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Section: Technical Manual	Subject Title: Hippurate Test	
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HIPPURATE TEST

Principle

This test determines the ability of bacteria to hydrolyse sodium hippurate. One of the end products, glycine is detected by the addition of ninhydrin reagent.

Reagents

Hippurate disks (store refrigerated)
Ninhydrin reagent
Sterile distilled water

Other Materials

Sterile tube (13 x 100mm)
Bacteriology loop
Sterile graduated pasteur pipette

Procedure

1. Place a Hippurate disk into a sterile tube and add 0.4 mL sterile water.
2. Heavily inoculate the tube with a loopful of the test organism.
3. Incubate at 35⁰C for 2 hours.
4. Following incubation add 5 drops of ninhydrin reagent to the tube and shake gently.
5. Reincubate tube for 10 minutes and read reaction.

Interpretation

Positive: Deep purple-blue colour

Negative: No colour change or light purple

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Precautions

1. A heavy inoculum is necessary to obtain a high concentration of enzyme.
2. Do not incubate longer than 30 minutes after addition of ninhydrin reagent because a false positive reaction could result.

Quality Control

Test with known positive and negative controls each time the test is preformed.

Positive: *Campylobacter jejuni* (ATCC 29428)

Negative: *Campylobacter coli* (CPI B7080)

Reference

1. Difco package insert.