
Ertapenem / Ertapenem + Boronic Acid Ezy MIC[™] Strips **EM141**
(ETP/ETP+)

(For KPC Detection)

Ertapenem (ETP): 0.125 – 8

Ertapenem + Boronic Acid (ETP+): 0.032 – 2

Antimicrobial Susceptibility Testing

For *In Vitro* Diagnostic use

Not for Medicinal Use

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

Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality. Infections caused by bacteria-producing Klebsiella pneumoniae carbapenemases (KPCs) are becoming an increasingly significant problem worldwide since the first detection of these enzymes greater than a decade ago. KPCs are an important mechanism of resistance for an increasingly wide range of Gram-negative bacteria and are no longer limited to K pneumoniae. KPC-producing bacteria are often misidentified by routine microbiological susceptibility testing and incorrectly reported as sensitive to carbapenems; however, resistance to the carbapenem antibiotic ertapenem is common and a better indicator of the presence of KPCs (1).

Although KPCs do not represent the first or the sole mechanism of carbapenem resistance, they are remarkable because they are often not detected by routine susceptibility screening and possess an exceptional potential for dissemination. In addition to the infection control challenges that have arisen, infections caused by these organisms present clinicians with serious treatment challenges, due to limited antibiotic options (1).

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 (2,4).

- **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 (2,4).

- **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 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 for 2-8 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. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. Please ignore resistant colonies while interpreting results.
4. 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 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
KPC positive strain	$\frac{\text{ETP}}{\text{ETP}+} = >8$ $\frac{\text{ETP}}{\text{ETP}+} = \geq 8$ $\text{ETP}+ < 2$	<p>When the ratio of the value obtained for Ertapenem (ETP) : the value of Ertapenem + Boronic acid (ETP+) is more than 8</p> <p style="text-align: center;">or</p> <p>If zone is observed on the side coated with Ertapenem + Boronic acid (ETP+) & no zone is observed on the side coated with Ertapenem, interpret the culture as KPC positive.</p>
KPC negative strain	$\frac{\text{ETP}}{\text{ETP}+} = \leq 8$	When the ratio of the value obtained for Ertapenem (ETP) : the value of Ertapenem + Boronic acid (ETP+) is less than or equal to 8
KPC (Non - conclusive)	$\frac{\text{ETP}}{\text{ETP}+} = \geq 8$ $\text{ETP}+ > 2$	<p>When no zone of inhibition is obtained on either side.</p> <p>In such cases resistance may be due to mechanisms other than KPC production. These have to be further investigated before reporting.</p>

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
<i>K. pneumoniae</i> ATCC BAA-1705	Mueller Hinton Agar	35-37°C for 18 hrs.	When the ratio of the value obtained for Ertapenem (ETP) : the value of Ertapenem + Boronic acid (ETP+) is more than to 8
<i>K. pneumoniae</i> ATCC BAA-1706	Mueller Hinton Agar	35-37°C for 18 hrs.	When the ratio of the value obtained for Ertapenem (ETP) : the value of Ertapenem + Boronic acid (ETP+) 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 -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 (3,4).

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.

References:

1. Arnold, R.S., Thom, K.A., Sharma, S., Phillips, M., Johnson, J.K, and Morgan, D. J. (2011). Emergence of *Klebsiella pneumoniae* Carbapenemase (KPC) Producing Bacteria. South Med J. 2011 January ; 104(1): 40–45.
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.

Packing:

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

- 1) Ertapenem/ Ertapenem + Boronic acid Ezy MIC™ Strips (10/30/60/90/120/150 Strips per pack)
- 2) Applicator sticks
- 3) Package insert

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.