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EM137 MBL-ESBL-AmpC
Co-existence Detection Ezy MIC® Kit
For phenotypic detection of MBL-ESBL - AmpC

For use only after MBL presence is confirmed

EM134 : MBL Plus ESBL Detection Ezy MIC Strip (EM134)
EM135 : AmpC Plus MBL Detection Ezy MIC Strip (EM135)

Introduction:
A useful tool for quantitative determination of susceptibility to extended spectrum antibiotics as well as phenotypic detection of beta-lactamase. The Phenotypic detection system comprises of a pre-defined quantitative gradient which is used to determine the Kirby Bauer Concentration (KBC)  of different antimicrobial agents against microorganisms as listed on appropriate agar media, following overnight incubation.

Ezy MIC® Strips Features and Advantages
Ezy MIC® Strips are enzyme detection strips
1) Ezy MIC® Strip is made out of paper on a non-porous material
2) Ezy MIC® Strips have received a horrible number of strips
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 off without opening the set of plate as most commonly used medium as medium in Muller Hinton Agar is aerobic.
5) Shots placed, Ezy MIC® strip is absorbed within 60 seconds and firmly adheres to the agar surface.
6) Unlike the plastic material, it does not form any bubbles underneath and there is no need to penalize the patients.

Background & Principle
Resistance to extended spectrum cephalosporin or carbapenems may arise from over expression of the naturally occurring cephalosporin or carbapenems. Extended spectrum cephalosporin or carbapenems are the most important mechanisms which result in the development of infection in most parts of the world. The occurrence and spread of MBLs in both the community and hospital settings, should raise the notion of a possible MBL production that warrants confirmation either phenotypically or genotypically.

So far there is no simple method to detect presence of ESBLS and AmpC enzymes when co-occur in a single isolate due to the fact that when AmpC enzyme is present, MIC values of ESBL and AmpC enzymes are often higher in the presence of ESBL and AmpC enzymes. Beside, variable levels of expression of these enzymes can lead to a failure in detection of one or both enzymes due to plasmid-mediated AmpC enzymes that are broader in spectrum than extended-spectrum beta-lactamase (ESBLs) or ESBLs enzyme. They are the drug of choice for the treatment of infections caused by beta-lactam resistant bacteria including those that produce extended-spectrum beta-lactam. The increased prevalence of ESBLs and AmpC enzymes in hospital settings has raised the notion of possible presence of MBL in these isolate. The impact of ESBLs and AmpC enzymes has necessitated the need for simple and rapid methods to detect them.

The other set of two strips named as MBL Plus ESBL Detection Ezy MIC Strip (EM134) and AmpC Plus MBL Detection Ezy MIC Strip (EM135) are used to detect the presence of MBLs and AmpC enzymes.

METHOD AND USE OF EZY MIC® STRIPS

Type of specimen
Pure cultures should be derived from specimens obtained patients prior to the initiation of antimicrobial therapy. Specimens can be derived from blood, urine, tissue, sputum, etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (EM134)

Clinical specimen collection, handling and processing
Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated material must be placed in a designated area.

Guidelines for preparation of the medium
Prepare the medium of choice from sterile dried powder according to the directions specified on the label. Pour the required medium into 45 °C and pour in to sterile Petri plates on a level surface, to a depth of 4 ± 0.2 mm and allow solidifying. Petri plates appear on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be placed on a level surface and can be used immediately for handling.

Preparation of inoculum
Obtain the inoculum. Carefully streak designated earings starting before obtaining susceptibility test. Transfer 0.5 ± 0.1 mL of culture of 104 - 106 colonies per 0.5 mL of 104 - 106 colonies per 0.5 mL of broth (EM131 + EM132) and incubate at 35 - 37 °C for 24 hours in an anaerobic atmosphere. Compare the inoculum to the standard inoculum of 104 - 106 colonies per 0.5 mL of broth. Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hours old, on 5 mL of brain heart infusion broth or 5 mL of brain heart infusion broth with 1% yeast extract and incubated at 35 - 37 °C for 24 hours in an anaerobic atmosphere. Compare the inoculum to the standard inoculum of 104 - 106 colonies per 0.5 mL of broth.

Note: Production of beta-lactamase is directly proportional to inoculum size.
5. Performance of Ezy MIC Strips depends on use of proper inoculum and control cultures, 4. Ezy MIC Strip should be used strictly according to procedures described herein. 3. This strip is intended only for agar diffusion method and not for broth dilution method. trained personnel.

These Strips should be tested either on ESBL & AmpC Non Conclusive strain or confirmed Quality Control :

4. If the ellipse intersects the strip in between 2 dilutions, read the IC value which is nearest to Reading of IC (Inhibitory Concentration) values: 

9. Ezy MIC strip should not be repositioned or adjusted once placed.

7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the 6. Lift the applicator along with attached Ezy MIC strip.


2. Oberoi, L., Singh, N., Sharma, P., Aggarwal, A. (2013). ESBL, MBL and AmpC Lactamases EM134 : EM135 : (No zone obtained on both side of EM134 as EDTA alone or ESBL in combination with Cloxacillin and ceftazidime are not found in any of our strains EM135 alone or that combination of ESBL, Cloxacillin and Ampc do not play any role in in-vitro resistance). EM134 : Zone on upper side. lower side may show small or no zone. EM135 : Zone on upper side No zone on lower side Interperation: No zone on both side of EM134 as ESBL alone does not have role in play whereas upper side of EM134 shows inhibitory zone to ESBL in combination with Clavulanic acid and inhibits both ESBL and MBL. Replace zone observed for EM135 as Clavulanic acid on upper side of strip gives inhibitory zone and clavulanic acid present in strip does not play any role.

EM134 : No zones on both side Interperation: MBL and Ampc enzymes are present.

EM134 : Zone on upper side. small hazy zone on lower side EM135 : Larger zone on upper side small hazy on lower side same as obtained on lower side of EM134.

Interperation: MBL, ESBL and Ampc enzymes are expressed.

EM135 : No zone on upper or lower side Interperation: MBL, ESBL and Ampc enzymes are expressed.

(Smaller zone of inhibition is observed on upper side of EM134 due to over production of ESBL. Similarly smaller zone is observed on lower side of EM135 due to over production of AmpC in combination with Clavulanic acid due to combined effect of ESBL, Clavulanic acid and inhibiting presence of MBL, ESBL and Ampc enzymes).