IFU

Instructions for Use

HIPPURATE DISKS

Cat. no. Z18	Hippurate Disks	25 disks/jar

INTENDED USE

Hardy Diagnostics Hippurate Disks are used to perform the sodium hippurate hydrolysis test. Glycine, an end product of hippurate hydrolysis, reacts with ninhydrin to produce a purple color. Hippurate is commonly used to presumptively identify *Gardnerella vaginalis*, *Listeria monocytogenes*, *Campylobacter jejuni,* and group B streptococci.

SUMMARY

The hippurate test is a qualitative test to detect hydrolysis of sodium hippurate by the enzyme, hippuricase. The test determines an organism's ability to enzymatically hydrolyze sodium hippurate.[8,9] The classical method for detection of this byproduct involved the use of ferric chloride indicator to detect benzoic acid, the first byproduct in the pathway. However, this method took 48 hours. Consequently, Hwang and Ederer utilized Ninhydrin Reagent (Cat. no. Z70) and developed a rapid method to detect hippurate hydrolysis by utilizing the end product glycine, the second byproduct in hippurate hydrolysis.[10] Jolly later specified using hippurate hydrolysis as one of the criteria for identifying *G. vaginalis*.[11] In the late 1980s, Cacho et al. further included use of the hippurate test to identify *Campylobacter* spp.[12] The rapid method has been shown to be as specific and as sensitive as the classical method.[10-12]

Hardy Diagnostics Hippurate Disks are impregnated with sodium hippurate. Hippuric acid is hydrolyzed by the enzyme hippuricase to glycine and benzoic acid. The addition of Ninhydrin Reagent (Cat. no. Z70) evokes a five-step biochemical reaction that begins with the deamination of glycine to form hydrindantin (a reduced form of ninhydrin), carbon dioxide, and ammonia. This step is followed by a condensation reaction using hydrindantin, ammonia, and residual ninhydrin to produce the final deep purple to violet complex indicative of a positive reaction.

FORMULA

Each HardyDisk™ Hippurate Disk is prepared by impregnating a controlled concentration of sodium hippurate onto a 3/8 inch diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC away from direct light. Disks should not be used if there are any signs of deterioration (discoloration) or contamination, or if the expiration date has passed. **Protect product from light and moisture.** Product is light, temperature, and moisture sensitive.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is not intended for the primary isolation of specimens. The product is used in conjunction with other biochemical tests to identify pure cultures from isolated organisms.

Method of Use: Concurrent inoculation of positive and negative control organisms is strongly recommended. Hippurate Disks are not sterile, but good aseptic technique should be followed to ensure unused disks are not soiled prior to use. Close the container tightly between uses and store as directed.

1. Allow disks to equilibrate to room temperature prior to opening the container.

2. Using clean forceps, place one disk into a small clean optically clear test tube. **Note**: use a separate tube for each isolate.

3. Add 0.5ml or ten drops of a neutral pH (6.5 to 7.2) sterile water to the tube.

4. Obtain a heavy inoculum of organism from 2 to 3 well-isolated colonies grown on a fresh 18-24 hour purity plate using a sterile loop or sterile cotton swab. Inoculate each tube with a separate organism. **Note**: the suspension should be milky and opaque. Avoid picking up agar when selecting colonies.

5. Incubate tubes at 35-37°C for at least 2 hours, but not more than 6 hours.

6. After incubation, place 4 drops of Ninhydrin Reagent (Cat. no. Z70) into the tube. Mix well and re-incubate tubes at 35-37°C.

7. Observe tubes at 10 minute intervals, but not more than a total of 30 minutes, for a deep purple or violet color change indicative of a positive reaction. **Note**: Incubation beyond 30 minutes may yield false-positive results.

INTERPRETATION OF RESULTS

The development of a deep purple or violet coloration indicates a positive test for hippurate hydrolysis. No purple coloration or a faint blue-gray color change indicates a negative test. Compare the reaction against a concurrent reaction from a known negative and positive control.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies

from pure culture for complete identification of bacteria and/or fungi.

HardyDisk™ Hippurate Disk should only be used on well-isolated fresh (18-24 hour) colonies obtained from a non-selective purity plate. Do not use on mixed cultures. Known positive and negative controls should be inoculated concurrently with each test to confirm the color intensity of each group.

Do not use pigmented colonies from a chromogenic plate, since chromogens in the medium may affect the colorimetric reaction of the test.

A faint blue-gray color change is inconclusive and should be disregarded. If results are inconclusive, repeat the test with a larger inoculum. Do not reincubate used disks or tubes.

Incubation of test cultures for more than 30 minutes after addition of Ninhydrin Reagent can yield false-positive results.

Optically clear tubes should be used in order to observe the color reaction.

Obtain colonies from plates with caution. Avoid picking up any agar with the colonies, as the medium contains proteins that may interfere with the results of the test.

HardyDisk™ Hippurate Disk cannot be used alone to determine hippurate hydrolysis. Use disks in conjunction with Ninhydrin Reagent (Cat. no. Z70) for the hippurate test.

The product is used in conjunction with other tests to identify pure cultures of an organism. Rapid sodium hippurate hydrolysis reactions may be used in the presumptive identification of group B beta-hemolytic streptococci, *G. vaginalis*, *L. monocytogenes*, and *C. jejuni*. Additional testing using pure cultures is recommended for complete identification.

Some strains of group D streptococci may give a weak positive result. Group D streptococci can be differentiated from group B by esculin hydrolysis. Only group D streptococci hydrolyze esculin (Cat. no. L29), yielding a blackening of the medium.

A negative test does not rule out *G. vaginalis* since rare biotypes that cause bacterial vaginosis can be hippurate negative.

Some members of the viridans streptococci may be hippurate positive. Perform additional tests for complete identification of non-hemolytic strains.

A small number of enterococci are beta-hemolytic and may hydrolyze hippurate, but are pyrrolidinyl-β-naphthylamide (PYR) positive. *S. agalactiae* (Group B Strep) is PYR negative.

A small percentage of *C. jejuni* strains are hippurate negative and use of other methods for complete identification are necessary.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as empty test tubes, sterile water, loops, swabs, applicator sticks, other culture media, incinerators, incubators, etc., as well as serological and biochemical reagents such as Ninhydrin Reagent (Cat. no. Z70), are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Reactions
Listeria monocytogenes ATCC® 7644	Positive; Purple color change
Streptococcus agalactiae ATCC® 12386	Positive; Purple color change
Streptococcus pyogenes ATCC® 19615	Negative; No color change

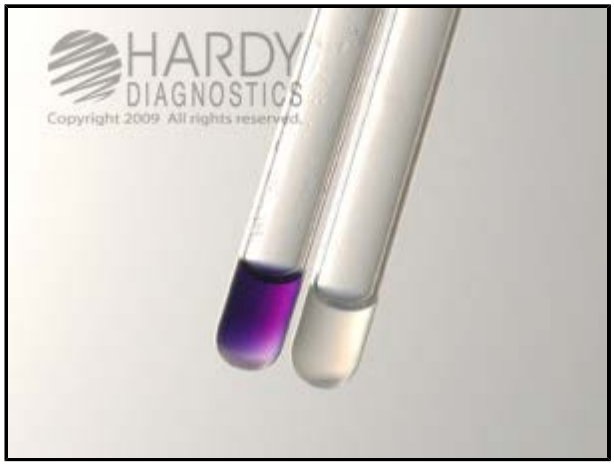
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

Note: Test each new lot with known positive and negative controls to confirm the intensity of the color reaction. Test isolates concurrently with controls thereafter.

PHYSICAL APPEARANCE

Hippurate Disks should appear white to light yellow in color.



Tubes showing positive (left tube inoculated with *Streptococcus agalactiae* ATCC® 12386) and negative (right tube inoculated with *Streptococcus pyogenes* ATCC® 19615) hippurate reactions. See procedure for test method.

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IFU-000820 [A]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]