

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date

**SOP-Number
MIC.033**

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Date

Title: CEFINASE BETA LACTAMASE TEST

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Annual Review	
By	Date

Title: CEFINASE BETA LACTAMASE TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: CEFINASE BETA LACTAMASE TEST

PURPOSE

Cefinase disks will be used for rapid detection of β -lactamase enzyme produced by some strains of *Haemophilus influenzae*, *Enterococcus* sp., *Neisseria gonorrhoeae*.

PRINCIPLE

The cefinase disk is impregnated with the chromogenic cephalosporin, nitrocefin. Isolated colonies of the test organism are applied to an area on a moistened disk. If β -lactamase is produced by the bacterium, the enzyme will hydrolyze the amine bond in the β -lactam ring of the cephalosporin resulting in the release of the chromogenic compound and development of a red colour in the area on the disk where the organism was applied. Organisms that produce β -lactamase are resistant to treatment with the β -lactam group of antibiotics (some examples are; penicillin, ampicillin and some cephalosporins).

SCOPE

This Standard Operating Procedure applies to technical staff in the microbiology laboratory who have been trained and are competent in the use and performance of this test.

MATERIALS

Cefinase disks (store at -20°C to 8°C in airtight container with dessicant)

NOTE: Change expiration date to 60 days from the date the package is opened.

Empty Petri dish or microscope slide

1 drop of distilled water per disk

Pipette for distilled water

Sterile loop or applicator stick

Isolated colonies of test organism on a non-selective (blood or chocolate) agar

Fine-tipped forceps cleaned with 70% alcohol wipe

QUALITY CONTROL

Frequency: every new lot number or shipment.

Controls/Acceptable Results:

Staphylococcus aureus ATCC 29213 = Positive

Haemophilus influenzae ATCC 10211 = Negative

Corrective Actions for Unacceptable QC Results:

1. Repeat test using fresh subcultures of control organisms and 5 disks for each control organism.
2. If any of the disks gives unsatisfactory results, do not use for patient isolates.
3. Arrange for replacement.

Title: CEFINASE BETA LACTAMASE TEST**Documentation:**

1. Record QC results on β -lactamase (Cefinase) QC sheet.
2. Record unacceptable results and corrective actions on a QC DEVIATION form and submit to supervisor for review.
3. Supervisor will review QC monthly.

PROCEDURE

1. Dispense cefinase disk using clean forceps, into empty Petri dish or onto microscope slide.
2. Moisten disk with 1 drop of distilled water. (Alternatively, you can use the lid of the Petri dish that contains the test organism and put the disk in a drop of condensation to avoid the need for adding distilled water.)
3. With sterile loop or applicator stick remove several well-isolated similar colonies and smear onto disk surface. Take care not to smear agar onto disk.
4. Observe disk for up to 5 minutes for development of a red colour where organism was applied.,
5. Record test results on POSITIVE CULTURE FORM.
6. Discard disk into Biohazard Waste container.

RESULTS AND INTERPRETATION

POSITIVE: Development of a red colour in the area where the organism was applied.

NEGATIVE: No colour change where organism was applied.

Organism	Result	Approximate reaction time	Interpretation
<i>H. influenzae</i>	Positive	1 minute	Resistant to ampicillin
<i>N. gonorrhoeae</i> and <i>Moraxella catarrhalis</i>	Positive	1 minute	Resistant to penicillin
<i>Enterococcus faecalis</i>	Positive	5 minutes	Resistant to penicillin and ampicillin

Negative results imply but do not guarantee susceptibility.

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LIMITATIONS

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

The cefinase β -lactamase test should be used as a rapid supplement but not a replacement for conventional susceptibility testing as there are other mechanisms of resistance to β -lactam antibiotics besides β -lactamase production. .

REFERENCES

BD BBL™ Paper Discs for the Detection of β -lactamase Enzymes, Package Insert, 2004, Becton Dickinson and Company, Sparks, MA, USA

APPENDIX

β -lactamase (Cefinase) QC Sheet