

## Department of Microbiology Hippurate Hydrolysis Test Procedure

### I. Purpose and Test Principle

The hippurate test can be used in the identification of *Campylobacter jejuni*, *Gardnerella vaginalis*, *Listeria monocytogenes* and *Streptococcus agalactiae*, by detecting the ability of the organism to hydrolyze sodium hippurate to benzoic acid and glycine by the action of the enzyme hippuricase. This rapid test employs ninhydrin as the indicator, which reacts with any protein or amino acid and, in this case detecting glycine.

### II. Specimen Information

Only well-isolated colonies should be used for testing. Inoculum should be taken from 18-24 h growth. Use a 48 h culture if growth is poor at 24 h.

### III. Reagents & Equipment

- Hardy Diagnostics Hippurate Test Tubes & Ninhydrin Indicator Solution: Upon receipt, store reagents at 2-30° C. Products should not be used if there are any signs of contamination or deterioration, if the expiration date has passed, or if the rehydrated mixtures do not appear clear and colorless. Do not use if the tube is cracked or the powder is not free-flowing. Do not expose to excessive heat or moisture.
- Disposable sterile inoculation loops or wooden applicator sticks
- Transfer pipettes
- Test tube rack
- Distilled water
- Aerobic incubator set at 35 ± 2°C

### IV. Procedure

- A. To a Hippurate Test tube, add 0.2ml (3-4 drops) of distilled water at a pH of 6.8-7.2.
- B. Using a **heavy** inoculum (a heaping 1-uL loop is adequate) from an 18-48 h culture, make a heavy suspension of the organism in the Hippurate Reagent with a standard inoculating loop.
- C. Incubate the tube for 2 h at 35 ± 2°C.
- D. During the incubation period, reconstitute the Ninhydrin Indicator Solution in the dropper bottle by adding 2 mL of distilled water at a pH of 6.8-7.2. Replace the cap tip and cap, and vigorously shake for 1 min. Let stand at room temperature for 30 min or until all the substrate has dissolved.  
**Note:** Care must be taken when handling the Ninhydrin Indicator Solution. Spilling reagent onto skin or clothing leads to the development of a blue color that does not wash off but will wear off the skin in 24 to 48 h.
- E. After the 2 h incubation period, add 2 drops of the Ninhydrin Indicator Solution to the Hippurate Reagent and organism mixture.
- F. Reincubate at 35 ± 2°C for 30 min. Observe the tubes at 10 min intervals for the appearance of a deep blue color, which is a positive test. The color change will usually appear in 10 to 15 min after the Ninhydrin Indicator Solution has been added.

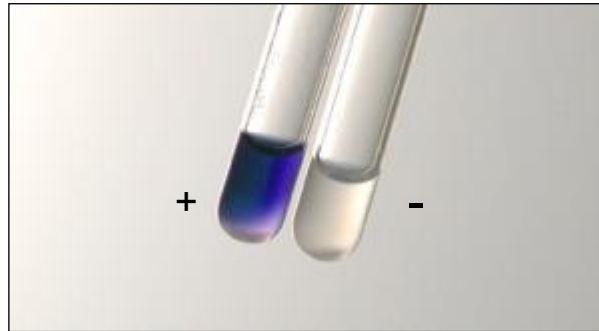
## V. Interpretation

### A. Positive Test

A positive test is indicated by the appearance of a deep blue color within 30 min after adding the ninhydrin.

### B. Negative Test

A negative reaction is indicated by a faint blue color change or no color change within 30 min after adding the ninhydrin.



## VI. Quality Control

Each new lot or shipment should be examined for product deterioration and tested with the following control strains.

<b>Control strain</b>	<b>Expected Results</b>
<i>Streptococcus agalactiae</i> ATCC 13813	Positive: dark blue color
<i>Streptococcus pyogenes</i> ATCC 19615	Negative: no color change

## VII. Limitations

- This test is part of an overall scheme of identification. Further biochemical testing may be necessary for definitive identification.
- Insufficient inoculum may result in erroneous results.
- False-positive results can occur if incubation with ninhydrin exceeds 30 min.
- When *Streptococcus* species are tested for their ability to hydrolyze hippurate, it must be remembered that not all group B streptococci are beta-hemolytic. In addition, a small number of group D streptococci are beta-hemolytic, and some hydrolyze hippurate as well.

## VIII. Verification of Test Method

This test was evaluated using a total of eight test strains. This included *Campylobacter jejuni* ATCC 33291 and a clinical *C. jejuni* isolate, *Gardnerella vaginalis* ATCC 14017 and ATCC 49145, two clinical strains of *Listeria monocytogenes*, *Streptococcus agalactiae* ATCC 13813, and *Streptococcus pyogenes* ATCC 19615. Except for the *S. pyogenes*, all test strains produced the expected positive hippurate results. The *S. pyogenes* strain did not produce a color change.

## **IX. References**

- A. Hardy Diagnostics online technical data: [Hippurate Test](#) (package insert version 120908md).
- B. Clinical Microbiology Procedures Handbook, 3<sup>rd</sup> ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

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Updates and Revisions: