MBL-ESBL-AmpC Co-existence Detection Ezy MIC Kit EM137

Antimicrobial Susceptibility Testing

For *In Vitro* Diagnostic use Not for Medicinal Use

EM134 :MBL plus ESBL Detection Ezy MIC™ Strip (ESBL+/ESBL)

ESBL +: Ceftazidime, Cefotaxime, EDTA + Clavulanic acid (0.032 - 4)

ESBL : Ceftazidime, Cefotaxime & EDTA (0.125 -16)

EM135:MBL plus AmpC Detection Ezy MIC™ Strip (AmpC+/AmpC)

AmpC +: Ceftazidime, Cefotaxime, Cloxacillin, EDTA + Clavulanic acid (0.032 - 4)

AmpC : Ceftazidime, Cefotaxime, Cloxacillin & EDTA (0.125 -16)

It is unique co-existence detection kit which contains 10 strips of **MBL Plus ESBL Detection Ezy MIC™ Strip (EM134)** which is coated with Ceftazidime, Cefotaxime, EDTA mixture + clavulanic acid on upper half with highest concentration tapering downward whereas lower half is coated with Ceftazidime, Cefotaxime, EDTA mixture in a reverse direction. and 10 strips of **MBL Plus AmpC Detection Ezy MIC™ Strip (EM135)** which is coated with Ceftazidime, Cefotaxime, Cloxacillin, EDTA mixture + clavulanic acid on upper half with highest concentration tapering downward whereas lower half is coated with Ceftazidime, Cefotaxime, Cloxacillin & EDTA mixture in a reverse direction.

.

Introduction:

Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents as well as phenotypic detection of bacteria. The phenotypic detection system comprises of a predefined quantitative gradient which is used to determine the Inhibitory Concentration (IC values) of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

Principle and Interpretation

The rapid emergence of antibiotic resistance among the hospital pathogens is a serious threat to the management of infectious diseases. β-lactam antibiotics are the most frequently used antimicrobials for empirical therapy. Production of β -lactamases is one of the strategies adopted by bacteria to develop resistance to β-lactam class of antibiotics (1). Often there is also coexistence of multiple beta-lactamase enzymes responsible for beta-lactam resistance in a single isolate, which further complicates treatment options. Resistance to extended spectrum cephalosporins may arise from over expression of the naturally occurring cephalosporinase or acquired beta-lactamases such as extended-spectrum β-lactamases (ESBL), AmpC β-lactamases (AmpC) and metallo-β-lactamases (MBL)(2). The selective pressures which are

generated by the indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as the ESBLs, AmpC β-lactamases and metallo-β-lactamases which have emerged as the most worrisome resistance mechanism which poses a therapeutic challenge to the health care settings (3).

AmpC beta-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of the *Enterobacteriaceae* and a few other organisms, where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and beta-lactamase inhibitor-beta-lactam combinations. In many bacteria, AmpC enzymes are inducible and can be expressed at high levels by mutation. Overexpression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone and is a problem especially in infections due to *Enterobacter aerogenes* and *Enterobacter cloacae*, where an isolate initially susceptible to these agents may become resistant upon therapy. Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal bla(AmpC) gene, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum beta-lactamase production in most parts of the world but may be both harder to detect and broader in spectrum. (4).

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., Ceftazidime, Cefotaxime, and Ceftriaxone) and monobactams (e.g., Aztreonam) but do not affect cephamycins (e.g., Cefoxitin, Cefotetan , Cefmetazole) or carbapenems (e.g., Meropenem, Imipenem, Ertapenem, Doripenem etc) (5). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. If an ESBL is detected, all penicillins, cephalosporins, and Aztreonam should be reported as resistant, even if *in vitro* test results indicate susceptibility. Carbapenems represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant. Due to their broad spectrum of activity and stability to hydrolysis by most -lactamase, the carbapenems have been the drugs of choice for treatment of infections caused by penicillin or cephalosporin resistant gram negative bacilli (6).

Metallo-β-lactamases (MBLs) are β-lactamase enzymes that hydrolyze and confer resistance on carbapenems, but are yet inhibited by chelating agents like ethylene-diamine tetra-acetic acid (EDTA).They are a type of carbapenemases that require zinc ion (Zn2+) as a cofactor for enzyme activity. MBLs have become a serious public health problem with catastrophic consequences for the treatment of bacterial related infections. Their emergence and uncontrolled spread has put the use of the carbapenems under threat. The carbapenems including imipenem, meropenem and ertapenem are broad spectrum antibiotics with high stability against most β-lactamase enzymes. They are the drug of choice for the treatment of infections caused by β-lactam resistant bacteria including those that produce extended spectrum enzymes. The uncommon reduced susceptibility of bacterial pathogens to MBLs as reported in some quarters is a call for concern. The growing resistance of organisms to the carbapenems is a risk to available antibiotics used for treating nosocomial infections (e.g. bacteremia, septicemia, and pneumonia in children). Their unprecedented presence in a clinical setting should be a source of worry to healthcare givers because they limit treatment options. The increased utility of the carbapenems in clinical medicine may have necessitated their emergence and spread. Early detection of MBL-producing bacteria is critical due to the worldwide increase in the occurrence, types and spread of MBLs in both the community and hospital settings, and a carbapenem-intermediate or resistant result arising from antibiotic susceptibility studies should raise the notion of a possible MBL production that warrants confirmation either phenotypically or genotypically (7) .

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (8,10).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (8, 10).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow solidifying. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).

Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, and streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

Note: Production of beta-lactamase is directly proportional to inoculum size.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

Reading of IC (Inhibitory Concentration) values:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin always read the MIC at the point of completion inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for IC determination at a point on the scale above which no resistant colonies are observed close to strip (within 1-3 mm distance from the strip).
5. If the ellipse intersects the strip in between 2 dilutions, read the IC value which is nearest to the intersection

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strips is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

QUALITY CONTROL & INTERPRETATION OF EM134

Interpretation:

Use following interpretive criteria for susceptibility categorization.

Report	Formula	Interpretative Criteria
MBL+ESBL Positive Strain	$\frac{\text{ESBL}}{\text{ESBL+}} => 8$	When the ratio of the value obtained for ESBL **:** the value obtained for ESBL+ is more than 8 or No zone is obtained for ESBL and Zone obtained in ESBL+
MBL Positive Strain (Only MBL present)	$\frac{\text{ESBL}}{\text{ESBL+}} =< 8$	When Ratio of the value obtained for ESBL: the value ESBL+ is less than or equal to 8.
MBL & ESBL (non-conclusive)	-	When no zone of inhibition is obtained on either side. In such cases resistance may be due to mechanisms other than MBL & ESBL production. These have to be further investigated before reporting.

Quality Control:

(This strip should be tested either on ESBL and AmpC non-conclusive strain or confirmed MBL positive Strain. Confirmed clinical isolates may be used as reference positive strain)
Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC cultures on suitable medium incubated appropriately.

Organism	Medium used	Incubation	Standard
K. pneumoniae ATCC 700603 (ESBL positive)	Mueller Hinton Agar	35-37°C for 18 hrs.	Ratio of the value obtained for (ESBL) : the value of (ESBL+) is more than 8 Or No zone is obtained for ESBL and zone obtained for ESBL+

QUALITY CONTROL & INTERPRETATION OF EM135

Interpretation:
Use following interpretive criteria for susceptibility categorization.

Report	Formula	Interpretative Criteria
MBL + AmpC + ESBL Positive Strain	$\frac{\text{AmpC}}{\text{AmpC+}} = > 8$	When the ratio of the value obtained for AmpC : the value of AmpC+ is more than 8 or No zone is obtained for AmpC and zone obtained for AmpC+
MBL + AmpC Positive Strain (ESBL is not present along with MBL)	$\frac{\text{AmpC}}{\text{AmpC+}} = \leq 8$	When Ratio of the value obtained for AmpC **:** the value AmpC+ is less than or equal to 8.
MBL + AmpC (Non - conclusive)	-	When no zone of inhibition is obtained on either side. In such cases resistance may be due to mechanisms other than MBL & AmpC production. These have to be further investigated before reporting.

Quality control:
(This strip should be tested either on ESBL and AmpC non-conclusive strain or confirmed MBL positive Strain. Confirmed clinical isolates may be used as reference positive strain)
Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC cultures on suitable medium incubated appropriately.

Organism	Medium used	Incubation	Standard
K. pneumoniae ATCC 700603 (ESBL positive)	Mueller Hinton Agar	35-37°C for 18 hrs.	Ratio of the value obtained for (AmpC) : the value of (AmpC+) is more than 8 Or No zone is obtained for AmpC and zone obtained for AmpC+

K. pneumoniae ATCC BAA-1144 (AmpC positive)	Mueller Hinton Agar	35-37°C for 18 hrs.	Ratio of the value obtained for (AmpC) : the value of (AmpC+) is less than or equal to 8

Storage & Shelf Life:

1. Once the consignment is received, store applicators at room temperature and Ezy MIC™ Strip container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check wether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Limitation of Test

Ezy MIC™ Strips provides in vitro MIC values, which provides only a possible insinuation of pathogens potential in In vivo susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Grover, N., Sahni, A. K., Bhattacharya, S. (2013). Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. *Med. J Armed Force India.* 69: 4-10.
2. Sivaraman, U., Josephn N.M; Kumari, K., Easow, J.M., Kumar, S., Stephen, S. Srirangaraj, S., Raj, S. (2011). Detection of extended spectrum beta lactamases, ampc beta lactamases and metallobetalactamases in clinical isolates of ceftazidime resistant *Pseudomonas aeruginosa. Braz. J. Microbiol.* vol.42 no.4.
3. Oberoi, L., Singh, N., Sharma, P., Aggarwal,A.(2013). ESBL, MBL and Ampc β Lactamases Producing Superbugs – Havoc in the Intensive Care Units of Punjab India. J Clin Diagn Res. 7(1): 70–73.
4. Jacoby, G.A. (2009) AmpC beta-lactamases. *Clin Microbiol Rev*. 22(1):161-82.
5. Grover, C.N., Sahni, B.A.K and Bhattacharya, C.S. (2013) Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. *Med. J. Armed force India.* 69:4-10
6. Wadekar, M. D., Anuradha, K., Venkatesha, D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. (2013) Int. J. Curr. Res. Aca. Rev. 1(13): 89-95.
7. Chika, E., Malachy, U., Ifeanyichukwu, I., Peter, E., Thaddeus, G., Charles, E. (2014). Phenotypic Detection of Metallo-β-Lactamase (MBL) Enzyme in Enugu, Southeast Nigeria. *American. J. Bio. Chem. Pharma. Sci.* 2 (2) : 2328 – 6814.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.

8. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
10. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
11. Performance standards of Antimicrobial Susceptibility Testing; Twenty Ninth Informational Supplement. M100-S29, Vol. 39, No.1, Jan 2019.

Packing:

Each Pack contains following material packed in air-tight container with a desiccator capsule.

Kit Content:

1. EM134-10ST: MBL Plus ESBL Detection Ezy MIC™ Strip
2. EM135-10ST: MBL Plus AmpC Detection Ezy MIC™ Strip
3. Package inserts
4. Applicator Sticks