Hippurate Hydrolysis Broth

M1054

Hippurate Hydrolysis Broth is recommended for detection of hippurate hydrolyzing bacteria.

Composition**

Ingredients	Gms / Litre
Heart infusion powder	10.000
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Sodium hippurate	10.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amounts in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Ayers and Rupp (1) discovered that haemolytic Streptococci from human and bovine sources could be differentiated by their ability to hydrolyze sodium hippurate (1). Facklam et al (2) 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 beta haemolytic group B Streptococci, from human beta haemolytic group B Streptococci (2). Differentiation of ß-haemolytic group B Streptococci from beta-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 (3).

Heart infusion and peptic digest of animal tissue provide essential nutrients required for bacterial metabolism. Sodium chloride maintains osmotic equilibrium. Sodium hippurate serves as the substrate for the measurement of hippurate hydrolysis. The amount of the precipitate is related to the degree of hippurate hydrolysis. Confirmed ß-haemolytic *Streptococcus* colonies are inoculated in this medium.

Hippurate Hydrolysis Test

Ferric Chloride : Ferric chloride: 12.0 gm

Test Reagents : Distilled water: 94.6 ml.

Concentrated hydrochloric acid: 5.4 ml

Add approximately 75 ml of distilled water to a 100 ml volumetric flask. With a transfer pipette, add 5.4 ml of HCl to the flask, running down the acid along the sides of the flask. Add 12 gram of ferric chloride. Dissolve by warming the flask gently, swirling the contents to mix well. Bring the volume up to 100 ml with distilled water. The solution appears orange in colour.

Inoculate tubes with 1 to 2 drops of 18 to 24 hours old pure broth culture of a confirmed beta-haemolytic Streptococcus or with one to two isolated colonies from an original isolation plate. Include an un-inoculated tube as a negative control and a positive control (*Streptococcus agalactiae*). Incubate tubes with loosened caps for 48 hours at 35 ± 2°C in an aerobic atmosphere. Following incubation, centrifuge all cloudy cultures and use the supernatant fluid in the test. Aseptically transfer a specific aliquot of culture (or its supernatant) to a small test tube. Add ferric chloride solution (0.2 ml of 12% $FeCl_3$ solution). Shake the tube immediately. Stand for 10-15 minutes before interpretation. If the test is positive, brown flocculant insoluble precipitate persists on shaking after 10 minutes i.e. hippurate is hydrolyzed. If the precipitate gets dissolved on shaking, hippurate is not hydrolyzed and the test is negative.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured, clear solution without any precipitate

Reaction
Reaction of 3.5% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M1054: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Hippurate Hydrolysis
Enterococcus faecalis ATCC 29212	50-100	luxuriant	negative reaction,precipitate if any, dissolves on shaking
Streptococcus agalactiae ATCC 4768	50-100	luxuriant	positive reaction, brown flocculant precipitate persisting on shaking after 10 minutes
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	negative reaction, precipitate if any, dissolves on shaking

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1.Ayers S. H. and Rupp P., 1922, J. Infect. Dis., 30:388.
2.Facklam R. R., Padula J. F., Thacker L. G., Wortham E. G., and Sconyers B. J., ,1974, Appl. Microbiol., 27:107.
3.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. 1, William and Wilkins, Baltimore.

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