

Paper discs for the detection of β -lactamase enzymes



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English

REF 231650

INTENDED USE

BD BBL™ Cefinase™ discs are intended for use in rapid testing of isolated colonies of *Neisseria gonorrhoeae*, *Staphylococcus* species, *Haemophilus influenzae*, enterococci and anaerobic bacteria for the production of β -lactamase.

SUMMARY AND EXPLANATION

The ability of certain bacteria to produce enzymes that inactivate β -lactam antibiotics, i.e., penicillins and cephalosporins, has long been recognized. Abraham and Chain in 1940 first recognized an enzymatic activity in extracts of *Escherichia coli* that inactivated penicillin.¹ Since then a large number of similar enzymes have been isolated from a number of bacterial species with somewhat different substrate specificities. Some selectively hydrolyze penicillin-class antimicrobics (i.e., penicillin G, ampicillin, carbenicillin) and have been described as penicillinases. Others selectively hydrolyze the cephalosporin-class antimicrobics (i.e., cephalothin, cephalexin, cephadrine) and have been described as cephalosporinases. Still other enzymes hydrolyze both cephalosporins and penicillins.²

A large number of β -lactamase-resistant penicillin and cephalosporin class antimicrobics have been developed by various pharmaceutical companies. One group includes the semisynthetic penicillins; methicillin, oxacillin, nafcillin and others, which are resistant to the penicillinase enzymes produced by staphylococci.³ A large number of cephalosporins have also been developed which have varying degrees of resistance to β -lactamases. These include the second-generation cephalosporins (cefotaxim, cefamandole and cefuroxime) and third-generation cephalosporins (cefotaxime, moxalactam, cefoperazone and others).⁴

Several clinical tests have been developed for the detection of β -lactamases. These tests provide rapid information predictive of the development of resistance. Interpretation of β -lactamase test results must consider: the sensitivity of the test for different classes of β -lactamase enzymes, the types of β -lactamases produced by different taxonomic groups of organisms and the substrate specificities of the different β -lactamases.

The most commonly used clinical procedures include the iodometric method, the acidometric method, and a variety of chromogenic substrates.⁵ The iodometric and acidometric tests are generally performed using penicillin as a substrate and, therefore, can only detect enzymes which hydrolyze penicillin. One of the chromogenic cephalosporins, PADAC (Calbiochem-Behring) has been found effective in detecting most of the known β -lactamases except for some of the penicillinases produced by staphylococci, and some β -lactamases produced by anaerobic bacteria.⁶ Another chromogenic cephalosporin, nitrocefin (Glaxo Research), has been found effective in detecting all known β -lactamases including the staphylococcal penicillinases.⁷⁻⁹

For many taxonomic groups of organisms, e.g., *Enterobacteriaceae*, the β -lactamase test is of little value because a diversity of β -lactamase enzymes with different substrate specificities may be produced within the group, or even within a single strain.¹⁰

In other bacteria, for example, penicillin-resistant *Neisseria gonorrhoeae*,¹¹ *Staphylococcus aureus*,^{12,13} *Moraxella catarrhalis*,¹⁴ and ampicillin-resistant *Haemophilus influenzae*,^{5,9,15} only one class of enzyme is produced by resistant strains. The β -lactamase test performed with these organisms enables a prediction of resistance to be made immediately after primary isolation, 18–24 hours prior to the time that growth-dependent susceptibility results would be available.

While the prevalence of β -lactamase-producing enterococci appears to be small, a low inoculum may result in strains going undetected by susceptibility-testing procedures, and routine screening by the nitrocefin disc procedure is recommended.¹⁶

With anaerobic bacteria, the relationship between the production of β -lactamase and resistance to β -lactam antimicrobics is complicated and somewhat similar to *Enterobacteriaceae*. β -lactamases are most commonly found within the *Bacteroides* species,¹⁷ however, β -lactamase-producing strains of *Clostridium butyricum*, *C. perfringens* and *Fusobacterium* sp. have been reported.^{18,19} Among the *Bacteroides* group, a variety of enzymes may be produced with different substrate specificities. The β -lactamases frequently found in strains of *Prevotella melaninogenica* and *P. oralis* are usually specific for penicillins (penicillinase),²⁰ whereas the β -lactamases frequently found in the *B. fragilis* group are cephalosporinases.^{21,22} A variety of cephalosporinases have been reported in the *B. fragilis* group and they include some very active enzymes which can hydrolyze some of the reportedly β -lactamase-resistant cephalosporins such as cefotaxime.^{23,24} Rare strains have been reported which hydrolyze at high rates all known β -lactams including cefotaxim.^{24,25}

Even though the β -lactamases produced by the *B. fragilis* group are most active against cephalosporins, most strains are found to be resistant to penicillin, carbenicillin and ampicillin in growth-dependent susceptibility tests.^{17,26} This finding suggests that the *B. fragilis* group may be intrinsically resistant to penicillins through factors such as permeability barriers,²² or that the β -lactamase is produced in quantities sufficient to overcome the relatively slow hydrolysis rate of the enzyme with penicillins. Evidence which tends to support a contributory role for β -lactamase in the resistance to penicillins is found in reports that the combination of clavulanic acid (a β -lactamase inhibitor) and penicillins is many times more active against *B. fragilis* than is the penicillin alone.²⁷

Whatever the cause or causes of penicillin resistance in *B. fragilis*, all strains should probably be considered resistant.²⁸ The other gram-negative anaerobic strains are probably susceptible to penicillin so long as they are β -lactamase negative.²⁸

PRINCIPLES OF THE PROCEDURE

The BD BBL Cefinase disc is impregnated with the chromogenic cephalosporin, nitrocefin. This compound exhibits a very rapid color change from yellow to red as the amide bond in the β -lactam ring is hydrolyzed by a β -lactamase. When a bacterium produces this enzyme in significant quantities, the yellow-colored disc turns red in the area where the isolate is smeared.

Although other penicillins and cephalosporins may be used as substrates for specific enzymes, nitrocefin has the wide spectrum of susceptibility and sensitivity of the commercially available β -lactams. It is not known to react with other microbial enzymes.²⁹

Each disc is used to test one bacterial strain for the presence of β -lactamase.

REAGENTS

BD BBL Cefinase discs impregnated with nitrocefin.

Warnings and Precautions

EU Only: users should report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

For *in vitro* Diagnostic Use. Do not reuse. For trained laboratory personnel.

These discs are not for use in susceptibility testing.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates and other contaminated materials must be sterilized by autoclaving before discarding.

Nitrocefin induces mutations in certain strains of bacteria (Ames test) and may be sensitizing. Ingestion, inhalation or contact with the skin or eyes should be avoided.

Storage Instructions

Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

Upon receipt, store unopened package at -20 to +8 °C. After use, the BD BBL Cefinase cartridge should be stored in any glass, air tight container containing desiccant and stored at -20 to +8 °C. Discard remaining BD BBL Cefinase discs 60 days after opening blister packaging. The expiration date on the cartridge applies only to discs intact in unopened blister packaging.

Indications of Deterioration

Do not use the cartridge if the discs appear orange or red in color.

SPECIMEN COLLECTION AND HANDLING

This procedure is not to be used directly with clinical specimens or other sources containing mixed microbial flora. The bacteria to be tested must first be isolated as separate colonies by streaking the specimen onto appropriate culture media plates.

PROCEDURE

Material Provided

BD BBL Cefinase discs, 50 discs per cartridge.

Materials Required But Not Provided

Ancillary reagents, quality control organisms and laboratory equipment as required for the procedure.

Test Procedure

1. Using a single disc dispenser, dispense the required number of discs from the cartridge into an empty Petri dish or onto a microscope slide.
2. Moisten each disc with one drop of purified water.
3. With a sterilized loop or applicator stick remove several well-isolated similar colonies and smear onto a disc surface.
4. Observe disc for color change.
5. Alternate procedure: Using forceps moisten disc with one drop of purified water and then wipe across colony.

User Quality Control

Control reference cultures should be run with each group of unknowns. The following organisms are recommended for use as test strains.

| Test Strain | Expected Results |
|--|------------------|
| <i>Staphylococcus aureus</i> ATCC® 29213 | Positive |
| <i>Haemophilus influenzae</i> ATCC 10211 | Negative |

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

RESULTS AND INTERPRETATION

A positive reaction will show a yellow to red color change on the area where the culture was applied. Note: color change does not usually develop over the entire disc. A negative result will show no color change on the disc.

For most bacterial strains a positive result will develop within 5 min. However, positive reactions for some staphylococci may take up to 1 hour to develop.

| Organism | Result | Approx. Reaction Time | Interpretation |
|---|----------|-----------------------|--|
| <i>Staphylococcus aureus</i> | Positive | 1 hour | Resistant to penicillin, ampicillin, carbenicillin and ticarcillin. Probably susceptible to cephalothin, methicillin, oxacillin, nafcillin and other penicillinase-resistant penicillins.* |
| <i>Haemophilus influenzae</i> | Positive | 1 min. | Resistant to ampicillin. Susceptible to cephalosporins.* |
| <i>Neisseria gonorrhoeae</i> and <i>Moraxella catarrhalis</i> | Positive | 1 min. | Resistant to penicillin. |
| <i>Enterococcus faecalis</i> | Positive | 5 min. | Resistant to penicillin and ampicillin |
| Anaerobic bacteria | Positive | 30 min. | Probable identification is <i>Bacteroides</i> species. Probably resistant to penicillin and may be resistant to cephalosporins including cefotaxime and rarely ceftiofur. |

* Susceptibility should be confirmed by growth-dependent susceptibility tests.

Negative results imply but do not guarantee susceptibility.

LIMITATIONS OF THE PROCEDURE

The efficacy of this test in predicting the β -lactam resistance of microorganisms other than *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, staphylococci, enterococci and certain anaerobic bacteria is unproven.

Resistance to β -lactam antibiotics has been on rare occasions reported in some of the above organisms without the production of β -lactamases.^{30,31} In these cases, resistance mechanisms such as permeability barriers have been postulated. Therefore, the β -lactamase test should be used as a rapid supplement and not a replacement for conventional susceptibility testing.

For some strains of staphylococci,¹³ particularly *S. epidermidis*, an inducible β -lactamase has been described that might result in a false-negative β -lactamase reaction with a strain which is resistant to penicillin or ampicillin.

SPECIFIC PERFORMANCE CHARACTERISTICS

In a comparative study of four methods for detecting β -lactamase activity in anaerobic bacteria, the following percentages of agreement with a "standard" employing nitrocefin-saturated filter paper were obtained: Cefinase, 100%; pyridine-2-azo-p-dimethylaniline cephalosporin, 96%; a penicillinase disc using bromocresol purple pH indicator, 72%; the slide iodometric technique, 78%.³²

AVAILABILITY

| Catalog Number | Description |
|----------------|-----------------------|
| 231650 | BD BBL™ Cefinase™, 50 |

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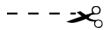





























Change History

| Revision | Date | Change Summary |
|----------|---------|--|
| 03 | 2018-09 | Added RxOnly and IVD symbols. |
| 04 | 2020-10 | Converted printed instructions for use to electronic format and added access information to obtain the document from bd.com/e-labeling . Added "Do not use if package is damaged" and Catalog Number symbols. Added associated catalog numbers. Updated Australian Sponsor address and added New Zealand Sponsor address. General formatting. |


Symbol Glossary

Some symbols listed below may not apply to this product.

US Customers only: For symbol glossary, refer to bd.com/symbols-glossary

| | | | |
|---|---|---|-------------------------------|
|  | Manufacturer |  | Peel |
|  | Use by date |  | Perforation |
|  | Catalog number |  | Do not use if package damaged |
|  | Authorized Representative in the European Community |  | Keep away from sunlight |
|  | <i>In vitro</i> diagnostic medical device |  | Cut |
|  | Temperature limit |  | Date |
|  | Batch code |  | Oil; Fluid |
|  | Contains sufficient for <n> tests |  | Keep away from light |
|  | Consult instructions for use |  | Hydrogen gas is generated |
|  | Do not reuse |  | Patient number |
|  | CE marking; Signifies European technical conformity |  | Fragile; handle with care |
|  | Serial number |  | Start panel sequence number |
|  | In vitro diagnostic (IVD) device for performance evaluation |  | End panel sequence number |
|  | Lower limit of temperature |  | Sequential numbering |
|  | Control |  | Prescription Use Only |
|  | Positive control | | |
|  | Negative control | | |
|  | Sterilized using ethylene oxide | | |
|  | Sterilized using irradiation | | |
|  | Biological risk | | |
|  | Caution | | |
|  | Upper limit of temperature | | |
|  | Keep away from rain | | |
|  | Collection time | | |



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