K310, K1310
HIPPURATE

DISCUSSION:

Key Hippurate tablets are used to test for sodium hippurate hydrolysis. Glycine, an end product of hippurate hydrolysis, reacts with ninhydrin to produce a dark blue color. This is valuable when testing *Streptococci* and *Gardnerella*. Most Group B streptococci will hydrolyze hippurate. Although some Group D enterococci also hydrolyze hippurate, they attack esculin and are rarely beta hemolytic.

MSDS:

Each tablet contains 4 mg. of sodium hippurate and inert fillers which are not known at this time to be hazardous. Ninhydrin reagent contains 50 mg. of ninhydrin in 0.4 ml. of solution with Dimethyl sulfoxide (DMSO). DMSO is harmful if inhaled, ingested, or absorbed through the skin, and may cause allergic reactions. In case of contact, flush eyes and skin with large amounts of water and remove any contaminated clothing. DMSO reacts violently with a large number of chemicals. Never add this reagent to anything except as recommended.

MATERIALS REQUIRED:

K310 is sold 50 tablets per package. K1310 is sold ready to use in tubes, 28 per package. Hippurate tablets require fresh 24 hour growth on media appropriate for the specimen. Consult a clinical microbiology manual for recommendations. The following items are required but not provided:

small test tubes (e.g. 12 x 75) (provided with K1310)
Inoculating Loop
Distilled water, pH 7.0-7.2
Concentrated Ninhydrin solution (Cat. # K475) or Hippurate developer (Cat. # K982311)

NINHYDRIN REAGENT:

Ninhydrin reagent K475 is sold separately. Prepare the Ninhydrin reagent by adding 0.4 ml. of the solution to 5 ml. of isopropyl alcohol. The diluted reagent has the same expiration date as the concentrate. Ninhydrin will stains hands and clothing so handle accordingly.

STORAGE:

Store tightly covered in a dry place at room temperature.

PROCEDURE:

(1)Place 0.4 ml. (about 10 drops) of distilled water in a small test tube. For K1310, simply add water to tablet in tube provided.

(2)Inoculate heavily with 8-10 isolated colonies, mixing well. The suspension should be milky and opaque. Avoid picking up agar with the loop.

(3) Add one Key Hippurate Tablet and incubate for at least 6 hours at 35-37^{o}C.

(4) After incubation, add 0.2 ml. of the diluted ninhydrin reagent and reincubate for 10 minutes. Observe for color change.

INTERPRETATION:

The appearance of an intense blue to blue/black color within 10 minutes is a positive test. A faint color should be disregarded. If results are inconclusive, the test must be redone with a longer incubation period. Reincubation of the already completed test will not give a more conclusive result.

QUALITY CONTROL:

Known positive (Group B strep ATCC 13813) and negative (Streptococcus pyogenes ATCC 19615) organisms should be run with each test group. Dispose of all used material in a manner appropriate for biohazardous material.

REFERENCES:

(1) Manual of Clinical Microbiology, 5th Edition, Chapter 122, “Reagents and Stains” and Chapter 48 “Gardnerella and other ill-defined Genera”. (2) Bailey and Scott’s Diagnostic Microbiology, Seventh Edition, Chapter 25 “Streptococci”.

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k310-0805