Hippurate Hydrolysis Broth

M1054

Intended Use:

Recommended for detection of hippurate hydrolyzing microorganisms from clinical and non-clinical samples.

Composition**

Ingredients	g / L
HI powder#	10.000
Peptone	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
Equivalent to Heart infusion powder

Directions

Suspend 35.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amounts in tubes or as desired. 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 ß-haemolytic group B Streptococci (2). Differentiation of ß-haemolytic group B Streptococci from ß-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).

HI powder and peptone 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.

Type of specimen

Clinical samples - Faeces

Specimen Collection and Handling:

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.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Upon addition of $FeCl_3$ reagent, tube must be shaken before interpretation of results as shaking aids along with excess of $FeCl_3$ in redissolving soluble hippurate and glycinate precipitates to give negative results. The failure to shake the tubes could result in false positive results if hippurate is not hydrolyzed (4).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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

Organism	Inoculum (CFU)	Growth	Hippurate Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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.
4. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, American Society for Microbiology, Washington, D.C.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD *In vitro* diagnostic medical device

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10°C
Storage temperature

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CE CE Marking

Do not use if package is damaged

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