# Meropenem with & without EDTA Ezy MIC™ Strip EM092
# (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 capable of detecting Enterobacteriaceae possessing MBLs (metallo beta lactamase) including strains with the recently discovered NDM-1 resistant gene. This Strip 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 adsorbs 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 b-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 b-lactamases. Metallo beta lactamase (MBL) belongs to a group b-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 b-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.

**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 isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **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 to solidify. 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, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood is recommended.
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 15-30 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 pre-spread 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.

**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 specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip 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:**

Use following interpretive criteria for susceptibility categorization.

| Report | Formula | Interpretative Criteria |
|---|---|---|
| MBL positive strain | MRP / MRP + EDTA = >8<br><br>MRP / MRP + EDTA = ≥256 / <64<br><br>MRP / IPM+ EDTA = ≥256 / <1 | When the ratio of the value obtained for Meropenem(MRP) : the value of Meropenem + EDTA (MRP +EDTA) is more than to 8<br>or<br>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 | MRP / MRP + EDTA =≤8<br>or<br>MRP / MRP + EDTA = ≤4 / <1 | When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP +EDTA) is less than or equal to 8<br>or<br>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. |
| MBL (non-determinative) | MRP / MRP + EDTA = ≥256 / >64 | When no zone of inhibition is obtained on either side.<br>In such cases resistance may be due to mechanisms other than MBL production. These have to be further investigated before reporting. |

**Quality Control**

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

| Organism | Medium used | Incubation | Standard |
|---|---|---|---|
| *S.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. |
| *K. pneumoniae* ATCC BAA-2146 | 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. |

**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 whether 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 (2, 3).

**Limitation of Test**

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens 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 test on the clinical use of an antibiotic in various therapeutic conditions.

**Packing:**

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

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