**Case - 6**

**EM134**: Very small zone observed on upper side (i.e. ESBL) side and no zone on lower side (i.e. AmpC) side.

**Interpretation:** MBL and ESBL enzymes are expressed together. A very small zone of inhibition is observed on upper side (i.e. ESBL) side of EM134 due to weak ESBL production whereas enhanced zone of inhibition is observed on upper side (i.e. AmpC) side of EM135 due to moderate AmpC production.

**EM135**: No zone on either side of the strip.

**Interpretation:** Non-Conclusion (Has to be further investigated for mechanism other than MBL, ESBL & AmpC or done deficiency).

**Case - 7**

**EM134**: No zone on either side of the strip.

**EM135**: No zone on either side of the strip.

**Interpretation:** Non-Conclusion (Has to be further investigated for mechanism other than MBL, ESBL & AmpC or done deficiency).

**Case - 8**

**EM134**: No zone obtained on both side.

**EM135**: Zone obtained on upper side i.e. AmpC+ side whereas no zone seen lower side i.e. AmpC- side.

**Interpretation:** MBL, ESBL and AmpC enzymes are expressed together (EDTA alone or EDTA in combination with clavulanic acid or in combination with clavulanic acid) but it does not have role to play. But EDTA in combination with clavulanic acid and clavulanic inhibit all three enzyme viz MBL, ESBL, AmpC and shows inhibitory zone on upper side (AmpC+) of EM135.

**Case - 9**

**EM134**: Clinical isolate (Interpret as MBL + ESBL + AmpC positive).

**EM135**: Clinical isolate (Interpret as MBL + ESBL + AmpC positive).

**Antimicrobial Susceptibility Testing For In Vivo Diagnostic use**

It is a unique phenotypic MBL & AmpC detection strip which is coated with mixture of 3 different antibiotics (EDTA & with & without clavulanic acid) on a single strip in a gradient manner in a reverse direction. On receipt store at -20 °C. Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated material must be disposed of by incineration before discarding (7,15).

**Guidelines for Preparation of the medium**

Prepare the medium of choice from starch medium powder according to the directions specified on the label of the vial. Follow the standard method to 4% TTC. Inoculate on the plate using a stab in the agar surface to achieve a density of 4.0 to 2.0 x 10^8 cfu/mL. All strains should appear on the surface of the medium following incubation do not matter, hence, once poured TM plated containing medium should not be dried on laminar flow and can be used immediately for swabbing.

**Preparation of inoculum**

Use only plate colonies. Selective by Gram staining before starting susceptibility test. Prepare E-5 performing colonies with a needle, wipe on loop to 5-10 microns directly onto M011 and incubate at 35-37°C for 18-24 hours until moderate turbidity develops. Compare the turbidity with standard turbidity with the standard turbidity chart. (3.0 McFarland). Compare the turbidity with the standard turbidity chart. (3.0 McFarland).

**Preparation of inoculum**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic colonies.
2. Prepare a 1% non-sterile cotton swab on a wooden applicator into the standard medium and at the desired level of density applied the open edge of the Ezy MIC strip using aseptic technique.
3. Briefly mix the upper agar surface of the plate with the swab twice, turning the plate at 90° angle between each mixing.
4. Emulate Ezy MIC™ strip along the agar surface at 35-37°C for 18-24 hours. Incubate the plate at 35-37°C for 18-24 hours. Incubate the plate at 35-37°C for 18-24 hours.
5. Lift the applicator along with attached Ezy MIC™ strip.
6. Plate the strip at a desired position on agar plate seeded with test culture. Gently turn the applicator clockwise to avoid dragging resistance. After use, the applicator dishwash and rinse by proxing the Ezy MIC™ strip with 70% alcohol.
7. Place the strip at a desired position on agar plate seeded with test culture. Gently turn the applicator clockwise to avoid dragging resistance. After use, the applicator dishwash and rinse by proxing the Ezy MIC™ strip with 70% alcohol.
8. Direct PRESS Ezy MIC™ strip Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.

**Ezy MIC™ strip does not lose the adsorbed strip in case of extended incubation.**

**Preparation of inoculum**

**METHOD AND USE OF Ezy MIC™ STRIPS**

**Type of specimen**

Pure cultures should be derived from specimens obtained after patients to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, sputum, urine, pus, CSF etc. Clinical specimens should not be sent in the test, which procedure, which includes preparation of inoculations (7, 15).

**Inoculum collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated material must be disposed of by incineration before discarding (7,15).

**Guidelines for Preparation of the medium**

Prepare the medium of choice from starch medium powder according to the directions specified on the label of the vial. Follow the standard method to 4% TTC. Inoculate on the plate using a stab in the agar surface to achieve a density of 4.0 to 2.0 x 10^8 cfu/mL. All strains should appear on the surface of the medium following incubation do not matter, hence, once poured TM plated containing medium should not be dried on laminar flow and can be used immediately for swabbing.

**Preparation of inoculum**

Use only plate colonies. Selective by Gram staining before starting susceptibility test. Prepare E-5 performing colonies with a needle, wipe on loop to 5-10 microns directly onto M011 and incubate at 35-37°C for 18-24 hours until moderate turbidity develops. Compare the turbidity with the standard turbidity chart. (3.0 McFarland). Compare the turbidity with the standard turbidity chart. (3.0 McFarland).
**Interpretation:**

Following illustrations and examples will help you in interpreting your results when EM134 & EM135 are simultaneously tested.

### Case - 1

**Case - 2**

<table>
<thead>
<tr>
<th>Project</th>
<th>Formula</th>
<th>Interpretation Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM134 + EM135 Positive Strain</td>
<td>EM134 + EM135 Positive Strain</td>
<td>Ratio of the value obtained for AmpC : value of AmpC+ is more than 8.</td>
</tr>
<tr>
<td>EM134 + AmpC Positive Strain (ESBL not present along with AmpC)</td>
<td>EM134 + AmpC Positive Strain (ESBL not present along with AmpC)</td>
<td>Ratio of the value obtained for AmpC : value of AmpC+ is more than 8. No zone is obtained for AmpC.</td>
</tr>
<tr>
<td>EM134 Positive Strain</td>
<td>EM134 Positive Strain</td>
<td>Ratio of the value obtained for AmpC : value of AmpC+ is less than or equal to 8.</td>
</tr>
</tbody>
</table>

**Case - 3**

<table>
<thead>
<tr>
<th>Project</th>
<th>Formula</th>
<th>Interpretation Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM134</td>
<td>EM134</td>
<td>EM134 is no zone on lower side i.e ESBL+ side whereas no zone seen on lower side i.e ESBL side</td>
</tr>
<tr>
<td>EM135</td>
<td>EM135</td>
<td>EM135 is no zone on both side i.e ESBL+ side and zone observed on upper side i.e on ESBL side</td>
</tr>
</tbody>
</table>

**Case - 4**

**References**