

Coli.ID

Catalog Number: K1539B

INTENDED USE:

Coli.ID is designed to aid in the rapid identification of *E. coli* from positive urine specimens and other clinical sites. Each disc will perform the **MUG**, **Indole** and **PYR** tests. Multiple samples may be applied to a single disc.

PRINCIPAL / DISCUSSION:

MUG: The enzyme B-glucuronidase hydrolyzes 4-methylumbelliferyl-B-D-glucuronide to 4-methylumbelliferone, which exhibits a bluish fluorescence under a 360-365 nm long wave UV light source (Woods lamp). Each disc contains approximately 10 ug of the substrate. The relationship of B-glucuronidase production with *E. coli* has been shown to be 97% positive. All other Escherichia species are MUG negative, including *E. coli* 0157:H7.

PYR: Enzymatic hydrolysis of the substrate pyrrolydonyl-B-naphtylamide releases free B-naphtylamine which is detected by the addition of PEP reagent. A positive PYR reaction is pink to red.

INDOLE: Indole is formed in bacterial cultures by the oxidation of one molecule of tryptophane to one molecule of indole. The color reaction is a coupling of one aromatic nucleus to another to give a colored byproduct. Key PEP Reagent (aka Cinnamaldehyde or Spot Indole) contains p-dimethyl-amino-cinnamaldehyde in weak hydrochloric acid. Indole reactions of organisms grown on any media containing tryptophane may interfere but will still produce predictable reactions. From such media, on positive PYR reactions, a negative indole reaction will range from dark pink or red. The development of a purple, green, or turquoise color indicates a positive indole reaction. A yellow color indicates a negative indole reaction.

MATERIALS REQUIRED:

Discs are provided 50 per container including reagent. This test requires a pure 24-48 hour culture of the organism on a non-fluorescing, tryptophan containing media such as TSA or blood agar. Items required but not provided: Purified water, pH 6.5 to 7.2; Microscope slide; Loop or sterile stick for harvesting; Woods lamp or equivalent.

LIMITATIONS:

Test discs are light sensitive. Discs may be used cold. Do not freeze reagent. Use only non-fluorescing, tryptophan containing media such as TSA or blood agar. Do not use MacConkey or EMB.

STORAGE:

Store discs and reagent tightly sealed in the original container in the dark at 2-8°C.

PROCEDURE:

- Place a disc onto a slide and moisten slightly. DO NOT use excessive water. The disc should be only wet enough to hold it in place on the slide.
- Smear a paste of the test organism onto the moistened paper disc.
- Incubate the inoculated disc in the dark at 35-37°C for 5 minutes.
- Observe the disc in a dark room under a long wave UV light for blue fluorescence.

INTERPRETATION: Blue fluorescence = positive MUG & No fluorescence = negative MUG.

- Immediately add 1 drop of PEP Reagent to the disc and wait at least 1 minute and no more than 2 minutes for color development.

INTERPRETATION: Colorless = negative; Blue/Green or Turquoise = Indole positive; Red/pink = PYR positive; Purple = BOTH Indole and PYR positive.

QUALITY CONTROL:

Each lot of Key **COLI.ID** should be checked with known positive and negative organisms. We recommend *E. coli* ATCC 25922 and *E. aerogenes* ATCC 13048 which will react as listed in the table below.

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

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