# Hippurate Hydrolysis Test

**DD035**

Hippurate Hydrolysis Test is used for detection of hippurate hydrolyzing bacteria, mainly Streptococcal species.

## Directions

Aseptically place hippurate disc in Brain Heart Infusion Broth (M210) inoculated with b haemolytic streptococci. Incubate at 35-37°C for 48 hours. Separate out the growth by centrifuging the broth. Add 2 ml of ferric chloride reagents to 2 ml of supernatant from the centrifuged culture tubes. Shake well and observe persistence of the precipitate formed even after 10 minutes.

Preparation of ferric chloride reagent :

Ingredients: Grams/100ml

Ferric chloride :12.0 gm

Distilled water : 94.6 ml

Concentrated hydrochloric acid : 5.4 ml

## Principle And Interpretation

Group B streptococci (*Streptococcus agalactiae*) and some enterococci can hydrolyze 1% aqueous sodium hippurate to produce glycine and sodium benzoate. Glycine is deaminated by the oxidizing agent ninhydrin which gets reduced and becomes purple. The test medium must contain only hippurate, since ninhydrin reacts with any free amino acids present (5,6).

Group B streptococci can thus be distinguished from Groups A, C, F and G which can not hydrolyze sodium hippurate. Some Group D and very few viridans streptococci can also hydrolyze sodium hippurate.

Ayers and Rupp (1) discovered that haemolytic streptococci from human and bovine sources could be differentiated by their ability to hydrolyze sodium hippurate (2). Facklam et al (3) modified the procedure for the presumptive identification of Group A, B and D streptococci. The ability of an organism to hydrolyze sodium hippurate is one of the tests that aid in the differentiation of bovine b-haemolytic Group B streptococci, from human b-haemolytic Group B Streptococcus species (2). Differentiation of b-haemolytic Group B streptococci from b-haemolytic Group A streptococci and non enterococcal Group D streptococci is also aided by the determination of hippurate hydrolysis by enzymatic activity to form benzoic acid as the end product (4).

## Quality Control

**Appearance**

Filter paper discs of 10 mm diameter bearing letters 'Hp' in continuous printing style.

**Cultural Response**

The Hippurate hydrolysis reaction is observed after an incubation at 35-37°C for 24-48 hours, of various bacteria with Hippurate differentiation discs, tested using Brain Heart Infusion Broth(M210).

**Cultural Response**

| Organism | Growth | Hippurate hydrolysis |
|---|---|---|
| *Enterococcus faecalis ATCC 29212* | luxuriant | negative : precipitate if any, dissolves on shaking |
| *Streptococcus agalactiae ATCC 4768* | luxuriant | positive : brown flocculant |

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|---|---|---|
| | | precipitate persisting on shaking after 10 minutes. |
| *Streptococcus pyogenes ATCC 19615* | luxuriant | negative : precipitate if any, dissolves on shaking |

## Storage and Shelf Life

Store at 2-8°C. Use before expiry date on the label.

## Reference

1.Ayers S.H. and Rupp P. (1922), J. Infect. Disease., 30:388.
2.Eaton A.D, Clesceri L.S., Greenberg. A.E, Rice E. W.,(Eds) 2005, Standard Methods for the Examination of Water and Wastewater, 21st edn, APHA. Washington. DC.
3.Facklam and Moody, 1970, Appl. Microbiol., 20:245.
4.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B. P.Simmons A (Eds.), Churchill Livingstone, Edinburgh.
5.Facklam, R.R. et al, 1974, Appl. Microbiol.,27:102-113
6.Forbes. A.B, Sahm. F.D, 2002, Bailey and Scott's Diagnostic Microbiology, 11th ed., The C.V. Mosby Co., St. Louis.

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