

Instructions for Use

NITROCEF MATCHBOOK™

Cat. no. Z108	Nitrocef Matchbook™	10 tests/pouch
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INTENDED USE

Hardy Diagnostics Nitrocef Matchbook™ sticks 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.⁽¹⁶⁾ 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⁾ Acidimetric methods produce acceptable results with *Haemophilus* spp., *N. gonorrhoeae* and staphylococci.⁽¹⁶⁾ Nitrocef, a chromogenic cephalosporin, can be used to test *Neisseria gonorrhoeae*, *Enterococcus* spp., *Moraxella (Branhamella) catarrhalis*, *Staphylococcus* spp., *Haemophilus influenzae* and some anaerobic bacteria, and has been found effective in detecting all known beta-lactamases.^(7-9,16) Nitrocef is the only reliable test for detecting beta-lactamase producing *Enterococcus* spp.⁽¹⁶⁾

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

Hardy Diagnostics Nitrocef Matchbook™ is intended for use in the rapid testing of isolated colonies of *Neisseria gonorrhoeae*, *Enterococcus* spp., *Moraxella (Branhamella) catarrhalis*, *Staphylococcus* spp., *Haemophilus influenzae* 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 taxonomic organisms (e.g., Enterobacteriaceae). This is because 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 -10°C. (frozen) away from direct light. The Nitrocef Matchbook™ sticks 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

1. Prior to inoculation, allow Nitrocef Matchbook™ sticks to equilibrate to room temperature.
2. Using care not to touch the pointed tip of the Nitrocef Matchbook™ stick that is impregnated with reagent, detach a single Nitrocef Matchbook™ stick from the base. Immediately replace the remaining matchsticks into the Mylar bag, reseal and place into freezer.
3. Briefly dip the pointed tip of the Nitrocef Matchbook™ stick in sterile deionized water. Alternately, condensation on the lid of a petri dish can be used to hydrate the tip as well. Do not over saturate the tip, which could dilute the reagent.
4. Using the Nitrocef Matchbook™ stick, "sweep" up 1-3 well isolated colonies of similar morphology, ensuring that the reagent on the pointed tip of the Nitrocef Matchbook™ stick is exposed to the cell paste.
5. Observe the inoculated tip for the development of an orange/red color.

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

INTERPRETATION OF RESULTS

A positive beta-lactamase result is denoted by a change in color of the tip of the Nitrocef Matchbook™ stick from its original yellow to orange or red. Most positive bacterial strains will produce a color change from yellow to orange or red 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 denoted by no color change (the tip of the Nitrocef Matchbook™ stick remains yellow). A negative result does not rule out resistance due to other mechanisms.⁽¹⁶⁾

LIMITATIONS OF THE PROCEDURE

Beta-lactamase detection with Nitrocef Matchbook™ 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 Matchbook™ at room temperature for extended periods of time. Replace unused matchsticks to 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 Matchbook™ to test members of the 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 Matchbook™ 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, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

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.

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

USER QUALITY CONTROL

It is recommended that each new lot of disks be tested with known positive and negative controls and retested each day of use thereafter.⁽¹⁵⁾

PHYSICAL APPEARANCE

Hardy Diagnostics Nitrocef Matchbook™ sticks should appear white with yellow to amber tips. There should be ten "Matchsticks" on the card.



Nitrocef Matchbook™ sticks (Cat. no. Z108). Showing negative (left) and positive (right). *Staphylococcus aureus* (ATCC® 25923) was tested with the strip on the left. *Haemophilus influenzae* (ATCC® 33533) was tested with the strip on the right. The red color development was indicative of beta-lactamase production.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: HardyDiagnostics.com

Email: TechnicalServices@HardyDiagnostics.com

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