K1139 STREPTOCOCCUS BOVIS SCREEN

PRINCIPLES/DISCUSSION:

The association of *Streptococcus bovis* (Biotype 1) with occurance of colonic cancers has been recognized for some time. Because of this connection it is increasingly important to correctly and quickly identify *Streptococcus bovis* in fecal samples. Key Scientific has combined a series of tests which will rapidly eliminate other similar organisms and leave *Streptococcus bovis* (Biotype 1) as the only likely identification.

Bacteria produce enzymes which hydrolyze various chromogenic and fluorescent substrates. This hydrolysis will produce a color reaction, some apparent immediately and others by the use of special equipment or the addition of color developers. The S-B screen makes use of these principles and, by combining non-interferring substrates, produces three reactions in one product, thus reducing the cost per test. The first test is p-nitrophenol ß-d-glucoside which is the substrate for esculin. Second is 4-MU-a-d-galactoside. The last test is pyrolidonyl aminopeptidase, frequently referred to as PYR.

MSDS/QUALITY CONTROL:

Each tablet consists of approximately 0.05 mgs. each of the applicable substrates with inert fillers and tableting compounds. None of the ingredients is hazardous in this form. Used tests should be disposed of in a manner suitable for biohazardous material. Test each lot number with organisms of known reactivity. See chart below for suggested strains.

MATERIALS REQUIRED:

S-B Screen is sold ready to use in test tubes, 26 tubes per bottle, with 1 vial of reagent. Usage requires 24 hour growth on media appropriate to the specimen. Consult a current reference manual for the correct media to use. The following items are also required but not provided:

Microbiology loop, needle, or sterile swab for harvesting
Distilled water, neutral pH
Wood's lamp KS1699 or equal

PROCEDURE:

Note: this test is intended for use with specimens that are known to be non-beta-hemolytic, catalase negative, Gram positive cocci in pairs or chains. Any other organisms will give erroneous conclusions.

1) Add at least 0.25 ml but not more than 0.5 ml (about 5 drops) of distilled water to the tube containing the tablet.

2) Inoculate heavily from a pure 24 hour culture. Use a visible paste of organism. The heavier the inoculum the brighter and faster the reaction: Mix well by using the loop to move the tablet around in the tube. It is not necessary for the tablet to dissolve.

3) Incubate for 2-4 hours @35-37C. If test cannot be read within 4 hours, incubate in the dark at 15-20C room temperature for 8 or more hours. Do not read after more than 24 hours as false positives may occur.

RESULTS:

(Read in the exact sequence listed, recording results, then refer to interpretation.)

1) **Nitro-phenol:** Observe for a yellow color indicating a positive test. Colorless is negative.

2) **4-MU**: Observe the tube for fluorescence by holding **under** a long-wave (360nm) ultra-violet light. Holding the light at any other angle could cause interference with true positive reactions. A positive 4-MU shows a **bright** bluish white fluorescence. Since some organisms tend to have a slight flourescence of their own, if pale fluorescence or fluorescence of a different color is noted, it is best to compare with a known positive as a control. **Disregard** pale fluorescence when the aminopeptidase reaction is positive; this pale fluorescence is related to the aminopeptidase reaction only.

3) **AMINOPEPTIDASE:** Perform the aminopeptidase test last by adding 2 drops of PEP reagent directly to the tube and reincubating for 2-5 minutes. Ambiguous results of the aminopeptidase may be intensified by vortexing a few seconds after addition of the reagent. Red or purple is positive. Green, turquoise, or yellow is negative. Brightest and easiest to read reactions are done by dipping a swab into the tube then dropping 1 drop of reagent onto the swab. Reaction will develop within 1 minute

INTERPRETATION:

The first result to consider is the PYR (aminopeptidase) test. Positive PYR specimens are not *Streptococcus bovis* (Biotype 1) and need no further consideration in this screen. These organisms require additional testing for identification. For PYR negative organisms, you should then look at the Esculin (p-nitrophenyl beta-glucosidase) results. Esculin negative organisms are not *Streptococcus bovis* (Biotype 1) and, as before, should be tested further. Esculin positive specimens are then reviewed for the last test, 4-MU a-galactosidase. *Streptococcus bovis* (Biotype 1) are positive for this test, while most viridans group streptococci are negative. The only organism which remains to cloud this picture is *Streptococcus salivarius*. This can easily be separated from *Streptococcus bovis* with an LAP test, (available from Key Scientific, K1378B) for which *Streptococcus bovis* is positive and *Streptococcus salivarius* is negative.

REFERENCES/FOOTNOTES:

(1) Manual of Clinical Microbiology, Fifth Edition

(2) Ruoff, Kathryn L., Samuel I. Miller, Carol V. Garner, Mary Jane Ferraro, and Stephen B. Calderwood. Bacteremia with Streptococcus bovis and Streptococcus salivarius: Clinical Correlates of More Accurate Identification of Isolates. Journal of Clinical Microbiology, Feb. 1989. p 305-308.

(3) Littel, Kenneth J. and Paul A. Hartman. Fluorogenic Selective and Differential Medium for Isolation of Fecal Streptococci. Applied and Environmental Microbiology, Feb. 1983. p. 622-627.

	ESC(2)	a-GAL(3)	PYR(1)
Streptococcus bovis ATCC 9809	+	+	-
Enterococcus faecalis ATCC 29212	+	-	+
Staphylococcus aureus ATCC 25923	-	V	-

( ) Numbers in parentheses indicate the order of consideration for interpretation of the test.

KEY SCIENTIFIC PRODUCTS, INC
1113 EAST REYNOLDS STREET
STAMFORD, TEXAS 79553
VOICE 800-843-1539
FAX 888-440-4208
WWW.KEYSCIENTIFIC.COM