

HiPer[®] Ezy MIC[™] Teaching Kit

Product Code: HTM006

Number of experiments that can be performed: 10

Duration of Experiment:

Day 1: Preparation of media and revival of strains

Day 2: Protocol

Day 3: Observations and Results

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
 - Store the culture stabs and EzyMIC[™] strips at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)



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Index

Sr. No.	Contents	Page No.
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	4
5	Materials Required But Not Provided	4
6	Storage	4
7	Important Instructions	5
8	Procedure	5
9	Observation and Result	6
10	Interpretation	7
11	Troubleshooting Guide	7

Aim:

To determine the minimum inhibitory concentration (MIC) for antimicrobial susceptibility

Introduction:

The minimum inhibitory concentration (MIC) of an organism is defined as the minimum amount of antibiotic required to inhibit the growth of the test organism over a specified time interval which is related to the growth rate of the bacteria. It is very important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. The determination of the MIC involves a semi-quantitative test procedure which measures the least concentration of an antimicrobial agent required to prevent the microbial growth. Ezy MIC™ Test is based on arraying a concentration gradient of each antibiotic on a strip where the concentration values are marked on both side of the strip so that one can easily locate corresponding concentrations.

Principle:

Minimum inhibitory concentration or MIC is a measure of the lowest concentration of an antimicrobial agent or antibiotic that is required to inhibit the visible growth of a microorganism after overnight incubation. MIC is the most basic laboratory measurement for the determination of antimicrobial activity against a microorganism. The MICs give a clue during a clinical therapy where low-level of microbial resistance is suspected. A microorganism is called resistant to a certain antimicrobial agent when the organism does not respond to it irrespective of the dosage. This is developed through mutation or plasmid exchange between bacteria of the same species. The term susceptible means that the microorganism is inhibited by a concentration of antimicrobial agent and is likely to respond to the treatment with that at the recommended dosage.

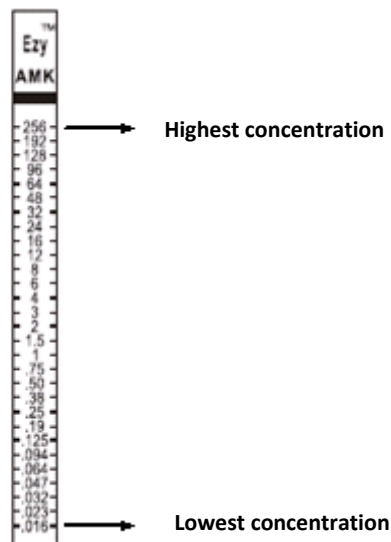


Fig 1: Ezy MIC™ strip is coated with an antibiotic in a concentration gradient manner

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 MIC in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation. The Ezy MIC™ strips are unique paper strip which is coated with antibiotics in a concentration gradient manner. This strip

is made of porous paper material and has MIC values printed on both sides identically. The antimicrobial agent is evenly distributed on either side of the strip and hence it can be placed on either ways on the agar surface. Once placed, the strip is adsorbed within 60 seconds and it firmly adheres to the agar surface. On application of the strip to the pre-inoculated agar, the antibiotic releases from the strip forming a defined concentration gradient in the area around the strip. After appropriate incubation of the plates, a zone of inhibition is formed on either side of Ezy MIC™ strip. The MIC is easily read as the value printed on the strip where the growth of the organism touches the strip. If the growth intersects on the line between two dilutions then the MIC is read as the value in the lower one. Ezy MIC™ Teaching Kit contains Ezy MIC™ strips which allow for the rapid and accurate determination of the MIC of an organism to an antibiotic for the improvement of patient management.

Kit Contents:

This kit can be used to determine the sensitivity of bacteria against various antibiotics.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 PR	
1.	TKC030	Culture Stab (<i>Staphylococcus aureus</i> ATCC29213)	1 No.	2-8°C
2.		Ezy MIC™ Strips		
a.	EM001	Amikacin	10*	2-8°C
b.	EM004	Azithromycin	10*	2-8°C
c.	EM017	Ciprofloxacin	10*	2-8°C
d.	EM029	Linezolid	10*	2-8°C
e.	EM060	Vancomycin	10*	2-8°C
3.	PW005	Sterile cotton swabs	55 Nos.	RT
4.	MV173	Mueller Hinton Agar	65 g	RT
5.	TKC376	Sterile Saline Solution	11 ml	2-8°C
6.	TKC375	Applicator sticks	10 Nos.	RT

* Ezy MIC™ Strips are provided in separate packs. Each pack contains 10 strips of the corresponding antibiotic.

Materials Required But Not Provided:

Glasswares: Sterile test tubes, sterile petriplates

Other requirements: Incubator, Micropipettes, Tips, Inoculation loop, Distilled Water

Storage:

HiPer® Ezy MIC™ Teaching Kit is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store culture stabs and MIC strips at 2-8°C. Other reagents can be stored at room temperature (15-25°C)

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Perform all the microbiological experiments under aseptic conditions.
3. **Preparation of Mueller Hinton (MH) Agar plates (150 ml):** Suspend 5.7 g of MH agar into 150 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 °C and pour 20 ml of media on sterile petri plates.
4. One applicator stick can be used multiple times.

Procedure:

Day 1: Revival of Strains

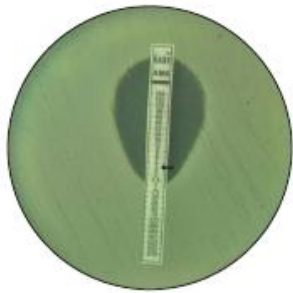
1. Open the vial containing culture and resuspend the cells with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto MH agar plate.
3. Incubate overnight at 37°C.

Day 2: Ezy MIC™ Test

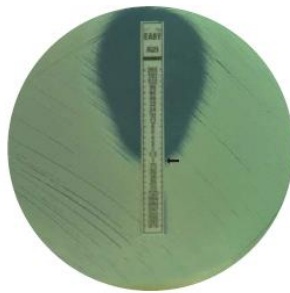
1. Take five MH agar plates and label them as 1 to 5.
2. Take a loopful of culture from the plate into a sterile test tube containing 1 ml of sterile saline. Mix the tube thoroughly.
3. Take a sterile cotton swab and dip it into test tube. While taking out the swab from the tube rotate the soaked swab firmly against the upper inside wall of the tube to get rid of excess fluid.
4. Spread the cotton swab containing the culture evenly onto the Mueller Hinton Agar plate labeled as 1.
5. Repeat step 4 for other plates i.e. plate number 2 to 5. While spreading make sure to streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
6. Remove Ezy MIC™ strips from cold storage and keep it at room temperature for 15 – 20 minutes before opening. Remove one applicator stick from the bag stored at room temperature.
7. Hold the applicator in the middle and gently press its broader sticky side on the centre of Amikacine Ezy MIC™ strip.
8. Lift the applicator along with attached Ezy MIC™ strip. Place the strip at a desired position on the Mueller Hinton agar plate labeled as 1 (swabbed with test culture). Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
9. Do not press the strip. Ezy MIC™ strip is adsorbed and will firmly adhere to the agar surface within 60 seconds. Once placed, the strip should not be repositioned or adjusted once placed.
10. Similarly, follow steps 6 to 8 for other antibiotics. Note down the plate number and the corresponding MIC strip that is impregnated.
11. Incubate the plates at 37° C and observe them after 16 - 18 hours (when the growth is sufficient).

Observation and Result:

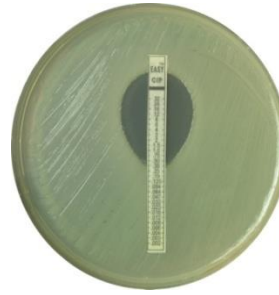
Observe the plates for MIC where the ellipse intersects the MIC scale on the strip and note down the corresponding values.



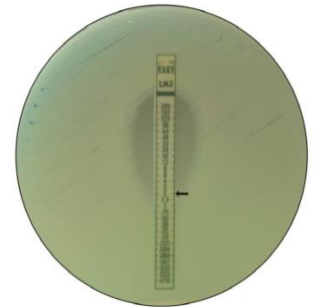
**Amikacin MIC: 1.5 mcg/ml
(Plate No. 1)**



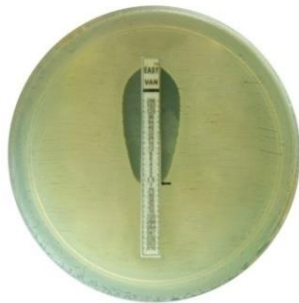
**Azithromycin MIC: 1.0 mcg/ml
(Plate No. 2)**



**Ciprofloxacin MIC: 1.5 mcg/ml
(Plate No. 3)**



**Linezolid MIC: 2.0 mcg/ml
(Plate No. 4)**



**Vancomycin MIC: 1.0 mcg/ml
(Plate No. 5)**

Fig 2: Plates showing MIC of different antibiotics against *S. aureus*

Reference MIC values for the antibiotics:

Antibiotics	Microorganisms to be tested	Std MIC values (mcg/ml)
Amikacin	<i>S. aureus</i> (ATCC 29213)	1.0 - 2.0 - 4.0
Azithromycin	<i>S. aureus</i> (ATCC 29213)	0.5 - 1.0 - 2.0
Ciprofloxacin	<i>S. aureus</i> (ATCC 29213)	0.12 - 0.25 - 0.5
Linezolid	<i>S. aureus</i> (ATCC 29213)	1.0 - 2.0 - 4.0
Vancomycin	<i>S. aureus</i> (ATCC 29213)	0.5 - 1.0 - 2.0

Use the following interpretive criteria for susceptibility categorization:

Microorganism Tested	Antibiotic Used	Interpretative Criteria		
		< S	I	>R
<i>S. aureus</i> (ATCC 29213)	Amikacin	16	32	64
<i>S. aureus</i> (ATCC 29213)	Azithromycin	2	4	8
<i>S. aureus</i> (ATCC 29213)	Ciprofloxacin	1	2	4
<i>S. aureus</i> (ATCC 29213)	Linezolid	4	-	8
<i>S. aureus</i> (ATCC 29213)	Vancomycin	2	4 - 8	16

S- Sensitive
I-Intermediate
R- Resistant

Interpretation:

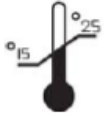
The appearance of the zone of inhibition around the antibiotic strip indicates that *S. aureus* is sensitive to the antibiotics tested here namely, Amikacin, Azithromycin, Ciprofloxacin, Linezolid and Vancomycin. The minimum inhibitory concentration of these antibiotics can be determined for *S. aureus* which in turn helps in selecting most appropriate treatment regimen.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	No clear zone of inhibition	Antibiotic may have been degraded	The EzyMIC™ strips should be stored at 2-8°C
		Uneven bacterial growth	Ensure that the culture is evenly streaked on the plate

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature



Do not use if package is damaged



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