Dual MRSA Detection EZY MIC Strip EM063

OXA : 0.064 – 8.0 mcg/ml
VAN : 0.19 -16.0 mcg/ml
Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use Not for Medicinal Use

It is a unique MIC determination paper strip which is coated with two different antibiotics on a single strip in a concentration gradient manner. The upper half has Oxacillin with a highest concentration tapering downwards and capable of showing MIC in the range of 0.064 – 8.0 mcg/ml, whereas lower half is similarly coated with Vancomycin concentration gradient in reverse direction to give MIC in the range of 0.19 – 16.0 mcg/ml.

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.

CLSI RECOMMENDATION FOR DETECTION OF OXACILLIN REASISTANCE

- Of the penicillinase-stable penicillins, Oxacillin is preferred for *in vitro* testing. Oxacillin is more resistant to degradation in storage and is more likely to detect heteroresistant staphylococcal strains. Oxacillin susceptibility results can be applied to the other penicillinase-stable penicillins like Cloxacillin, Dicloxacillin, Methicillin, Flucloxacillin and Naficillin.
- Addition of 2% NaCl is required for dilution testing of Oxacillin to improve the detection of heteroresistant MRSA.
- The use of direct colony suspension method for preparation of inoculum is necessary.
- Incubate tests to detect MRS for full 24 hours at 35± 2°C when using Oxacillin (testing at temperature above 35°C may not detect MRS).

CLSI RECOMMENDATION FOR VANCOMYCIN SENSITIVITY TEST

High molecular weight antibiotics such as Vancomycin do not diffuse in concentration gradient manner while diffusing through the agar medium when the disc susceptibility test is employed. The Antimicrobial Susceptibility Testing using disc diffusion test does not differentiate vancomycin-susceptible isolates of *S.aureus* from Vancomycin intermediate isolates, nor does the test differentiates among Vancomycin–susceptible, intermediate, and resistant isolates of coagulase-negative staphylococci, all of which may give similar size zones of inhibition.
CLSI therefore recommends that MIC test should be performed to determine the susceptibility of all isolates of staphylococci to Vancomycin .

Usefulness of Vancomycin Ezy MIC™ strip

1) Besides obtaining accurate MIC values for Gram- positive cultures, VISA (Vancomycin Intermediate *Staphylococcus aureus*) can be detected when isolated colonies appear within the zone of inhibition of Vancomycin particularly when 1.0 MacFarland inoculum is used and MIC is read on full 48 hrs incubation. The sensitivity of the method can be further enhanced for better detection of VISA/ VRSA (Vancomycin Resistant *Staphylococcus aureus* / hVISA (Hetro Vancomycin Intermediate *Staphylococcus aureus*) using BHI agar with higher inoculum and 48 hr incubation.

USEFULNESS OF DUAL MRSA EZY MIC STRIPS

1) MSSA/E can be detected when culture shows sensitivity to both Oxacillin and Vancomycin in terms of MIC values.
2) MRSA/E can be identified when culture is resistant to Oxacillin but sensitive to Vancomycin.
3) hVISA can be detected when isolated colonies appear within the zone of inhibition of Vancomycin when 1.0 McFarland inoculum is used and the MIC is read on full 48 hrs incubation. The sensitivity of the method can be further enhanced for better detection of VISA/ VRSA/ hVISA, using BHI agar with higher inoculum and 48 hr incubation.
4) Hetro Oxacillin resistant nature of the strain can be easily observed based on Oxacillin resistant colonies appearing in the zone of inhibition.
5) In short, Dual MRSA Detection EZY MIC strip possesses the ability to detect MSSA/E, MRSA/E, hVISA, VRSA and any combination of these resistant mechanisms.
6) Please note that Oxacillin resistance in some rare cases can also be observed due to other mechanism other than *mec*A. Tests for *mec*A or the protein expressed by *mec*A, the penicillin-binding protein 2a (PBP2a, also called PBP 2'), are the most accurate methods for prediction of resistance to Oxacillin in MRSA strains and can be used to confirm results for isolates of staphylococci from serious infections.

METHOD AND USE OF EZY MIC™ STRIPS

- **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 yields 10^5 - 10^6 cells/ml).
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 (with added 2% NaCl for detection of MRSA or MRSE.)
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.

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 Oxacillin, Vancomycin, Gentamicin and other members of β-lactams class of drugs, 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 hetero nature 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: Amikacin 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.

Interpretation:

Use following interpretive criteria for susceptibility categorization of Oxacillin

When testing	**Interpretative Criteria**		
	≤ S	**I**	**≥ R**
S.aureus and *S.lugdunesis*	2	-	4
Coagulase-negative Staphylococci except *S.lugdunesis*	0.25	-	0.5

Use following interpretive criteria for susceptibility categorization of Vancomycin.

When testing	**Interpretative Criteria**		
	≤ S	**I**	**≥ R**
Staphylococcus	2	4 - 8	16
Coagulase negative *Staphylococci* spps. and *Enterococci*	4	8 -16	32*
S.pneumoniae, Streptococcus spps. Beta haemolytic group, *Streptococcus* spps. Viridans group	1	-	-

*Using, Dual MRSA Detection EZY MIC strip, MIC determination for *E. faecalis* ATCC 29212 can not be established since highest concentration is 8.0 mcg/ml.

QUALITY CONTROL

Quality control of Ezy MIC™ Strips 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 Oxacillin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.125 – 0.25 – 0.5
E.faecalis ATCC 29212*	Mueller Hinton Agar	35-37°C for 18 hrs.	8 – 16 - 32
S.aureus ATCC 43300 (MRSA) *	Mueller Hinton Agar + 2 % NaCl	35-37°C for 24 hrs.	16 -32 – 64 (No zone would be obtained for MRSA on side coated with oxacillin as the highest concentration is 8 mcg/ml)

* Using Oxacillin-Vancomycin Ezy MIC™ Strip MIC determination for *E.faecalis* ATCC 29212 & *S.aureus* ATCC 43300 can not be established since highest concentration is 8.0 mcg/ml

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

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.5 – 1.0 – 2.0
E.faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	1.0 – 2.0 – 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.125 – 0.25 – 0.5

References:

1. Performance standards of Antimicrobial Disc Susceptibility Tests, M100- S21 CLSI Vol. 31 No.1, Jan 2011.

Storage and Shelf-life:
Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20 or below. Use before expiry date on the label.

Packing:
Each Pack contains following material packed in air-tight plastic container with desiccator's capsule.

1) Oxacillin-Vancomycin Ezy MIC™ strips (30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

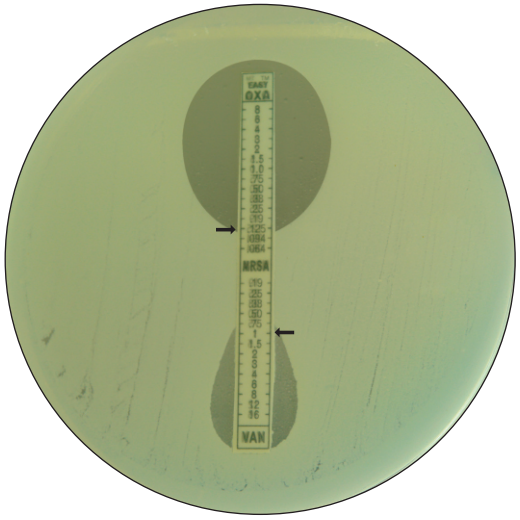

Fig. 1: *Staphylococcus aureus* ATCC 29213
Showing MIC : 0.125mcg/ml for Oxacilin
& MIC : 1mcg/ml for Vancomycin

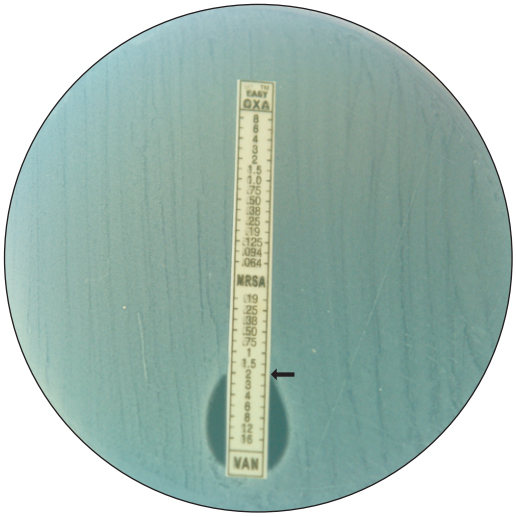

Fig. 2: *Enterococcus faecalis* ATCC29212
Showing MIC of 2mcg/ml for Vancomycin
& MIC of > 8 mcgIml for Oxacillin