**Improved ESBL detection Ezy MIC Strips**

**Storage & Shelf Life:**
- 1) Once the consignment is received, store applicators at room temperature and Ezy MIC Strips 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 if 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.(3,4)

**Limitation of Test**
Ezy MIC Strips provide in vitro MIC values, which provides only a possible insinuation of pathogens potential in its 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:**

**Packing:**
Each Pack contains following material packed in sealed glass vial with a desiccator capsule.

- 1) Improved ESBL detection Ezy MIC Strips (10/30/60/90/120/150 Strips per pack)
- 2) Applicator sticks
- 3) Package insert

**Zone of inhibition of Improved ESBL Ezy MIC strip (EM132)**

**Fig. 1:** Escherichia coli ATCC 25922
Ratio of MIX + = 0.75 = 7.9 = ESBL negative

**Fig. 2:** Klesbiella pneumoniae ATCC 700603
Ratio of MIX + = > 8 = ESBL positive

**Fig. 3:** Clinical Isolate
Ratio of MIX + = Non Conclusive

**Ref.:**
EM132

**Antimicrobial Susceptibility Testing**

**For In Vitro Diagnostic use**

It is a unique Phenotypic ESBL detection strip which is coated with mixture of 3 different antibiotics with & without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime (Mixture) + Clavulanic acid with highest concentration tapering downwards, whereas lower half is similarly coated with Ceftazidime & Cefotaxime (Mixture) in a concentration gradient in 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 at different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation. Ezy MIC Strips exhibit a number of advantages over existing plastic strips.

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 exhibits several advantages over existing plastic strip.
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., Ceftazidime, Cefotaxime, and Ceftriaxone) and monobactams (e.g., Aztreonam) but do not affect cephamycins (e.g., Cefotetan and Cefoxitin) 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 for isolates not confirmed as ESBLs.

Other isolates of Enterobacteriaceae, such as Salmonella species and P. mirabilis, and isolates of P. aeruginosa produce ESBLs. Through screening of P. mirabilis for ESBL 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**

**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 origin. The media from blood, urine, faeces, pus, etc. Direct specimens should not be employed in this test. Refer to the directions specified on the label. Cool the sterilized molten medium appropriately.

**Guideline 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 and lamellar 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 under light conditions. 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 maker of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above.
2. Dip a sterile non-toxic cotton swab onto a wooden applicator onto the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Stir the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each stirring.
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 center 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 repolished or adjusted once placed.
10. Place the test 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-lactamase class of drugs, Amikacin, Vancocmycin, Gentamicin always read the MIC at the point of completion inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glasses.
4. Isolated colonies, microcolonies and haze 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 MIC 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 specimens 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.

**Interpretation:**

The following interpretive criteria for susceptibility categorization:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium used</th>
<th>Incubation Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae, K. oxytoca and E. coli</td>
<td>Mueller Hinton Agar</td>
<td>35-37°C for 18 hrs.</td>
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</tbody>
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