Beta-Lactamase Detection in *Neisseria Gonorrhoeae, Hemophilus Influenzae, Enterococcus,* and *Staphylococcus*

I. Principle

Since beta-lactamase production can not be reliably detected by routine susceptibility testing, a nitrocefin (Cefinase) test is performed to detect the enzymes known as beta-lactamases that hydrolyze the beta-lactam ring of penicillins and cephalosporins.

The cefinase disc is impregnated with the chromogenic cephalosporin, nitrocefin. This compound exhibits a very rapid color change from yellow to red when the amine 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 has been smeared.

Some staphylococci may require induction (exposure to a beta-lactam agent) to increase the production of the enzyme to detectable levels.

II. Reagents

A. Cefinase discs impregnated with nitrocefin
B. Microscope slide
C. Sterile water
D. Loop or applicator stick
E. 0.85% NaCI
F. 0.5 McFarland standard

III. Quality Control

A. Quality control is performed on each new lot or shipment with the following organisms:

Test Strains	Expected Result
H. influenzae ATCC 35056	positive (red color)
H. influenzae ATCC 49247	negative (yellow color)

B. If controls do not display expected results, repeat testing and notify supervisor.

IV. Procedure for *N. gonorrhoeae*, *H. influenzae* and *Enterococcus*

A. Dispense the disc from the cartridge onto a microscope slide.
B. Moisten the disc with 1 drop of sterile water.
C. Smear several well-isolated colonies onto the disc surface with a loop or applicator stick.
D. Observe the disc for 5 min for a color change.

V. Procedure for *Staphylococcus*

Select organism from a routine Kirby Bauer test and proceed with step “F” below or perform the following:

A. Using a sterile cotton swab, select 4 or 5 isolated colonies of *Staphylococcus* sp., and emulsify them in 0.85% NaCI. Adjust the turbidity to a 0.5 McFarland standard.

B. Within 15 min of adjusting the inoculum, dip a sterile cotton swab into the inoculum, and rotate it against the wall of the tube above the liquid to remove excess inoculum.
C. Swab the entire surface of a BAP 3 times, rotating the plate approximately 60° between each streaking to ensure even distribution. Rim the inside perimeter of the plate.
D. Apply a 1 μg oxacillin disk to the inoculated BAP. Apply gentle pressure with a sterile stick to ensure complete contact of the disk with the agar.
E. Incubate the plate for 16-18 h at 35°C in ambient air within 15 min of applying the disc.
F. Following incubation, dispense a cefinase disc from the cartridge onto a microscope slide.
G. Moisten the disc with 1 drop of sterile water.
H. Using a sterile loop, remove several colonies from the edge of the zone of inhibition around the 1 μg oxacillin disc, and smear the organism onto the surface of the cefinase disc.
I. Observe the disc for a color change for 60 min.

VI. Results and Interpretation

A. A positive reaction is a color from yellow to red on the area where the organism was applied.
B. A negative result is no color change on the disc.
C. For most bacterial strains, a positive result will develop within 5 min. Organism specific times are as follows:

	Result	Approx. reaction time
Staph aureus	+	60 min
H. influenzae	+	1 min
N. gonorrhoeae	+	1 min
M. catarrhalis	+	1 min
Anaerobic bacteria	+	30 min
Enterococcus faecalis	+	5 min

VII. Limitations

Resistance to beta-lactam antibiotics occurs in some of the above organisms without the production of beta-lactamases. Therefore, the beta-lactamase test should be used as a supplement to and not a replacement for conventional susceptibility testing.

VIII. References

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B. Isenberg, H.D., Clinical Microbiology Procedures Handbook. 1992. Vol 1. ASM, Washington, D.C., p. 5.1.1-5.1.30.

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