

K9102 EC SPOT TESTS

DISCUSSION:

EC SPOT TESTS (XGAL/MUG discs) are useful in the speciation of *Staphylococcus* as well as a confirmatory spot test for *Escherichia coli*. Among the Enterobacteriaceae, only this organism is positive for these two tests and indole. The discs detect the presence of enzymes which hydrolyze various chromogenic substrates:

1. The primary test is beta galactosidase (same as ONPG) in the form of XGAL. Organisms containing the enzyme hydrolyze this substrate, releasing a blue color.
2. Enzymes acting on the second test (MUG) release fluorescent methylumbelliferone (4MU) which can be observed under a Wood's lamp.
3. The end product of the deamination of tryptophan is indole which will react with an aldehyde reagent to form a colored end product. Members of the *Providencia* and *Proteus* species will also oxidize tryptophan to indole-pyruvic acid. Tryptophan has not been added to the disc because TSA based blood agar contains the tryptophan necessary for spot tests. The indole can also be read directly from the plate when taken from this source. Spot Indole reagent has been included for this purpose.

MATERIAL SAFETY DATA:

K9102 discs contain 5-bromo-4-chloro-3-indolyl-beta-d-galactosidase and MUG (4-methyl-umbelliferyl-β-D-glucuronide). Neither of the ingredients is hazardous in this form. No special handling precautions are required for using the product, however, as with any laboratory test, good laboratory practices should be observed.

MATERIAL REQUIRED:

Discs are packed 50 per bottle with spot indole (PEP) provided. Usage requires 24 hour growth. Consult a current reference manual for the correct media to use. Each lot of discs should be checked for performance prior to use. We suggest *E. coli*, ATCC# 25922 (all positive) and *Proteus mirabilis* ATCC #43071 (all negative). The following items are also required but not provided:

- Microbiology loop or needle
- Distilled water, neutral pH
- Long-wave fluorescent light KS1699 or equal

PROCEDURE: (From 5% or 10% blood agar plates)

- 1) Place the disc into a small non-fluorescent test tube.
- 2) Add 2 drops of neutral pH water.
- 3) Incubate @32-38°C 15 minutes.

INTERPRETATION:

Read in the exact order listed.

- 1) Observe for a blue color indicating a positive test for XGAL. Colorless is negative.
- 2) Observe the disc or tube for fluorescence, using a long-wave ultra-violet light (~360nm). A positive 4-MU shows a bright blue fluorescence. **Note:** A "pink" fluorescence, which is not positive, may occur for colonies taken from MacConkey. Look for only blue-white fluorescence.
- 3) If the test is done from TSA + blood, perform the indole test by adding 1 drop of PEP (spot indole) reagent directly to the tube or disc. Observe between 1 and 2 minutes (no longer) for a blue or purple positive. (XGAL positive organisms, such as *E. Coli* will be a deep magenta from the mixing of the 2 reactions.) XGAL negative, indole positive organism may be blue/green however, red or yellow is negative Indole. Note that Indole-pyruvic acid positive organisms (*Proteus*, *Morganella*, *Providencia* or *Klebsiella* sp.) may also turn a purple color with this reagent. The indole test is easiest to interpret when done on a separate piece of filter paper to eliminate the XGAL cross reaction.

SPECIES	TEST		
	XGal	MUG	IND
<i>Cedecea sp.</i>	+	-	-
<i>Citrobacter sp.</i>	+	-	+a
<i>Edwardsiella sp.</i>	-	-	+
<i>Enterobacter sp.</i>	+	-	-
<i>Escherichia coli</i>	+	+b	+
<i>Escherichia sp.</i>	+	-	+
<i>Hafnia alvei</i>	-	-	-
<i>Klebsiella sp.</i>	+c	-	-d
<i>Kluyvera sp.</i>	+	-	+
<i>Leminorella sp.</i>	-	-	-
<i>Morganella morg.</i>	-	-	+
<i>Proteus sp.</i>	-	-	-e
<i>Providencia sp.</i>	-	-	+
<i>Rahnella sp.</i>	+	-	-
<i>Salmonella sp.</i>	-f	V	-
<i>Serratia sp.</i>	+	-	-
<i>Shigella sonnei</i>	+	+	-
<i>Shigella sp.</i>	-	V	V
<i>Yersinia sp.</i>	V	-	V

- a) *C. freundii* is negative.
- b) *E. coli* 0157 is MUG negative.
- c) *K. rhinoscleromatis* is negative.
- d) *K. oxytoca* and *K. ornithinolytica* are positive.
- e) *P. vulgaris* may be weakly positive.
- f) *Salmonella arizonia* is positive.



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