Antimicrobial Susceptibility Testing
For In Vitro Diagnostic use
Not for Medicinal Use

It is a Phenotypic ESBL detection strip which is coated with Ceftazidime with & without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime + Clavulanic acid with highest concentration tapering downwards, whereas lower half is similarly coated with Ceftazidime in a concentration gradient in reverse direction.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml 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

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., Cefotaxime, Ceftazidime, and Ceftriaxone) and monobactams (e.g., Aztreonam) but do not affect cephemycins (e.g., Cefoxitin and Cefotetan) or carbapenems (e.g., Meropenem or Imipenem). 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

Please refer disclaimer Overleaf
CLSI has developed initial micro dilution screening tests using selected antimicrobial agents (1). Following antimicrobial concentration is to be used for initial screen test:

For *K. pneumoniae*, *K. oxytoca* and *E. coli* use Cefpodoxime 4µg/ml or Ceftazidime 1 µg/ml or Aztreonam 1µg/ml or Cefotaxime 1µg/ml or Ceftriaxone 1µg/ml and for *P.mirabilis* use Cefpodoxime 1µg/ml or Ceftazidime 1 µg/ml or Cefotaxime 1µg/ml (The use of more than one antimicrobial agent for screening improves sensitivity of detection).

Growth at or above the screening concentrations may indicate ESBL production i.e. for *K. pneumoniae*, *K. oxytoca* and *E. coli* MIC of ≥ 8 µg/ml for Cefpodoxime and ≥ 2 µg/ml for Ceftazidime, Aztreonam, Cefotaxime and Ceftriaxone while a MIC of ≥ 2 µg/ml for Cefpodoxime, Ceftazidime and Cefotaxime for *P.mirabilis* is indicative of ESBL production.

Strains of *Klebsiella* spp. and *Escherichia coli* that produce ESBLs may be clinically resistant to therapy with penicillins, cephalosporins, or Aztreonam, despite apparent *in vitro* susceptibility to some of these agents.

CLSI recommends performing phenotypic confirmation of potential ESBL-producing isolates by testing both Cefotaxime and Ceftazidime, alone and in combination with Clavulanic acid (1). Testing can be performed by the broth micro dilution method or by disk diffusion. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 should be used for quality control of ESBL tests (1).

For dilution method; the culture is to be reported as ESBL positive if a ≥ 3 twofold concentration decrease in MIC for antibiotic tested in combination with clavulanic acid vs its MIC when tested alone is obtained.

However, the phenotypic confirmatory test does not detect all ESBLs. Some organisms with ESBLs contain other β-lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. Currently, detection of organisms with multiple β-lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing.

If an isolate is confirmed as an ESBL-producer by the CLSI-recommended phenotypic confirmatory test procedure, all penicillins, cephalosporins, and Aztreonam should be reported as resistant. This list does not include the cephemycins (Cefotetan and Cefoxitin), which should be reported according to their routine test results. If an isolate is not confirmed as an ESBL-producer, current recommendations suggest reporting results as for routine testing. Do not change interpretations of penicillins, cephalosporins, and Aztreonam for isolates not confirmed as ESBLs.

Other isolates of *Enterobacteriaceae*, such as *Salmonella* species and *P. mirabilis*, and isolates of *P.aeruginosa* produce ESBLs. Though screening of *P. mirabilis* for ESBL production is recommended only when it is deemed clinically relevant (e.g. bacteremic isolate). The decision to perform ESBL screening tests to all urine isolates should be made on an institutional basis, considering prevalence, therapy, and infection control issues.
METHOD AND USE OF EZY MIC™ STRIPS

- **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 for 2-8 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.

- **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.

Interpretation:
Use following interpretive criteria for susceptibility categorization.

<table>
<thead>
<tr>
<th>Report</th>
<th>Formula</th>
<th>Interpretative Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL positive strain</td>
<td>CAZ &lt; 8</td>
<td>When the ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is more than 8 or No zone is obtained for CAZ and Zone obtained in CAZ+</td>
</tr>
<tr>
<td></td>
<td>CAZ+</td>
<td></td>
</tr>
<tr>
<td>ESBL negative strain</td>
<td>CAZ ≥ 8</td>
<td>When Ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is less than or equal 8.</td>
</tr>
<tr>
<td></td>
<td>CAZ+</td>
<td></td>
</tr>
<tr>
<td>ESBL (non-conclusive)</td>
<td>CAZ &lt; 8</td>
<td>When no zone of inhibition is obtained on either side. In such cases resistance may be due to mechanisms other than ESBL production. These have to be further investigated before reporting.</td>
</tr>
<tr>
<td></td>
<td>CAZ+</td>
<td></td>
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</tbody>
</table>

QUALITY CONTROL

Quality control of Ezy MIC™ Strips is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium used</th>
<th>Incubation</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae ATCC 700603</td>
<td>Mueller Hinton Agar</td>
<td>35-37°C for 18 hrs.</td>
<td>When the ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is more than 8</td>
</tr>
</tbody>
</table>
### References:

### Storage and Shelf-life:
Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20°C or below. Use before expiry date on the label.

### Packing:
Each Pack contains following material packed in air-tight plastic container with a dessicator capsule.
1. Ceftazidime/ Ceftazidime + Clavulanic acid Ezy MIC™ Strip (10/30/60/90/120/150 Strips per pack)
2. Applicator sticks
3. Package insert

### Zone of inhibition of Ceftazidime/ Ceftazidime + Clavulanic acid Ezy MIC™ strip (EM098) for standard ATCC cultures

<table>
<thead>
<tr>
<th>E.coli ATCC 25922</th>
<th>Mueller Hinton Agar</th>
<th>35-37°C for 18 hrs.</th>
<th>When Ratio of the value obtained for Ceftazidime (CAZ) : the value of Ceftazidime in combination with Clavulanic acid (CAZ+) is less than or equal 8.</th>
</tr>
</thead>
</table>

**Fig. 1:** *Escherichia coli* ATCC 25922  
Ratio of CAZ = \( \frac{1.5}{1.0} = 1.5 \) = ESBL negative

**Fig. 2:** *Klebsiella pneumoniae* ATCC 700603  
Ratio of CAZ = \( \frac{R}{R} = > 8 \) = ESBL positive

**Fig. 3:** *Escherichia coli* Hospital Isolate  
Ratio of CAZ = \( \frac{R}{R} = \text{Non Conclusive} \)

### Disclaimer:
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