HIPPURATE BROTH

INTENDED USE
Remel Hippurate Broth is a liquid medium recommended for use in qualitative procedures for determination of the ability of an organism to hydrolyze sodium hippurate.

SUMMARY AND EXPLANATION
In 1922, Ayers and Rupp used an enriched medium containing hippuric acid to investigate the ability of bovine and human β-hemolytic streptococci to split hippuric acid into benzoic acid and glycine.[1] In 1951, Leuthardt described hippuricase, the enzyme responsible for the hydrolysis of hippurate.[2] Braunstein et al. found the hippurate hydrolysis test to be useful for identification of group B streptococci (Lancefield group B).[3] Facklam et al. recommended using the hippurate hydrolysis test in conjunction with bile esculin and 6.5% sodium chloride for identification of β-hemolytic streptococci belonging to groups A, B, and D.[4]

PRINCIPLE
Peptones and heart infusion supply nitrogenous compounds and amino acids necessary for the growth of streptococci. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Dextrose is an energy source which also stimulates the production of streptococcal antigenic hemolysin. Disodium phosphate and sodium carbonate are buffers which counteract the effects of acid produced during the fermentation of dextrose and prevent inactivation of the hemolysin. Hippuricase hydrolyzes sodium hippurate in the medium to form benzoic acid and glycine. The addition of ferric chloride (Hippurate Hydrolysis Reagent) results in ferric ion combining with benzoic acid to form an insoluble precipitate, ferric benzoate.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	10.0 g	Sodium Carbonate	2.5 g
Meat Peptone	10.0 g	Dextrose	2.0 g
Sodium Hippurate	10.0 g	Sodium Chloride	2.0 g
Heart Infusion	3.1 g	Disodium Phosphate	0.4 g
		Demineralized Water	1000.0 ml

pH 7.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate Hippurate Broth with 1 to 2 drops from a pure, 18-24 hour broth culture of the test isolate or with 1-2 isolated colonies from a primary isolation plate.
2. Positive and negative controls should be inoculated and incubated with each run of test isolates. Use a known culture of group B *Streptococcus* for the positive control. A known culture of group A *Streptococcus* or uninoculated tubes of Hippurate Broth can be used for the negative control. Inoculate and incubate one tube of Hippurate Broth for the positive control and 5 tubes for the negative control.
3. Incubate tubes aerobically with caps loosened at 33-37°C for 24-48 hours.
4. Following incubation, centrifuge all tubes with growth (cloudy broth). The supernatant fluid is used for the test.
5. Before adding Hippurate Hydrolysis Reagent (12% Ferric Chloride, R21221), determine the amount of reagent to add by means of the following procedure:
    a. Transfer 0.8 ml from a negative control tube to a small tube labeled #1. Add 0.2 ml of Hippurate Hydrolysis Reagent and immediately shake gently. Allow the tube to stand 10-15 minutes before reading the result.
    b. If negative, the initial precipitate will clear within 15 minutes, indicating ferric ion is in excess. The reagent can be used without further titration. The appropriate amount of reagent to add to each test isolate and control tube is 0.2 ml.
    c. If positive, the initial precipitate will not clear within 15 minutes, indicating ferric ion is not in excess. The reagent must be titrated to determine the appropriate amount to add.
    d. If titration is necessary, transfer 1.0 ml of negative control broth to each of 4 small tubes (labeled #2 through #5).
    e. Add Hippurate Hydrolysis Reagent to each tube as follows: 0.2, 0.3, 0.4, and 0.5 ml to tubes #2 through #5, respectively.
    f. Let stand 10-15 minutes with occasional shaking. The smallest amount of reagent producing a clear solution (indicating excess ferric ion) is the amount to add to each test isolate and control tube.
6. Aseptically transfer 0.8 ml of supernatant from each tube of broth (test isolate or positive control) to a small test tube.
7. Add the appropriate amount of reagent (determined in the titration procedure) to each small tube, test isolates and positive control.
8. Examine tubes for the production of a brown, flocculant, insoluble precipitate that persists on shaking.

INTERPRETATION OF THE TEST
Positive Test - A brown, flocculent, insoluble precipitate that persists on shaking

Negative Test - No precipitate or formation of a precipitate that dissolves on shaking

QUALITY CONTROL
All lot numbers of Hippurate Broth have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Streptococcus agalactiae ATCC® 12386	Ambient, 18-24 h @ 33-37°C	Positive
Streptococcus pyogenes ATCC® 19615	Ambient, 18-24 h @ 33-37°C	Negative

(Continued on back)

LIMITATIONS

1. When using 12% Ferric Chloride (Hippurate Hydrolysis Reagent), positive and negative controls must be tested concurrently with the test isolates. If an uninoculated Hippurate Broth is used for a negative control, the tube must be incubated along with the test isolates.[6]
2. If using the hippurate hydrolysis test for presumptive identification of group B streptococci, test only β-hemolytic colonies which are catalase-negative, gram-positive cocci morphologically characteristic of streptococci.[1,5]
3. After addition of 12% Ferric Chloride, shake the tubes before interpreting results. Shaking facilitates dissolution of soluble hippurate and glycinate precipitate to produce negative results. Failure to shake tubes may result in false-positive results.[5]
4. This test depends on relative solubilities of hippurate and glycine precipitate; the final concentration of iron is critical and titration of Hippurate Broth and 12% Ferric Chloride is essential before addition of reagent to the test isolate tubes.[7]
5. This test is only part of the overall scheme for identification of group B streptococci. Additional testing may be required for definitive identification of the test isolate. Consult appropriate references for further instructions.[8]
6. Organisms other than group B streptococci can produce positive results in Hippurate Broth.

BIBLIOGRAPHY

1. Ayers, S.H. and P. Rupp. 1922. J. Infect. Dis. 30:388-399.
2. Summer, J.B. and K. Myrback. 1951. The Enzymes: Chemistry and Mechanisms of Action. Vol. 1. Academic Press, New York, NY.
3. Braunstein, H., E.B. Tucker, and B.C. Gibson. 1969. Am. J. Clin. Pathol. 51:207-213.
4. Facklam, R.R., J.F. Padula, L.G. Thacker, E.C. Wortham, and B.J. Sconyers. 1974. Appl. Microbiol. 27:107-113.
5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
6. Blazevic, D.J. and G.M. Ederer. 1975. Principles of Biochemical Tests in Diagnostic Microbiology. Wiley & Sons, Baltimore, MD.
7. Cowan, S.T. 1974. Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd ed. Cambridge University Press, Cambridge, UK.
8. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock. 2011. Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

IFU 61150, Revised July 23, 2014 Printed in U.S.A.

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