**Antimicrobial Susceptibility Testing**

For In Vitro Diagnostic use

Ezy MIC Strip is a unique phenotypic ESBL & AmpC detection strip which is coated with mixture of 3 different antibiotics with & without cephalosporins on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefixime & Clavulanic Acid Mixture + Ceftazidime with highest concentration tapering downwards whereas lower half is similarly coated with Ceftazidime, Cefixime & Clavulanic Acid Mixture in a concentration gradient manner in reverse direction.

**Introduction**

Ezy MIC strip is useful for quantitative determination of susceptibility of bacteria to antibiotics as well as phenotypic detection of beta-lactamases. The phenotypic detection system comprises of a preformed quantitative gradient which is used to determine the Inhibitory Concentration (IC50) of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC strip exhibits several advantages over existing plastic strip:

1. Ezy MIC strip is made up of porous paper material unlike plastic nonporous 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 used.
5. Once placed, Ezy MIC strip is absorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike plastic material, it does not form air bubbles underneath and hence there is no need to press the strip into place.

**Principle and Interpretation**

AmpC beta-lactamases are usually important cephalosporinases encoded on the chromosomes of many of the Enterobacteriaceae and a few other organisms, where they mediate resistance to cephalosporins, carbapenems, most penicillins, and beta-lactamase inhibitor beta-lactam combinations. In many bacteria, AmpC enzymes are inducible and can be repressed by acylated homoserine lactones (AHL) of the LuxI–LuxR family of signal transduction systems. All AmpC enzymes are beta-lactamases acting on beta-lactam antibiotics. However, unlike the plasmid-encoded AmpC enzymes, AmpC enzymes are associated with the chromosomal DNA and are constitutively produced.

The presence of ESBL producing organisms in a clinical infection can result in treatment failure if one of the above factors does not exist. ESBLs can be difficult to detect because they have a broad level of activity against various cephalosporins. Thus, the choice of which cephalosporins to test for an organism is critical. If an isolate is ESBL producing, all penicillins, cephalosporins, and carbapenems (Doripenem) are expected to be resistant. However, in some instances, the ESBL producers are resistant to carbapenems.

**How to Test**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. Specimens can be of bacterial or fungal isolates derived from blood, sputa, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer appropriate procedure which includes preparation of inocula (1).

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. Swab the entire agar surface of the plate with the swab three time, turning the plate to the 60° angle between each swabbing.

3. Remove Ezy MIC strip container from cold and keep it at room temperature for 15 minutes before opening.

4. 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 agar surface.

5. Once the strip is placed, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

6. DO NOT PRESS EZY MIC STRIP. Within 60 seconds, Ezy MIC strip will be adsorbed to the agar surface and will firmly adhere to the agar surface.

**Guidelines for preparation of the medium**

Use pure colonies. Confirm by Gram staining before susceptibility test. Transfer 4-5 similar colonies with a sterile needle or loop to 5 ml Tryptone Soya Broth (YSW) and incubate at 30-35°C for 24-48 hours under 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 turid suspension at 620 nm).

**Clinical specimen collection, handling and processing**

Follow appropriate procedure for handling specimens as per established guidelines. After use, contaminated materials must be disposed of in autoclaving before discarding (1).

**Preparation of inoculum**

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**Ezy MIC and Ezy MIC<sup>TM</sup> are both trademarks owned by HiMedia Laboratories Pvt. Limited, pertaining to the product logos of MIC test strips.**
Reading of IC (Inhibitory Concentration) Values:

1. Read the plates only when sufficient growth is seen.
2. Read the value where the ellipse intersects the scale on the strip.
3. This strip is intended only for agar dilution method and not for broth dilution method.
4. Ezy MIC™ Strips should be used strictly according to procedures described herein.
5. 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 IC value at a point on the scale above which no resistant colonies are observed close to the strip (within 1-3 mm distance from the strip).
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. Place the unused strips back to recommended temperature.

QUALITY CONTROL

1. Ezy MIC™ Strips are intended for in-vitro diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strips should only be used by at least semi-trained personnel.
3. This strip is intended only for agar dilution method and not for broth dilution method.
4. Ezy MIC™ Strips 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 careful. 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.

Limitation of Test

Ezy MIC™ Strips provides in vitro MIC values, which provides only a possible minimization of pathogen’s 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 tests on the clinical use of an antibiotic in various therapeutic conditions.

References:


Storage & Shelf Life:

1. Once the consignment is received, store applicator at room temperature and Ezy MIC™ Strip container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strips left over from opened package must be kept dry.
4. Strips should be stored at room temperature and in a dry place.
5. Check the batch number and expiry date and make sure they 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 (5,6).

Interpretation:

Following illustrations and examples will help you in interpreting your results when EM132 & EM133 are simultaneously tested.

**Case - 1**

**EM132**: No zone obtained on both the side.
**EM133**: Equal zone upper and lower side (Ratio < 0.8)

**Interpretation**: AmpC – ve (Only AmpC present & ESBL Absent)

**Case - 2**

**EM132**: No zone obtained on both the side.
**EM133**: Zone for MIX+ greater than zone for MIX- (Ratio of MIX+ / MIX- > 1)

**Interpretation**: Both ESBL & AmpC enzymes are present (ESBL under expressed, AmpC over expressed)

**Case - 3**

**EM132**: No zone obtained on both the side.
**EM133**: Zone obtained on MIX+ side whereas no zone seen on MIX- side

**Interpretation**: Both ESBL & AmpC enzymes are present.

(If both ESBL & AmpC are expressed equally, Clevulanic acid and present on upper side of EM132 inhibits only ESBL while Cloxacinillin in lower side of EM133 inhibits only AmpC. However, when both Cloxacinillin and Clevulanic acid are present on upper side of EM133, inhibitory zone is observed as both ESBL & AmpC are inhibited.)