IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

M.O.T. DISCS

Cat. no. K9101	50 discs/bottle

PRINCIPLES/DISCUSSION

It is known that approximately 50% of all clinical isolates and 80% of gram-negative bacilli are from the family *Enterobacteriaceae*, the most common of which is *Escherichia coli*.[1] Most*Enterobacteriaceae* can be recognized by their reactions to a few chemical compounds. M.O.T. discs provide three tests (ONPG, Indole and MUG) needed to identify approximately 80% of 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 beta-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 ortho-nitrophenol-beta-D-galacto-pyranoside liberating ortho-nitrophenol with its characteristic yellow color.

MUG: Most *Escherichia coli* have the enzyme b-glucuronidase. This enzyme reacts with 4-methylumbelliferyl-beta-D-glucuronide and releases 4-methylumbelliferone which is fluorescent under long-wave ultra-violet light. Rare strains of *Salmonella*, *Shigella*, and *Yersinia* may also possess the enzyme but these organisms are easily differentiated by their lack of ability to ferment lactose.

Indole: The oxidation of tryptophane forms indole which is indicated by the formation of a red ring in the supernatant layer after the addition of Kovac's reagent.

ACTIVE INGREDIENTS/MSDS

Each disc is impregnated with a solution of 4-methylumbelliferyl-beta-D-Glucuronide, tryptophane, and ONPG in water. None of these are known at this time to be hazardous.

MATERIALS PROVIDED

M.O.T. discs are sold 50 per bottle. Consult a current reference manual for the correct media to use.

MATERIALS REQUIRED BUT NOT PROVIDED

- 24 hour growth on media
- Small non-fluorescent test tubes
- Microbiology loop or needle
- Distilled water, neutral pH
- Long-wave fluorescent light

Standard microbiological supplies and equipment such as loops, other culture media, swabs, UV lamps, applicator sticks, incinerators, handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with

compatible UV lamp (Cat. no. EA160), and incubators, etc., as well as serological and biochemical reagents, are not provided. A long-wave, 365 nm, UV light source is not provided.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PROCEDURE

1. Place one M.O.T. disc into a small non-fluorescing tube and add 2-3 drops of sterile water.

2. Inoculate the tube with growth from a Blood Agar (Cat. no. A10) or MacConkey (Cat. no. G35) plate. Asingle 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 two-four hours. The test will begin to dry and be rendered invalid after four hours. ONPG can be read at one hour is a positive is easy to see. However, results are brighter and easier to read after longer incubation. Negatives must be held 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 ultra-violet light. A positive MUG shows a **bright** blue fluorescence and indicates *Escherichia coli*. Some 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 one-two drops of Kovac's reagent to the tube. For a positive test, a red ring will form at the surface of the liquid within five minutes.

After tests are completed, refer to the chart for identification. Only *E. coli* is positive for all three tests. Negative results on any of the three tests may require futher testing to identify the organism.

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect broth fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect broth fluorescence. See 'CAUTION' above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may

be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over tubes, approximately 3 to 4 inches (7 to 10cm) away.
3. MUG positive cultures will fluoresce bright blue. See Interpretation section below..
4. Fluorescence will fade over time.

Organism	**MOT / OMPI**			
	ONPG	MUG	IND	PYR
Cedecea spp.	+	-	-	-
Citrobacter spp.	+	-	+ [e]	+
Edwardsiella spp.	-	-	+	-
Escherichia coli	+	+ [f]	+	-
Escherichia spp.	+	-	+	+
Hafnia alvei	-	-	-	-
Klebsiella spp.	+ [a]	-	- [b]	+
Kluyvera spp.	+	-	+	-
Leminorella spp.	-	-	-	-
Morganella morganii	-	-	+	-
Proteus spp.	-	-	+ [c]	-
Providencia spp.	-	-	+	-
Salmonella spp.	- [d]	V	-	-
Serratia spp.	+	-	-	+
Shigella sonnei	+	+	-	-
Shigella spp.	-	V	V	-
Yersinia spp.	V	-	-	+

a	*Klebsiella rhinoscleromatis* is negative.
b	*Klebsiella oxytoca* and *Klebsiella ornithinolytica* are positive.
c	*Proteus vulgaris* may be weakly positive.
d	*Salmonella arizonia* is positive.
e	*Citrobacter freundii* is negative.
f	*Escherichia coli* O157 is MUG negative.

LIMITATIONS

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

Showing Positive ONPG Reaction
Escherichia coli (ATCC® 25922) suspension with M.O.T. Disc (Cat. no. K9101). Incubated aerobically for 1 hour at 35ºC. The yellow color development was indicative of a positive reaction (ONPG positive). *E. coli* was transfered from Blood Agar (Cat. no. A10) after an aerobic incubation at 35ºC. for 24 hours.

Showing Negative ONPG Reaction
Proteus mirabilis (ATCC® 12453) suspension with M.O.T. Disc (Cat. no. K9101). Incubated aerobically for 1 hour at 35ºC. No yellow color development was indicative of a negative reaction (ONPG negative). *P. mirabilis* was transfered from Blood Agar (Cat. no. A10) after an aerobic incubation at 35ºC. for 24 hours.

Left: Positive MUG Reaction; *Escherichia coli* (ATCC® 25922) suspension with M.O.T. Disc (Cat. no. K9101).
Right: Negative MUG Reaction; *Proteus mirabilis* (ATCC® 12453) suspension with M.O.T. Disc (Cat. no. K9101).

Organisms were incubated aerobically for 1 hour at 35ºC. Shown under long-wave UV light. Fluorescence is indicative a positive MUG test. Organisms were transfered from Blood Agar (Cat. no. A10) after an aerobic incubation at 35ºC. for 24 hours.

Showing Positive Indole Reaction
Escherichia coli (ATCC® 25922) suspension with M.O.T. Disc (Cat. no. K9101). Incubated aerobically for 1 hour at 35ºC. Red color development after addition of five drops Kovacs Indole Reagent (included) was indicative of a positive indole reaction. *E. coli* was transfered from Blood Agar (Cat. no. A10) after an aerobic incubation at 35ºC. for 24 hours.

Showing Negative Indole Reaction
Proteus mirabilis (ATCC® 12453) suspension with M.O.T. Disc (Cat. no. K9101). Incubated aerobically for 1 hour at 35ºC. No red color development after addition of five drops Kovacs Indole Reagent (included) was indicative of a negative indole reaction. *P. mirabilis* was transfered from Blood Agar (Cat. no. A10) after an aerobic incubation at 35ºC. for 24 hours.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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