Micafungin Ezy MIC™ Strip (MYC) (0.002-32 mcg/ml) EM121

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use Not for Medicinal Use

It is a unique MIC determination paper strip which is coated with Micafungin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.002 mcg/ml to 32 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of yeast and fungi to antifungal 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 or Mueller Hinton Agar, 2% glucose with methylene blue 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.
7. **The strips give reproducible MIC values that are equivalent to the standard reference MIC obtained by Broth dilution performed as per guidelines with less efforts.**

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 Mueller Hinton Agar, 2% glucose with methylene blue (M1825) from dehydrated powder according to the directions specified on the label. Alternately, prepare Mueller Hinton Agar with added 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (this could be added pre or post sterilization). 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 ± 0.2mm 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**

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Please refer disclaimer Overleaf

- **Test Procedure**

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye i.e. Mueller Hinton Agar, 2% glucose with methylene blue (M1825) as mentioned above.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) 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. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
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. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniform and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation
4. Isolated colonies, pinpoint microcolonies and hazes may appear within the zone of inhibition frequently and they should be ignored. In such cases, consider reading for MIC determination at a point on the scale at which prominent reduction of growth is seen.
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: Micafungin showing reading of 0.38 mcg/ml should be rounded up to next concentration ie. 0.5 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 $\geq$ 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.

When testing	Incubation	Interpretative Criteria		
		≤ S	I*	≥ R
Candida albicans	35-37°C for 24 hrs.	0.25	0.5	1.0
Candida glabrata	35-37°C for 24 hrs.	0.06	0.12	0.25
Candida tropicalis	35-37°C for 24 hrs.	0.25	0.5	1.0
Candida krusei	35-37°C for 24 hrs.	0.25	0.5	1.0
Candida parapsilosis	35-37°C for 24-48 hrs.	2.0	4.0	8.0
Candida guilliermondii	35-37°C for 24 hrs.	2.0	4.0	8.0

*: the ability to successfully treat infections with isolates for which the MIC results are in the intermediate category. The available data do not permit the MIC results to be clearly categorized as either "susceptible" or "resistant"

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 Micafungin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
C. parapsilosis ATCC 22019	Mueller Hinton Agar, 2% Glucose with 0.5 mg/ml Methylene Blue	35-37°C for 24-48 hrs.	0.5 – 1.0 – 2.0 – 4.0
C. krusei ATCC 6258	Mueller Hinton Agar, 2% Glucose with 0.5 mg/ml Methylene Blue	35-37°C for 24 hrs.	0.12 – 0.25 – 0.5

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at 2-8°C, for prolonged use store below -20°C.
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:

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. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume
5. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Edition. Vol.28 No.14, April- 2008 CLSI document M27-S3.
6. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. Vol.32 No.17, December 2012 CLSI document M27-S4.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Micafungin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert