IFU

Instructions for Use

NITROCEF DISKS™

Cat. no. Z7301	Nitrocef Disks™	50 disks/vial

INTENDED USE

Hardy Diagnostics Nitrocef Disks™ are intended for use in the rapid testing of isolated colonies of *Neisseria gonorrhoeae, Moraxella* (*Branhamella*) *catarrhalis, Staphylococcus* spp., *Haemophilus influenzae, Enterococcus* spp., and some anaerobic bacteria for the production of beta-lactamase.[11-13]

SUMMARY

It has long been recognized that certain bacteria possess the ability to produce enzymes that inactivate beta-lactam antibiotics. Some hydrolyze penicillin class antimicrobics and are described as penicillinases. Others hydrolyze the cephalosporin class antimicrobics and are described as cephalosporinases. Some bacteria produce enzymes that hydrolyze both cephalosporins and penicillins.[5]

Rapid beta-lactamase tests can yield clinically relevant information earlier than an MIC or disk diffusion test. Several clinical tests have been devised to detect beta-lactamases. These tests include the iodometric method, the acidometric method, and chromogenic substrates. [6] Iodometric methods are suitable for testing *N. gonorrhoeae*.[16] Acidometric methods produce acceptable results with *Haemophilus* spp., *N. gonorrhoeae* and staphylococci.[16] Nitrocefin, a chromogenic cephalosporin, can be used to test *Neisseria gonorrhoeae, Moraxella* (*Branhamella*) *catarrhalis*, Staphylococcus spp., *Haemophilus influenzae*, *Enterococcus* spp., and some anaerobic bacteria, and has been found effective in detecting all known beta-lactamases.[7-9,16] Nitrocefin is the only reliable test for detecting beta-lactamase producing *Enterococcus* spp.[16]

Nitrocef Disks™ are impregnated with nitrocefin, a chromogenic cephalosporin. As the amide bond in a beta-lactam ring is hydrolyzed by a beta-lactamase, nitrocefin changes color from yellow to red. Bacteria which produce beta-lactamase in significant amounts produce this yellow to red color change on the Nitrocef Disk™. These beta-lactamases are capable of inactivating "penicillinase-labile-penicillins", such as amoxicillin, ampicillin, penicillin, carbenicillin, mezlocillin, and piperacillin.

Nitrocef Disks™ are intended for the rapid testing of isolated colonies of *Neisseria gonorrhoeae, Moraxella* (*Branhamella*) *catarrhalis, Staphylococcus* spp., *Haemophilus influenzae, Enterococcus* spp., and anaerobic bacteria of the genera *Bacteroides, Clostridium, Porphyromonas, Fusobacterium*, and *Prevotella*.[11-13,19] The beta-lactamase test is of little value for many organisms (e.g., *Enterobacteriaceae*) as organisms within a taxonomic group, or even a single strain, can produce a diversity of enzymes with different substrate specificities.[10]

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at less than -10ºC. (frozen) 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. After use, the remaining disks should be stored in the provided vial with an air tight seal.

The expiration dating 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 quality control incubation times.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

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

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

1. Using sterile forceps, remove a Nitrocef Disk™ from the vial and place it on an empty petri dish or microscopic slide. Immediately place the remaining unused disks into the freezer.

2. Prior to inoculation, allow the Nitrocef Disk™ to equilibrate to room temperature.

3. Moisten each disk with one drop of sterile deionized water. Alternatively, condensation on the lid of a petri dish can be used to hydrate the disk by transferring a drop with a sterile loop. Do not over saturate the disk, which could dilute the reagent.

Note: Water is critical to the development of the color reaction, if the disk begins to dry out it may be necessary to rehydrate the reaction area of the Nitrocef Disk™ with a small amount of water.

4. With a sterilized loop or applicator stick remove a well-isolated colony and spread it on the disk surface. An alternate procedure requires the use of sterile forceps to wipe a pre-moistened disk across a colony surface. Regardless of which method is used, ensure that the disk surface is exposed to the cell paste.

5. Observe the inoculated disk for the development of an orange/red color.

INTERPRETATION OF RESULTS

A positive beta-lactamase result is recorded when the Nitrocef Disk™ changes in color from its original yellow to orange or red. Most positive bacterial strains will produce a color change within 5 minutes. Some staphylococci, however, may take up to 60 minutes for a positive result.

A positive beta-lactamase result predicts the following:

1. Resistance to penicillin, ampicillin and amoxicillin among *Haemophilus* spp., *N. gonorrhoeae* and *M. catarrhalis*.[16]

2. Resistance to penicillin, as well as acylamino-, carboxy-, and uriedo-penicillins among staphylococci and enterococci.[16]

A negative beta-lactamase result is recorded when the Nitrocef Disk™ remains yellow in color. A negative result does not rule out resistance due to other mechanisms.[16]

LIMITATIONS OF THE PROCEDURE

Beta-lactamase detection with the Nitrocef Disk™ should not entirely replace conventional susceptibility test methods, as other factors also influence the results of such tests, and on occasion intrinsic resistance to beta-lactam antimicrobials has not been correlated with production of beta-lactamase.[14]

Do not over saturate the tip, which could dilute the reagent. Do not leave Nitrocef Disks™ at room temperature for extended periods of time. Place unused disks in the freezer immediately after use.

Detection of beta-lactamase activity in staphylococci may take up to one hour. Induction of the enzyme may also be required, this can be done by testing growth from the zone margin around an oxacillin disk.[16]

A negative result does not rule out resistance due to other mechanisms.[16]

Do not use Nitrocef Disks™ to test members of *Enterobacteriaceae*, *Pseudomonas* species or other aerobic, gram-negative bacilli because the results may not be predictive of susceptibility to the beta-lactams most often used for therapy.[16]

Do not use the Nitrocef Disks™ for organisms where penicillin resistance is not due to beta-lactamase production, such as *Streptococcus pneumoniae* and viridans streptococci.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, culture media, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Results
Haemophilus influenzae ATCC® 33533	Beta-lactamase positive (Orange/Red)
Staphylococcus aureus ATCC® 25923	Beta-lactamase negative (Yellow)
Staphylococcus aureus ATCC® 43300	Beta-lactamase positive (Orange/Red)
Moraxella (Branhamella) catarrhalis ATCC® 25240	Beta-lactamase negative (Yellow)

USER QUALITY CONTROL

Test each batch, lot, and shipment for positive and negative reactivity for reagents.[20]

PHYSICAL APPEARANCE

Nitrocef Disks™ should appear as 0.25 inch (in diameter) filter paper disks imprinted with ß, and white to yellow in color.

Showing positive (left disk) and negative (right disk) reactions for Nitrocef Disks™ (Cat. no. Z7301).
Each disk was moistened with deionized water and growth from cultures of *Haemophilus influenzae* (ATCC® 33533) and *Moraxella (Branhamella) catarrhalis* (ATCC® 25240) was applied to the left

and right disks, respectively. The orange/red color development was indicative as beta-lactamase positive. The yellow color development was indicative as beta-lactamase negative.

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

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