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Section: Technical Manual	Subject Title: Beta-Lactamase (Cefinase) Test	
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BETA-LACTAMASE (CEFINASE) TEST

Principle

Cefinase discs are intended for use in rapid testing of isolated colonies of *Neisseria gonorrhoeae*, *Staphylococcus* species, *Enterococcus* species, *Hameophilus influenzae* and anaerobic bacteria for the production of beta-lactamase.

The Cefinase disc is impregnated with the chromogenic cephalosporin, Nitrocefin. This compound exhibits a very rapid colour change from yellow to red as the amide bond in the beta lactam ring is hydrolyzed by a beta-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 beta lactams. It is not known to react with other microbial enzymes.

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

Materials

Cefinase discs impregnated with Nitrocefin.

Procedure

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

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Interpretation

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

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

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

Controls: *Staphylococcus aureus* (ATCC 29213): Positive
Haemophilus influenzae (ATCC 10211): Negative

Reference

1. Murray P.A., et al. Manual of Clinical Microbiology, 7th ed. 1999.