Technical Information

Imipenem with & without EDTA Ezy MIC Strips (IPM+EDTA/IPM) (Imipenem + EDTA: 1-64 mcg/ml) (Imipenem : 4- 256 mcg/ml)

Product Code: EM1078

Antimicrobial Susceptibility Testing — Not for medical use
For *In Vitro* Diagnostic use

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

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 (DM1011) 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 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.

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 strips are 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	IPM / IPM+ EDTA = >8 IPM / IPM+ EDTA = ≥256 / <64 IPM / IPM+ EDTA = ≥256 / <1	When the ratio of the value obtained for Imipenem (IPM) : the value of Imipenem + EDTA (IPM+EDTA) is more than to 8 or If zone is observed on the side coated with Imipenem+EDTA & no zone is observed on the opposite the side coated with Imipenem, interpret the culture as MBL positive.
MBL negative strain	IPM / IPM+ EDTA = ≤8 IPM / IPM+ EDTA = ≤4 / <1	When the ratio of the value obtained for Imipenem (IPM) : the value of Imipenem + EDTA (IPM+EDTA) is less than or equal to 8 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.
MBL (non-determinative)	IPM / IPM+ EDTA = ≥256 / >64	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.

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
K. pneumoniae ATCC BAA-2146	Mueller Hinton Agar	35-37°C for 18 hrs	When the ratio of the value obtained for Imipenem (IPM): the value of Imipenem + EDTA (IPM+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 Imipenem (IPM): the value of Imipenem + EDTA (IPM+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 Strip 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) Imipenem with & without EDTA Ezy MIC Strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Further Reading

1. Varaiya, A., Kulkarni, N., Kulkarni, M., Bhalekar, P., Dogra, J.(2008). Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients. Indian J Med Res 127, April 2008, pp 398-402.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Performance standards of Antimicrobial Susceptibility Testing; Twenty Ninth Informational Supplement. M100-S29, Vol. 39, No.1, Jan 2019.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
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