add 2-3 drops of sterile water.
2) Inoculate the tube with growth from a Blood agar or MacConkey plate. A single colony is sufficient, but a denser suspension will react faster. Mix well by using the loop to move the disc around in the tube.
3) Incubate for 2-4 hours. The test will begin to dry and be rendered invalid after 4 hours. Positive MUG and ONPG can be read in as little as one hour but you must hold negatives for the full four hours.

INTERPRETATION OF RESULTS:

1) **ONPG:** Observe for a yellow color indicating a positive test.
2) **MUG**: Observe the tube for fluorescence, using a long-wave uv light. A positive MUG shows a **bright** blue fluorescence and indicates *Escherichia coli*. Some organisms and/or test tubes fluoresce so disregard other colors or pale fluorescence. If in doubt, compare the fluorescence of the completed test to an identical tube of inoculated water only.
3) **Indole**: After observing the other tests, add 1-2 drops of Kovac's reagent to the tube. For a positive test, a red ring will form at the surface of the liquid within 5 minutes.

After tests are completed, refer to the chart for identification. Only E. coli is positive for all 3 tests. Negative results on any of the three tests require further testing to identify the organism. Other rapid tests are included in the chart for your convenience, and are available from Key Scientific.

	MOT TEST				CONFIRMATION					
NAME	ONPG	MUG	IND	PPA	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)	–	–

a) *K. rhinoscleromatis* is negative.
b) *K. oxytoca* and *K. ornithinolytica* are positive.
c) *K. ornithinolytica* is positive.
d) *K. ozaenae* and *K. rhinosclermatis* 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.

REFERENCES/FOOTNOTES:

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

For quick *E. coli* confirmation (from Blood Agar only) use only 1 drop of water and harvest several colonies. Mix well then incubate 15 minutes.

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