remel

HIPPURATE HYDROLYSIS REAGENT
(12% Ferric Chloride)

INTENDED USE
Remel Hippurate Hydrolysis Reagent is recommended for use in qualitative procedures to determine 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 test the ability of β-hemolytic streptococci to split hippuric acid into benzoic acid and glycine.[1] In 1951, Leuthardt identified hippuricase as the enzyme responsible for hippurate hydrolysis.[2] In 1969 Braunstein et al. found the hippurate hydrolysis test to be useful in the identification of *Streptococcus agalactiae* (Lancefield group B).[3] Facklam et al. reported identification of groups A, B, and D β-hemolytic *Streptococcus* could be obtained using hippurate hydrolysis in combination with bile esculin and 6.5% sodium chloride.[4]

PRINCIPLE
The bacterial enzyme hippuricase hydrolyzes sodium hippurate present in the medium to form benzoic acid and glycine. The addition of ferric chloride results in the ferric ion combining with benzoate to form an insoluble precipitate, ferric benzoate.

REAGENTS (CLASSICAL FORMULA)*

Ferric Chloride (CAS 10025-77-1)	120.0 g
Hydrochloric Acid (CAS 7647-01-0)	54.0 ml
Demineralized Water (CAS 7732-18-5)	946.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
DANGER! CORROSIVE, may cause burns or irritation to skin, eyes or respiratory tract. Avoid breathing vapor and eye/skin contact.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Read and follow directions carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Protect product from light.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.[5]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Test tubes, (7) Centrifuge, (8) Pipette, (9) Hippurate Broth (REF R061150).

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 as 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 determine the amount 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 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, flocculent, insoluble precipitate that persists on shaking.

INTERPRETATION

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 Hydrolysis Reagent have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control 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	Aerobic, 18-24 h @ 33-37°C	Positive
Streptococcus pyogenes ATCC® 19615	Aerobic, 18-24 h @ 33-37°C	Negative

LIMITATIONS

1. When Hippurate Hydrolysis Reagent is used, positive and negative controls must be tested concurrently with the test isolates. If an uninoculated Hippurate Broth is used as a negative control, the tube must be incubated along with the test isolates.[7]
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.[1,6]
3. 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.[1,8]
4. After addition of Hippurate Hydrolysis Reagent, shake 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.[6]

BIBLIOGRAPHY

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PACKAGING

REF R21221, Hippurate Hydrolysis Reagent .... 25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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