K9760 CLOSTRIDIUM SCREEN

Some bacteria produce enzymes which hydrolyze various chromogenic substrates. When these substrates are bound to another base, the hydrolysis will produce a color reaction, some apparent immediately and others by the use of special equipment or the addition of color developers. ThIs screen makes use of these principles and, by combining non-interferring substrates, produce two chromogenic reactions in one product, reducing the cost per test.

1. The primary tests are nitro-phenol bound substrates. Organisms containing the necessary enzymes hydrolyze these substrates, releasing the yellow nitro-phenol which is visible without addition of any reagent. The tablet contains O-Nitrophenol-ß-d-galactopyranoside (ONPG) and the disc contains p-Nitrophenol phosphate (alkaline phosphatase) (PO4)

2. The second tests are aminopeptidase tests. Organisms containing the enzymes necessary for hydrolysis of the applicable arylamide release free beta-naphthylamide which is detected by the addition of aminopeptidase (PEP) reagent. The tablet contains L-pyroglutamic acid α-naphthylamide (PYR) and the disc contains L-proline α-naphthylamide (PRO) .

MATERIAL SAFETY DATA:

Each tablet consists of approximately 0.5 mgs. of the applicable substrate with inert fillers and tableting compounds. Each disc consists of approximately 0.5 mgs. of the applicable substrate in an inorganic solvent. None of the ingredients is hazardous in this form.

MATERIALS REQUIRED:

Each screen is sold ready to use in test tubes, 25 per pack. Usage requires 24-48 hour growth on appropriate media. The following items are also required but not provided:

Microbiology loop, needle, or sterile swab for harvesting
Distilled or purified water
K2375 PEP reagent sold separately.

PROCEDURE:

Caution: This test is intended for use with organisms which are anaerobic Gram positive spore forming rods. Testing any other type of organism may lead to erroneous conclusions.

1) Add at least 0.25 ml but not more than 0.5 ml (about 5 drops) of water to the tube containing the tablet and 2-3 drops of water to the tube containing the disc.

2) Inoculate heavily from fresh growth on a plate or slant. Mix well by using the loop to move the tablet or disc around in the tube. It is not necessary for the tablet to dissolve.

3) Incubate aerobically at 35-37c for 2 hours.

INTERPRETATION:

(Read and interpret in the exact sequence listed)

1) **Nitro-phenol tests:** Observe for a yellow color indicating a positive test. Colorless is negative. Inconclusive reactions may be enhanced by adding 1 drop of 1% KOH solution.

2) **Aminopeptidase:** After recording both nitro-phenol tests, perform the aminopeptidase tests by adding 2 drops of PEP reagent directly to each well in the tube and reincubating for 15 minutes. Ambiquous 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.

3) If the tests for ONPG, PYR, and PO4 are all positive, and the test for PROLINE is negative, you may make a presumptive identification of *Clostridium perfringens*. If the first three are negative, and the PROLINE is positive, the specimen is presumptively *Clostridium difficile*. Any other combinations of positive and negative will require further testing for identification.

QUALITY CONTROL:

Each lot of tablets should be tested with known positive and negative organisms. Key suggests *Clostridium perfringes* ATCC 13124 positive for ONPG, PYR, and PO4 and *Clostridium difficile* ATCC 9689 positive for PRO. Each organism serves as negative control for the tests for which it is not positive Dispose of all used material in a manner appropriate for biohazardous material.

REFERENCES:

(1) Manual of Clinical Microbiology, Fifth Edition

(2) Kilian, M and Bulow, P. 1976. Rapid Diagnosis of Enterobacteriacae, Acta path. microbio. Scan, Sect B, 84:245-251

(3) Wadsworth Anaerobic Bacteriology Manual, 5th Edition, 1993, Glucosidase tests, page 152.

KEY SCIENTIFIC PRODUCTS
1113 EAST REYNOLDS ST.
STAMFORD, TEXAS 79553
WWW.KEYSCIENTIFIC.COM
Voice 800-843-1539
Fax 888-440-4208