K1202 C.E.A.L. TEST

DISCUSSION:
Although they are not phylogenetically related, *Erysipelothrix*, *Arcanobacterium*, and bacteria belonging to the genus *Listeria* are physiologically very similiar to *Corynebacterium* and are frequently misidentified as such. Traditional methods of separating these organisms can take 24 to 48 hours and the rare media required makes the cost high. These organisms have been shown to have the ability to produce enzymes which hydrolyze various chromogenic and fluorogenic substrates (1). The detection of these enzymes is possible in a much shorter time frame allowing rapid separation of the organisms. One CEAL tablet contains 3 tests to separate these organisms to their correct genus in a single tube: p-nitrophenol ß-d-mannopyranoside, 4 MU ß-d-fucopyranoside, and proline ß-naphthylamide. All *Listeria spp*. produce the enzyme mannosidase while the only *Corynebacterium* to do so is *Corynebacterium aquaticum*. (*Listeria spp.* can be further separated from this organism by BANA (trypsin)* which is sold separately.) The demonstration of this enzyme activity is readily visible by a color change to yellow when the ortho-nitrophenol has been liberated. The hydrolysis of 4MU-ß-d-fucopyranoside releases fluorescent blue methyumbelliferone which is visible under a long-wave UV light (Wood's Lamp). *Corynebacterium spp*. do not fluoresce while both *Arcanobacterium* and *Erysipelothrix* do. These two species are separated by proline ß-naphthylamide. The hydrolysis of proline by *Arcanobacterium spp.* releases free ß-naphthylamine thus differentiating it from *Erysipelothrix spp.* which will not hydrolize this substrate. The hydrolysis is detected and shown by a color change to red after adding PEP reagent.

MATERIAL SAFETY DATA:
Each of the tablets contain 0.05 mg. of the above substrates in a Sodium Chloride and Dicalcium phosphate base: None of these is hazardous in this application. Wipe up spills or discard inoculated product in a manner appropriate for microbiological hazard. Spills of uninoculated material may be cleaned with a paper towel and discarded as normal trash.

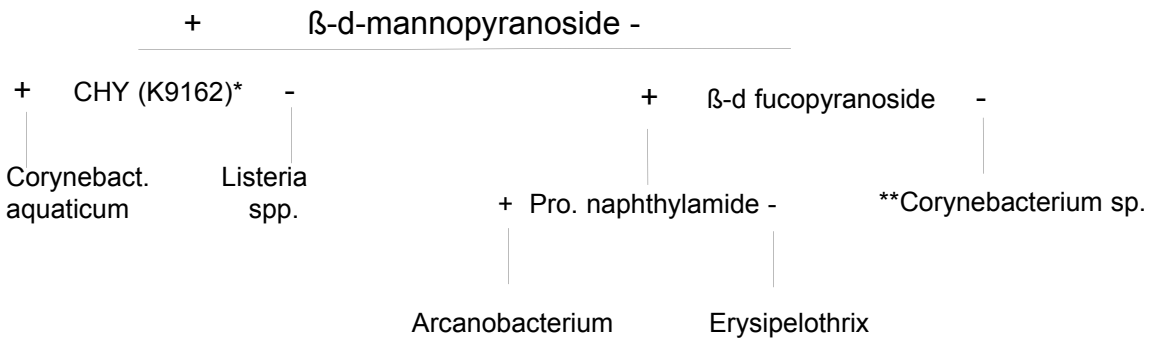
MATERIALS REQUIRED:
C.E.A.L. Tablets are sold 20 tubes per pack with PEP reagent provided. The tests require fresh 24 hour growth on culture media. Consult the Manual of Clinical Microbiology for recommended media for the specimen. The following items are required but not provided:

- Distilled water, pH 7.0 to 7.2
- Inoculating loop
- Wood's Lamp (KS1699 $40.00)

SETUP:
1) Label the tube then add 5 drops of water.
2) Inoculate heavily to achieve a turbidity of at least #3 McFarland then incubate uncovered for 4 hours at 32-37^{O} C.

INTERPRETATION:
Use the flow chart for identification.
(1) The appearance of a bright yellow color at any time during the 4 hours is a positive mannosidase test. No change at 4 hours is negative.
(2) Hold the tube under a Wood's lamp to observe for blue fluorescence in a positive test. No fluorescence is negative.
(3) After the first two reactions are recorded, add 3 drops of PEP reagent and reincubate 15 minutes. Any shade of pink or red is positive. Yellow is considered negative. Weak positives can be verified by observing under a Wood's lamp for a bright red/orange fluorescence.

REFERENCES:
(1) Kampfer, Peter. 1992. Differentiation of Corynebacterium Spp., Listeria spp., and related Organisms by Using Fluorogenic Substrates. J. Clin. Microbiol. 30:1067-1071



**2 strains have variable reactions on ß d fucopyranoside. These will be NAG (K1463) negative while Arcanobacterium and Erysipelothrix are NAG positive. K9162 and K1463 sold separately.

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