TITLE: ß-Lactamase (Cefinase)	Revision Date: 20-April-2018	Issue Date: 20-April-2016
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Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by:	

PRINCIPLE:

Certain organisms have acquired the capacity to produce enzymes (ß-lactamases) that inactivate ß-lactam antibiotics. Several tests are available to detect the presence of ß-Lactamase, by using different principles. The chromogenic method, as described below, is considered to be the preferred method.

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 ß-lactam ring is hydrolyzed by a ß-lactamase. When a bacterium produces this enzyme in significant quantities, the **yellow** coloured disc turns **red** in the areas where the isolate is smeared.

PURPOSE:

To rapidly test for the production of ß-lactamase in colonies of *Enterococcus faecalis*, *Neisseria gonorrhoeae, Staphylococcus aureus. Moraxella catarrhalis, Haemophilus influenzae* and anaerobic bacteria.

SAMPLE INFORMATION:

Type	Well isolated colonies

REAGENTS and/or MEDIA:

	Reagent #1	Reagent #2
Type	Cefinase discs	Sterile water
Volume	One disc per bacterial strain	1 drop/disc
Stability	Do not use past expiry date	
Storage Requirements	-20°C-8°C in air tight container with desiccant	

SUPPLIES:

- Wooden sticks

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

Avoid contact of the Cefinase discs with eyes or skin and refer to MSDS sheet for Cefinase disc prior to use.

QUALITY CONTROL:

Quality control is set up each day the test is performed using the following control organisms. A QC order is generated in the TQC system.

Positive: *Staphylococcus aureus* ATCC 29213

Negative: *Haemophilus influenzae* ATCC 10211

PROCEDURE INSTRUCTIONS:

Step	Action
Testing a Pure Culture for ß-lactamase Production	
1	To order a ß-lactamase in the system, order **LIS CODE: ^BL**
2	Using forceps dispense the required number of discs from the cartridge into an empty petri dish or onto a microscope slide.
3	Moisten each disc with 1 drop of sterile distilled water – do NOT flood the disc with water.
4	With a sterilized loop or applicator stick, remove several well-isolated similar colonies and smear onto a disc surface.
5	Observe disc for colour change within appropriate time-frame for organism tested. See chart under “Result Interpretation”.
Alternate Procedure	
1	Using forceps moisten disc with one drop of sterile water and then wipe across colony.

LIMITATIONS OF THE PROCEDURE:

Primary isolation medium (e.g. modified Thayer-Martin) may grow ß-lactamase producing bacteria in addition to the pathogen, which may result in a false positive test. Therefore, ensure that the culture being tested is pure.

A negative ß-lactamase result **does not imply** sensitivity to Penicillin or Ampicillin.

INTERPRETATION OF RESULTS:

POSITIVE	**Red** colour development at site of inoculation
NEGATIVE	No colour change

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. Refer to the following chart for expected reaction times.

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

REFERENCES:

- BBL, B. (2004/06). Paper Disc for the Detection of ß-Lactamase Enzymes. *Package Insert*

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	03Nov10	Initial Release	ML Dufresne
1.1	31Jul13	Illustrations added	A Darrach
1.2	07Mar14	Changed to document control number MIC50300	C Russell
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations	C. Russell