Meropenem with & without EDTA Ezy MIC™ Strips EM092
(MRP+EDTA/MRP)
(Meropenem + EDTA: 1-64 mcg/ml)
(Meropenem : 4- 256 mcg/ml)

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

It is a unique Phenotypic MBL detection strip which is coated with mixture of Meropenem + EDTA and Meropenem on a single strip in a concentration gradient manner. The upper half has Meropenem+ EDTA with highest concentration tapering downwards, whereas lower half is similarly coated with Meropenem 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

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most beta-lactamases, the carbapenems have been the drug of choice for treatment of infections caused by penicillin-or cephalosporin-resistant Gram-negative bacilli especially, extended spectrum β-lactamase (ESBL) producing Gram-negative infections. The carbapenems available for use in India are Imipenem and Meropenem. However, Carbapenem resistance has been observed frequently in non fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp. Resistance to carbapenems is due to carbapenem hydrolyzing enzymes-carbapenemase among the others. These carbapenemase are

class B metallo β –lactamases. Metallo beta lactamase (MBL) belongs to a group β -lactamase which requires divalent cations of zinc as co-factors for enzyme activity. These have potent hydrolyzing activity not only against carbapenem but also against other β -lactam antibiotics. The genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. The genes responsible for MBL production may be chromosomally or plasmid mediated and hence poses a threat of spread of resistance by gene transfer among the Gram-negative bacteria. Thus, MBL-producing *Pseudomonas aeruginosa* isolates have been reported to be important causes of nosocomial infections . The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial chemotherapy.
Various methods have been recommended for screening MBL. These include the modified Hodge test, double disc synergy test using Meropenem and EDTA discs and dilution methods using Meropenem with and without EDTA. For interpretation using dilution methods refer to the interpretation section.

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 ± 0.2mm 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 for2-8 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 value where the ellipse intersects the scale on the strip.
3. For bactericidal drugs such as Amikacin, Vancomycin, Gentamicin and members of β-lactams class of drugs, always read the value at the point of complete 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 IC values determination 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).
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.

Report	**Formula**	**Interpretative Criteria**
MBL positive strain	$\frac{MRP}{MRP+EDTA} = >8$ $\frac{MRP}{MRP+EDTA} = \frac{\geq 256}{<64}$ $\frac{MRP}{MRP+EDTA} = \frac{\geq 256}{<1}$	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP+EDTA) is more than to 8 or If zone is observed on the side coated with Meropenem+EDTA & no zone is observed on the opposite the side coated with Meropenem, interpret the culture as MBL positive.
MBL negative strain	$\frac{MRP}{MRP+EDTA} = \leq 8$	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP+EDTA) is less than or equal to 8

MBL (non-determinative)	MRP / MRP+ EDTA = ≥256 / >64 MRP / MRP+ EDTA = ≤4 / <1	When no zone of inhibition is obtained on either side. In such cases resistance may be due to mechanisms other than MBL production. These have to be further investigated before reporting. or If the zones obtained are below the lowest concentration on both the sides, the strain has to be tested with concentrations below the lowest concentration on the strips before reaching to any conclusion.

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.

Organism	**Medium used**	**Incubation**	**Standard**
Stenotrophomonas maltophila ATCC 13636	Mueller Hinton Agar	35-37°C for 18 hrs.	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP+EDTA) is more than 8
Pseudomonas aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP+EDTA) is less than or equal to 8

References:

1. Performance standards of Antimicrobial Disc Susceptibility Tests, M100 S21 CLSI Vol. 31 No.1, Jan 2011.

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) Meropenem with & without EDTA Ezy MIC™ Strips (30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert