IFU

Instructions for Use

NOVOBIOCIN DIFFERENTIATION DISKS

Cat. no. Z7291	Novobiocin Differentiation Disk, 5ug	50 disks/cartridge

INTENDED USE

HardyDisk™ Novobiocin Differentiation Disks are useful for presumptively distinguishing *Staphylococcus saprophyticus* from other coagulase-negative staphylococci in clinical specimens.

SUMMARY

Coagulase-negative staphylococci (CoNS) are among those organisms that have traditionally been considered skin contaminants, and their recovery from cultures doesn't always indicate presence of disease. Therefore, little attention had been paid to the pathogenic potential of this group of bacteria until recently. By the mid-1970's, microbiologists were becoming aware that CoNS could indeed be pathogenic, especially in compromised hosts.[10] Today, *S. saprophyticus* has proven to be an important uropathogen.[9] It is second only to *E. coli* as the most common cause of cystitis and acute urinary tract infection (UTI) in healthy, young adult women.[5] *S. saprophyticus* tends to adhere to uroepithelial cells more often and more successfully than other staphylococcal species, this is believed to partially explain the organism's frequent role in urinary tract infections.[5,8]

The HardyDisk™ Novobiocin Differentiation Disks are useful in presumptively distinguishing *S. saprophyticus* from other CoNS. Other human staphylococcal species that are novobiocin-resistant (*S. cohnii*, *S. xylosus*, *S. pulvereri*) are rarely isolated from patients.[2-5] The novobiocin susceptibility test can be done using a plate method (18-24 hour test) or a tube method (5 hour test). A study conducted by Harrington and Gaydos in 1984, concluded that the novobiocin tube test is an acceptable method when performed using Tryptic Soy Broth (TSB), 3ml, and has the advantage of taking only 5 hours.[6] While the plate method is the more common approach of the two, both procedures are outlined in the section entitled "Procedure".

FORMULA

Each HardyDisk™ Novobiocin Differentiation Disk is prepared by impregnating 5ug of novobiocin onto high quality 6mm diameter filter paper disks.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8ºC. away from direct light. The disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

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 primary isolation of patient specimens. It should be used only with cultures of isolated organisms. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Plate Method:

1. Allow disks to equilibrate to room temperature.

2. Using a pure 18-24 hour culture, prepare a suspension, equivalent to a McFarland 0.5 opacity standard, in Tryptic Soy Broth (TSB, Cat. no. R30), Sterile Water (Cat. no. U85), or Brain Heart Infusion (BHI) Broth (Cat. no. R15).

3. Inoculate Mueller Hinton Agar (Cat. no. G45), Blood Agar, 5% (Cat. no. A10), or Tryptic Soy Agar (TSA, Cat. no. G60) plate with a sterile swab to obtain confluent growth.

4. Aseptically apply one novobiocin disk onto the inoculated agar surface and lightly press down to ensure full contact with the medium.

5. Incubate aerobically for 18-24 hours at 35-37ºC.

6. Measure (in millimeters) the diameter of the zone of inhibition around the novobiocin disk, and record as susceptible or resistant. See section below, entitled "Interpretation of Results."

Tube Method:

1. Using a pure 18-24 hour culture, **lightly** inoculate two 3ml TSB tubes (Cat. no. R41) with the organism to be tested. The inoculum should be light, with no visible turbidity.

2. Immediately after inoculation, add one novobiocin disk to one of the tubes and shake for 5-10 seconds. The tube without the novobiocin disk will be the control tube.

3. Incubate both tubes aerobically at 35-37ºC. for up to 5 hours, or until the control tube (without novobiocin disk) shows turbidity comparable to that of a McFarland 0.5 opacity standard.

4. Check for turbidity in the tube containing the novobiocin disk as compared with the control tube, and record as susceptible or resistant. See section below, entitled "Interpretation of Results".

INTERPRETATION OF RESULTS

Plate Method:

Sensitive - A zone of inhibition greater than 16mm.[2]
Resistant - A zone of inhibition less than or equal to 16mm.[2]

Tube Method:

Sensitive - Turbidity is less than the control tube.
Resistant - Turbidity is more than or equal to the control tube.

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.

The novobiocin disk is not helpful and can give misleading results if it is performed on isolates other that those from urinary specimens.

Occasional human isolates that are not *S. saprophyticus*, *S. cohnii* subsp., or *S. xylosis* may also be resistant to novobiocin.[5]

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, 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	Inoculation Method*	Incubation			Results (Plate/Tube Method)
		Time	Temperature	Atmosphere	
Staphylococcus saprophyticus ATCC® 15305	*	*	35-37°C	Aerobic	**Resistant -** Plate: Zone is less than or equal to 16mm Tube: Turbidity is more than or equal to control tube
Staphylococcus epidermidis ATCC® 12228	*	*	35-37°C	Aerobic	**Sensitive -** Plate: Zone is greater than 16mm Tube: Turbidity is less than control tube

* Refer to the document "Inoculation Procedures for Media QC" for more information.

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.

PHYSICAL APPEARANCE

HardyDisk™ Novobiocin Differentiation Disks are 6mm (in diameter) filter paper disks with the letters NB5 printed on both sides and should appear white in color.

Novobiocin-resistant
(zone of inhibition ≤16mm).

Staphylococcus saprophyticus (ATCC® 15305) growing around a HardyDisk™ Novobiocin Differentiation Disk (Cat. no. Z7291). Incubated aerobically on TSA (Cat. no. G60) for 24 hours at 35ºCC.

Novobiocin-sensitive
(zone of inhibition > 16mm).

Staphylococcus epidermidis (ATCC® 12228) inhibition zone around a HardyDisk™ Novobiocin Differentiation Disk (Cat. no. Z7291). Incubated aerobically on TSA (Cat. no. G60) for 24 hours at 35ºCC.

REFERENCES

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

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