

Hippurate Hydrolysis

Intended Use:

Aids in the differentiation of β -hemolytic *Strep. agalactiae* from other β -hemolytic streps. It should not be used with all streps, only the β -hemolytic streps of human and bovine origin. It is critical for the identification of *Campylobacter jejuni*.

Principle:

Hippurate acid is hydrolyzed to benzoic acid and glycine by the enzymatic action of hippuricase. The glycine end product is detected by the addition of ninhydrin reagent.

Test Procedure:

1. Add 4-6 drops of sterile water to a small tube of sodium hippurate.
2. Heavily inoculate the hippurate tube.
 - The tube should be cloudy looking after inoculation, turbid.
3. Incubate in a 37°C for 24 hours.
4. Carefully add 2-3 drops of the ninhydrin solution down the side of the tube to form an overlay over the cell mixture.
 - Do **NOT** mix the solutions.
5. Set aside for 5-10 minutes.

Results:

Development of a deep purple color within 5-10 minutes is a positive result.

Limitations:

- A faint purple color is considered a negative result.
- This test is only useful when distinguishing β -hemolytic streptococcus of human or bovine origin.
- The hippurate solution deteriorates in 7 days at 4°C.
- The ninhydrin solution deteriorates in 6 months.
- Hippurate hydrolysis is also found in some species of other genera, such as *Bacillus*, *Cornebacterium*, *Enterobacteriaceae*, and others.