Technical Information

Oxacillin-Vancomycin Ezy MICTM Strip

OXA: 0.064 - 8.0 mcg/ml

VAN: 0.19 -16.0 mcg/ml

Product Code: EM1063

Antimicrobial Susceptibility Testing — Not for medical use

For *In Vitro* Diagnostic 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 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.

CLSI RECOMMENDATION FOR DETECTION OF OXACILLIN RESISTANCE

1.Of the penicillinase-stable penicillin, 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 penicillin like Cloxacillin, Dicloxacillin, Methicillin, Flucloxacillin and Naficillin.

2.Addition of 2% NaCl is required for dilution testing of Oxacillin to improve the detection of heteroresistant MRSA.

3.The use of direct colony suspension method for preparation of inoculum is necessary.

4.Incubate plates 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

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 (Hetero Vancomycin Intermediate Staphylococcus aureus) using BHI agar with higher inoculum and 48 hr incubation.

USEFULNESS OF Oxacillin-Vancomycin 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) Hetero 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 mecA. Tests for mecA or the protein expressed by mecA, 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

- **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 fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood is recommended.
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.

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 and Quality Control (As per CLSI Guidelines)

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Interpretative Criteria		
	≤S	I	≥R
S. aureus and *S. lugdunensis*	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 spp	2	4-8	16
Coagulase negative *Staphylococci* spps. and *Enterococcus*			

Phone : +91-11-49404040 (100 Lines)
E-mail : sales@cdhfinechemical.com

Coagulase negative *Staphylococci* spps. and *Enterococcus*	4	8-16	32
S.pneumoniae, Streptococcus spps. Beta haemolytic group, *Streptococcus* spps. Viridans group	1	-	-

*Using, Oxacillin-Vancomycin EZY MIC strip, MIC determination for *E. faecalis* ATCC 29212 cannot be established since highest concentration is 8.0 mcg/ml.

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.

Table 2: 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	8.0 – 16.0 – 32.0
S.aureus ATCC 43300 (MRSA)	Mueller Hinton Agar + 2 % NaCl	35-37°C for 24 hrs.	16.0 -32.0 – 64.0

*Using, Oxacillin-Vancomycin EZY MIC strip, MIC determination for *E. faecalis* ATCC 29212 & *S.aureus* ATCC 43300 cannot 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	0.12– 0.25 – 0.5

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 desiccator capsule.
1) Vancomycin - Cefoxitin Ezy MIC Strips (30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Further Reading

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. 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.
4. 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.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
- **Central Drug House 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 of diagnostic reagents extra.
- 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.
- Do not use the products if it fails to meet specifications for identity and performance parameters.