ニンヒドリン試薬

RML
#21-238 (½)

Technical Information

HIPPURATE DISK AND NINHYDRIN REAGENT
~~TI NO. 21085~~

25ml

USE: This product is used to detect the hydrolysis of sodium hippurate by beta hemolytic streptococci, Gardnerella vaginalis, and Campylobacter jejuni.

HISTORY: This rapid test for hippurate hydrolysis was developed by Hwang and Ederer.[1] Greenwood and Pickett found that 90% of the Gardnerella vaginalis they studied hydrolyzed hippurate.[2] The hippurate test was used by Harvey to differentiate between Campylobacter jejuni and Campylobacter intestinalis.[3]

PRINCIPLE: One of the products of the hydrolysis of sodium hippurate is the amino acid, glycine. This can be detected by ninhydrin. In the hydrolysis of hippurate, ammonium and carbon dioxide are formed as well as an aldehyde and a reduced ninhydrin, hydrindantin, to form a blue-purple color, thus indicating a positive test.

CLASSICAL FORMULAE:*

Sodium hippurate	10.0 g	Deionized water	1000.0 ml
Ninhydrin Reagent:			
Acetone	500.0 ml	Butanol	500.0 ml
Ninhydrin	35.0 g		

*Adjusted as required to meet performance standards.

PRECAUTIONS: 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 their use. Directions should be read and followed carefully. Avoid contact with skin. Rinse thoroughly with water.

STORAGE: This product is ready for use and no further preparation is necessary. The product should be stored in its original container at 2-8C until used. Do not freeze or overheat. Allow product to come to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION: This product should not be used if there is (1) evidence of dehydration (2) the product is contaminated (3) the color has changed (4) the expiration date has passed.

PROCEDURE: The usual clinical microbiology equipment is required for procedures involving this product.

INSTRUCTIONS: Add 0.1ml of sterile water to a small plastic test tube (12 x 75mm) and emulsify 1-3 colonies of the organism to be tested. Place a Rapid Hippurate Disk in the mixture and incubate two hours at 35-37C. Following incubation, add 2 drops of Ninhydrin Reagent (REMEL #21-238) to the test tube, mix and reincubate for up to 30 minutes. The development of a blue to purple color indicates a positive reaction. Some negative tests may impart a light gray color but this is easily distinguished from a more intense blue color of a positive test.

MATERIALS REQUIRED BUT NOT SUPPLIED: (1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, anaerobic jars or candle jars, (4) Supplemental media, (5) Quality control organisms, (6) Supplemental reagents.

USER QUALITY CONTROL: Use "Instructions" procedure to inoculate the media with Streptococcus pyogenes ATCC 19615 and Streptococcus agalactiae ATCC 12386.

RESULTS:

ORGANISM	RESULT
Streptococcus pyogenes ATCC 19615	negative (no color change)
Streptococcus agalactiae ATCC 12386	positive (blue-purple color)

LIMITATIONS:
1. Some strains of group D streptococci will give a positive rapid hippurate test. A bile esculin test will differentiate group B streptococci from group D streptococci.
2. A positive and negative control should be run with each group of tests to better correlate the color changes produced by both positive and negative results.
3. Use of glass test tubes may result in false-negative reactions.

remel
12076 SANTA FE DRIVE-LENEXA, KANSAS 66215—Phone (913)888-8939 Wats (800)255-6730 Ks Wats (800)332-4377

RML
#21-238(3/2)

BIBLIOGRAPHY:
1. Hwang, Mei-Na and Ederer, G.M., Rapid Hippurate Hydrolysis Method for Presumptive Identification of Group B Streptococci, J. Clin. Micro., Jan., p.114-15, 1975.
2. Greenwood, J.R., and Pickett, M.J., Salient Features of Haemophilus Vaginalis, J. Clin. Micro., Feb., p.200-204, 1979.
3. Harvey, Sydney, Hippurate Hydrolysis by Campylobacter fetus, J. Clin. Micro., April, 1980.
4. Cumitech 3, Practical Quality Control Procedures for the Clinical Microbiology Laboratory, ASM, 1976.
5. Finegold, S.M., W.J. Martin and E.G. Scott, Bailey and Scott's Diagnostic Microbiology, 5th edition, The C.V. Mosby Co., St. Louis, 1978.
6. Lennette, E.H., A. Balows, W.J. Hausler, Jr. and H.J. Shadomy, Manual of Clinical Microbiology, 4th edition, 1985.

Rev 3/24/88
mw

(Disk 10)

remel

NINHYDRIN REAGENT
TI No. 21238

INTENDED USE
REMEL's Ninhydrin Reagent is recommended for use in qualitative procedures to detect the hydrolysis of sodium hippurate by beta hemolytic streptococci, *Gardnerella vaginalis*, and *Campylobacter jejuni*.

SUMMARY AND EXPLANATION
The hippurate hydrolysis test determines the ability of group B streptococci, as well as other bacteria, to enzymatically hydrolyze sodium hippurate.[1,2,3,4] Hwang and Ederer employed the ninhydrin reagent in a rapid method to detect hippurate hydrolysis by the end product glycine.[5]

PRINCIPLE
Hippuric acid is hydrolyzed by the enzyme hippuricase to glycine and benzoic acid. Ninhydrin evokes a five step reaction beginning with the deamination of glycine to form hydrindantin (a reduced form of ninhydrin), carbon dioxide, and ammonia. A condensation reaction occurs with hydrindantin, ammonia, and residual ninhydrin to produce the final purple colored complex.

REAGENTS (CLASSICAL FORMULA)*

Acetone (CAS 67-64-1)	500.0 ml
Butanol (CAS 71-36-3)	500.0 ml
Ninhydrin (CAS 485-47-2)	35.0 g

*Adjusted as required to meet performance standards.

PRECAUTIONS
WARNING! FLAMMABLE. keep away from heat, sparks and flame. May cause irritation to skin, eyes and 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 their use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE
This product is ready for use and no further preparation is necessary. The product should be stored in its original container at 2-8°C until used. Allow product to come 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 AND TRANSPORTATION
Specimens should be collected and handled following recommended guidelines.[6]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Plastic test tube, (7) Hippurate Disk (REMEL #21-085), (8) Pipettes, (9) Forceps, (10) Sterile water.

PROCEDURE
1. Add 0.1ml of sterile water to a small plastic test tube.
2. Emulsify 1-3 colonies of the organism to be tested in the water.
3. Using forceps, place a Hippurate Disk in the mixture. At the same time, test a positive and negative control.
4. Incubate aerobically for 2 hours at 35-37°C.
5. Dispense 2 drops of Ninhydrin Reagent into the test tube and mix.
6. Reincubate the tube aerobically for up to 30 minutes at 35-37°C.
7. Observe for a blue to purple color development.

INTERPRETATION OF THE TEST
Positive Test - a blue to purple color development within 30 minutes
Negative Test - a light gray color development or no color change within 30 minutes

QUALITY CONTROL
All lot numbers of the Ninhydrin 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
Gardnerella vaginalis ATCC® 14018	Aerobic, 30 min. @ 35°C	Positive
Streptococcus agalactiae ATCC® 12386	Aerobic, 30 min. @ 35°C	Positive
Streptococcus pyogenes ATCC® 19615	Aerobic, 30 min. @ 35°C	Negative

LIMITATIONS
1. A positive and negative control should be run with each group of tests to better correlate the color changes produced by both positive and negative results.
2. Plastic tubes should be utilized as glass tubes may yield a false negative result.
3. Incubation of the culture for more than 30 minutes after the Ninhydrin Reagent has been added may yield false-positive results.

BIBLIOGRAPHY

1. Greenwood, J.R. and M.J. Pickett. 1979. J. Clin. Microbiol. 9:200-204.
2. Jolly, J.L.S. 1983. J. Clin. Pathol. 36:476-478.
3. Cacho, J.B., P.M. Aguirri, A. Hemanz, and A.C. Velasco. 1989. J. Clin. Microbiol. 27:359-360.
4. Facklam, R.R., J.F. Padula, L.G. Thacker, E.C. Wortham, and B.J. Sconyers. 1974. Appl. Microbiol. 27:107-113.
5. Hwang, Mei-Na and G.M. Ederer. 1975. J. Clin. Microbiol. 1:114-115.
6. Balows, A., W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy. 1991. Manual of Clinical Microbiology. 5th ed. ASM, Washington, D.C.
7. Baron, E.J., L. R. Peterson, and S.M. Finegold. 1994. Bailey and Scott's Diagnostic Microbiology. 9th ed. Mosby, St. Louis, MO.
8. MacFaddin, J.F. 1980. Biochemical Tests for the Identification of Medical Bacteria. 2nd ed. Williams & Wilkins, Baltimore, MD.

Revised 8/15/95 Printed in U.S.A. CAS (Chemical Abstracts Service Registry No.)

12076 Santa Fe Drive, Lenexa, KS 66215-3594 800-447-3641 (Technical Service) 800-447-3635 (Order Entry)

MATERIAL SAFETY DATA SHEET

SECTION I-IDENTIFICATION

Product Name: Ninhydrin Reagent
Product No.: 21-238
Formula: Mixture

SECTION II-HAZARDOUS INGREDIENTS

Material:	Acetone	Butyl Alcohol
CAS #:	67-64-1	71-36-3
% Weight	50	50
NFPA Rating:	Health - 1 Flammability - 3 Reactivity - 0	Health - 1 Flammability - 3 Reactivity - 0

SECTION III-PHYSICAL DATA

	Acetone	Butyl Alcohol
Boiling Point:	133°F (56°C)	244°F (118°C)
Vapor Pressure:	180mm Hg @ 68°F (20°C)	5mm Hg @ 68°F (20°C)
Vapor Density: (air = 1)	2.0	2.6
Appearance and Odor:	Clear, distinct odor.	Clear,colorless solution, characteristic odor.
Melting Point:	-139°F (95°C)	-128°F (89°C)
Specific Gravity:	0.79	0.8
% Volatile by Volume	100	100
Evaporation Rate : (BuAg=1)	6.0	0.46

SECTION IV-FIRE AND EXPLOSION DATA

	Acetone	Butyl Alcohol
Flash point:	-4°F (20°C)	84°F (29°C)
Explosive Limits:	LEL 2.5 UEL 13.0	LEL 1.4 UEL 11.3

Extinguishing media: Carbon dioxide, dry chemical, water spray or foam.
Special Fire-fighting Procedures: Flammable, keep away from heat, sparks or flame. Wear self-contained breathing apparatus and protective equipment.
Unusual Fire and Explosive Data: Highly flammable. Explosive vapor-air mixture may form above 84°F (29°C).

SECTION V-HEALTH HAZARD AND EMERGENCY

	Acetone	Butyl Alcohol
OSHA TWA:	750ppm or 1800 mg/m^3	100 ppm or 300 mg/m^3
OSHA STEL:	1000 ppm or 2400 mg/m^3	Data not available.
OSHA Ceiling:	Data not available.	50 ppm or 150 mg/m^3
ACGIH TWA:	750 ppm or 1780 mg/m^3	50 ppm or 152 mg/m^3
ACGIH STEL:	1000 ppm or 2380 mg/m^3	Data not available.
ORAL RAT LD_{50}	5800 mg/kg	3484 mg/kg

Acute Health Effects: Avoid breathing vapor or eye/skin contact.

Primary Routes of Entry:
Inhalation: Remove victim to fresh air. If not breathing, give artificial respiration. Call physician.
Eye/skin Contact: Flush immediately with copious amounts of water for at least 15 minutes. Remove contaminated clothing. Call physician.
Ingestion: If victim is conscious, induce vomiting. Call physician immediately.
Carcinogen Potential: The components specified are not regulated by IARC, NTP or OSHA as carcinogens or potential carcinogens.
Target Organ Effects: Acetone - Prolonged contact may cause irritation to skin, eyes and respiratory tract. Butyl Alcohol - Prolonged contact may cause drying and cracking of the skin.
Medical Conditions Aggravated by Exposure: Persons with pre-existing eye, skin or respiratory disorders may be more susceptible to the effects of this substance.

SECTION VI-REACTIVITY

Stability: Stable under normal conditions.
Incompatibility: Alkali metals, strong oxidizers, aluminum, chromic anhydride, chromium trioxide.
Hazardous Polymerization: Will not occur.
Hazardous Decomposition Products: Carbon dioxide, carbon monoxide.
Conditions to Avoid: Heat, open flames, incompatible materials.

SECTION VII-SPILL AND DISPOSAL

Spill Procedures: Wear protective equipment as specified in Section VIII. Remove all sources of ignition. Contain spill and absorb with inert material. Scoop up and place in an approved hazardous waste container.
Disposal Procedures: Ensure compliance with all local, state and federal regulations or contact an approved and licensed disposal agency.

SECTION VIII-PROTECTION DATA

Eyes: Chemical safety goggles.
Gloves: Chemical resistant gloves.
Respiratory: Not required under normal conditions of use with ventilation.
Ventilation: Mechanical exhaust, fume hood.
Other: Protective apron or gown as required.

SECTION IX-HANDLING AND STORAGE

Store at 36-46°F (2-8°C) away from all sources of ignition. Keep container closed.

SECTION X-MISCELLANEOUS DATA

This information is believed to be correct, however no warranties are made with respect to this information and REMEL assumes no liability resulting from use. Make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of this product and personnel safety and health. Data for each component is specified where applicable as mixtures have not been tested.