It is a unique MIC determination paper strip which is coated with Ceftizoxime on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the test organism.

**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.

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

Please refer disclaimer Overleaf
**Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood. For *Haemophilus* spp, *Haemophilus Test Agar Base* (M1259) with added supplement (FD117) is to be used.

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.

**MIC Reading:**

1. Read the plates only when sufficient growth is seen.

2. Read the MIC where the ellipse intersects the MIC scale on the strip.

3. For bactericidal drugs such as Ceftriaxone and other members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin always read the MIC at the point of completion 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 heterogeneity of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).

5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.

6. Always round up these values to the next two-fold dilution before categorization. For example: Ceftriaxone showing reading of 0.75 mcg/ml should be rounded up to next concentration ie. 1.0 mcg/ml.

7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.

8. When growth occurs along the entire strip, report the MIC as ≥ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

**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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium used</th>
<th>Incubation</th>
<th>Interpretative Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤ S</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>35-37°C for 18 hrs.</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>other non-Enterobacteriae</td>
<td>35-37°C for 18 hrs.</td>
<td>8</td>
<td>16-32</td>
</tr>
<tr>
<td>Haemophilus spp</td>
<td>35-37°C for 20-24hrs at 5% CO₂</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>N.gonorhoeae</td>
<td>35-37°C for 20-24hrs at 5% CO₂</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>35-37°C for 20-24hrs under anaerobic condition</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

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.

Following are the reference MIC values (mcg/ml) range for Ceftizoxime.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium used</th>
<th>Incubation</th>
<th>Std. Quality limits (mcg/ml)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus ATCC 29213</td>
<td>Mueller Hinton Agar</td>
<td>35-37°C for 18 hrs.</td>
<td>2.0 - 4.0 - 8.0</td>
<td></td>
</tr>
<tr>
<td>E.coli ATCC 25922</td>
<td>Mueller Hinton Agar</td>
<td>35-37°C for 18 hrs.</td>
<td>0.03 - 0.06 - 0.12</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>Mueller Hinton Agar</td>
<td>35-37°C for 18 hrs.</td>
<td>16.0 - 32.0 - 64.0</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae ATCC 49619</td>
<td>Mueller Hinton Agar w/ 5% Sheep Blood</td>
<td>35-37°C for 20-24hrs at 5% CO₂</td>
<td>0.12 - 0.25 - 0.5</td>
<td></td>
</tr>
<tr>
<td>H.influenzae ATCC 49247</td>
<td>Haemophilus Test Medium</td>
<td>35-37°C for 20-24hrs at 5% CO₂</td>
<td>0.06 - 0.12 - 0.25 - 0.5</td>
<td></td>
</tr>
</tbody>
</table>

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.
References:

Packing:
Each Pack contains following material packed in sealed glass vial with a desiccator capsule.
1) Ceftizoxime 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.