

# K710310 HIPPURATE DISCS

## **DISCUSSION:**

Key Hippurate discs are used to test for sodium hippurate hydrolysis. Glycine, an end product of hippurate hydrolysis, reacts with ninhydrin to produce a dark blue color. Hippurate is commonly used to presumptively identify *Gardnerella vaginalis*, *Listeria monocytogenes*, *Campylobacter jejuni* and Group B Streptococci.

## **MSDS:**

Each disc contains approximately 4 mg. of sodium hippurate which is not known 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:**

K710310 is sold 50 discs per package. Hippurate tests 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), Inoculating Loop, Distilled or Purified Water, 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 stains hands and clothing so handle accordingly.

## **STORAGE:**

Store tightly covered at 2-8C.

## **PROCEDURE:**

- (1) Drop 1 disc into a small test tube and add 4 drops of neutral pH water (6.5 to 7.2).
- (2) Inoculate heavily with 8-10 isolated colonies, mixing well. The suspension should be milky and opaque (McFarland #3 or greater). Avoid picking up agar with the loop.
- (3) Incubate for 2 hours at 35-37°C.
- (4) After incubation, add 1-2 of the diluted ninhydrin reagent and reincubate for 1-15 minutes. Observe for color change.

## **INTERPRETATION:**

The appearance of an intense blue to blue/black color within 15 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:**

Any known positive and negative organism may be used and should be run on each lot#. We use Group B strep ATCC 13813, (positive) and (*Streptococcus pyogenes* ATCC 19615, (negative).

## **DISPOSAL:**

Dispose of all used material in a manner appropriate for bio-hazardous 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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