

A Multi-Site Evaluation of Two New Echinocandins, Anidulafungin (AND), and Micafungin (MF) on a Colorimetric MIC Susceptibility Test Plate Compared with the CLSI M27 Reference Broth Micro Dilution (BMD) Plate for Antifungal Susceptibility Testing

C.C. Knapp¹, N.Holliday¹, A. Miskov¹, S.B. Killian¹, M.A.Ghannoum², M.A. Pfaller³, S.A. Messer³, D.Diekema³, R. Ramani⁴, V.Chaturvedi⁴

1. TREK Diagnostic Systems, Cleveland, OH 2. Case Western Reserve University, Cleveland, OH 3. University of Iowa College of Medicine, Iowa City, IA 4. New York State Department of Health, Albany, NY.

ABSTRACT

Background: Micafungin (MF) (Astellas Pharma US, Inc., Deerfield, IL) and anidulafungin (AND) (Pfizer Pharmaceuticals, Groton, CT) are glucan synthesis inhibitors of the echinocandin structural class. MF is used for the prevention of *Candida* infections in patients undergoing hematopoietic stem cell transplantation or the treatment of esophageal candidiasis. AND is used for the treatment of esophageal and invasive candidiasis. A multi-site evaluation was undertaken to determine the performance of the Sensititre® YeastOne® susceptibility plate (TREK Diagnostic Systems, Cleveland, OH) with MF and AND compared to the CLSI BMD.

Materials and Methods: 100 clinical and 100 challenge strains of *C. spp.* were tested at 3 sites comparing the performance of MF and AND on the Sensititre YeastOne plate with CLSI BMD. The ranges tested for MF and AND were (0.008-16µg/ml). CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

Results: Clinical isolate comparisons of the Sensititre YeastOne plate to the CLSI BMD resulted in 95% essential agreement (EA) before repeat testing and 100% EA after repeat testing +/- 2 log₂ dilutions. Challenge isolates resulted in 89% EA before repeat testing and 100% EA after repeat testing +/- 2 log₂ dilutions. *C. tropicalis* caused lower percentage agreements due to difficult endpoint determination before repeat testing. After repeat testing (in triplicate) the MICs were determined to be within +/- 2 log₂ dilutions and EA resulted in 100%.

Conclusions: This evaluation indicates that the Sensititre YeastOne plate with MF and AND is equivalent to the CLSI BMD and is a potential method for susceptibility testing of MF and AND.

INTRODUCTION / OBJECTIVES

To evaluate MIC results with two new Echinocandins, anidulafungin and micafungin, using the Sensititre® YeastOne® colorimetric Antifungal Susceptibility Plate compared with the CLSI M27 frozen reference method at three different test sites.

Sensititre YeastOne test plates are designed for use in determining quantitative antifungal susceptibilities (MIC's) of non-fastidious yeasts.

MIC results for anidulafungin and micafungin were determined with the YeastOne plate at 24 hours using a 2-fold serial dilution range of 0.008-16µg/mL with alamarBlue® as a colorimetric indicator.

MATERIALS & METHODS

Testing was performed at:

- University of Iowa College of Medicine (M. A. Pfaller, S. A. Messer, D. Diekema)
- New York State Department of Health (R. Ramani, V. Chaturvedi)
- Case Western Reserve University (M. A. Ghannoum)

Organisms Tested:

- 200 clinical and challenge isolates were tested at three sites and consisted of the following:

Organism	Number of Isolates Tested	
	Organisms Tested	
	Clinical	Challenge
<i>Candida parapsilosis</i>	20	10
<i>Candida krusei</i>	20	7
<i>Candida lusitanae</i>	20	5
<i>Candida glabrata</i>	5	24
<i>Candida tropicalis</i>	10	16
<i>Candida albicans</i>	20	38
<i>Candida species</i>	5	N/A
Total	100	100

MATERIALS & METHODS cont.

Antimicrobials Tested

Antimicrobials Tested	Range Tested	Supplied By
Anidulafungin	0.008-16 µg/mL	Pfizer Pharmaceuticals Groton, CT
Micafungin	0.008-16 µg/mL	Astellas Pharmaceuticals Deerfield, IL

QUALITY CONTROL

The recommended CLSI M27 quality control isolates were tested daily and performed within the specified manufacturer's ranges for both micafungin and anidulafungin.

Quality Control Strains

Candida parapsilosis ATCC 22019
Issatchenkia orientalis (Candida krusei) ATCC 6258

SUSCEPTIBILITY TESTING METHODS

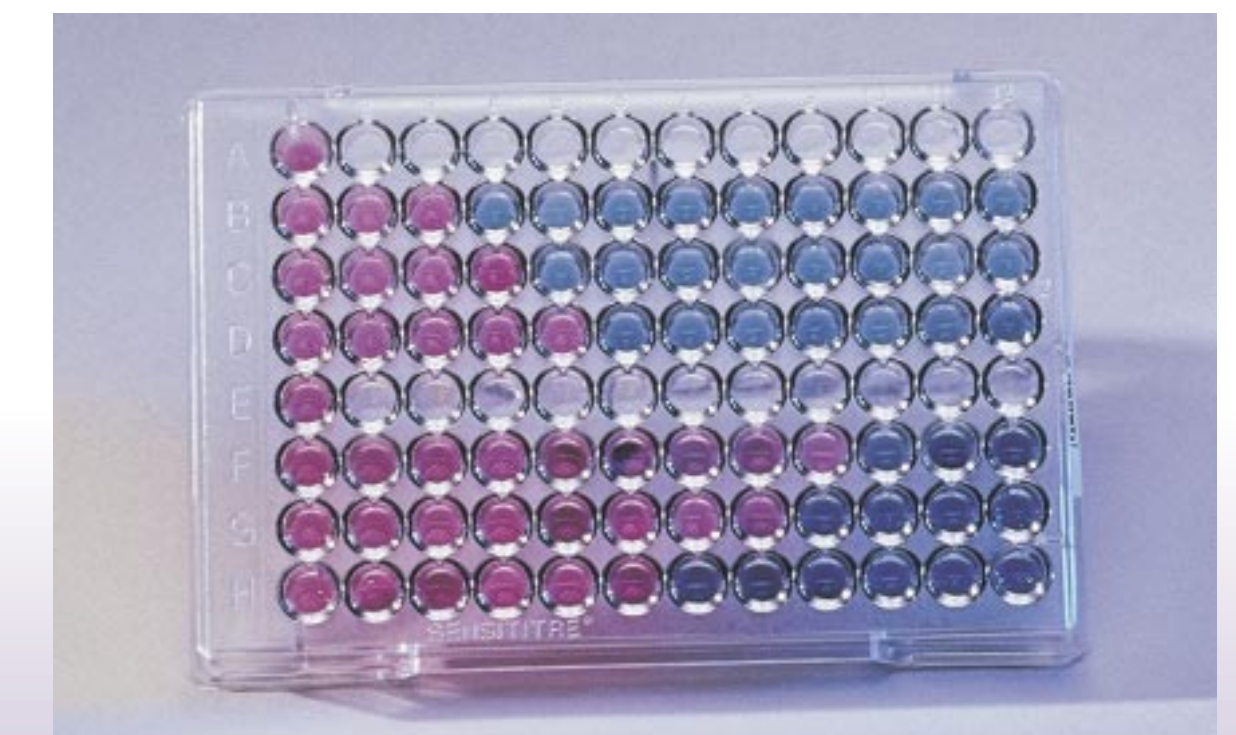
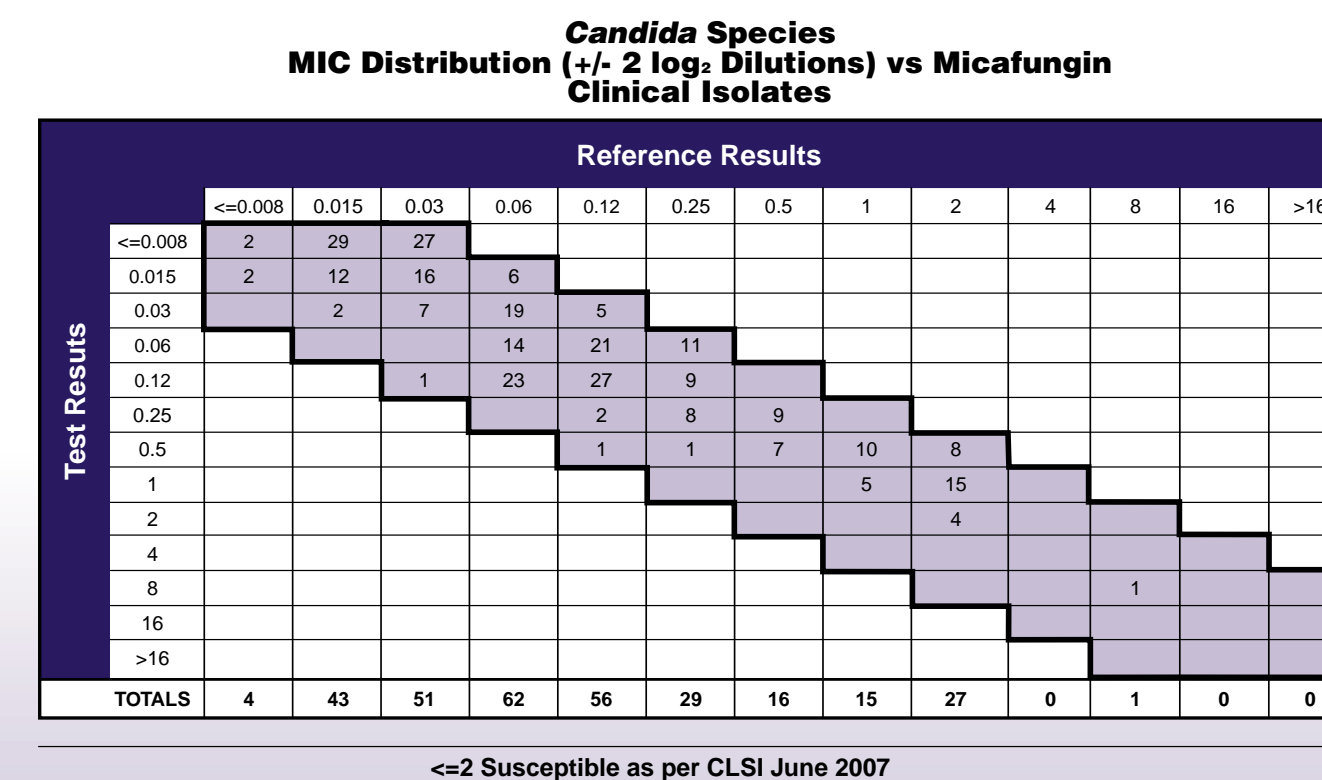
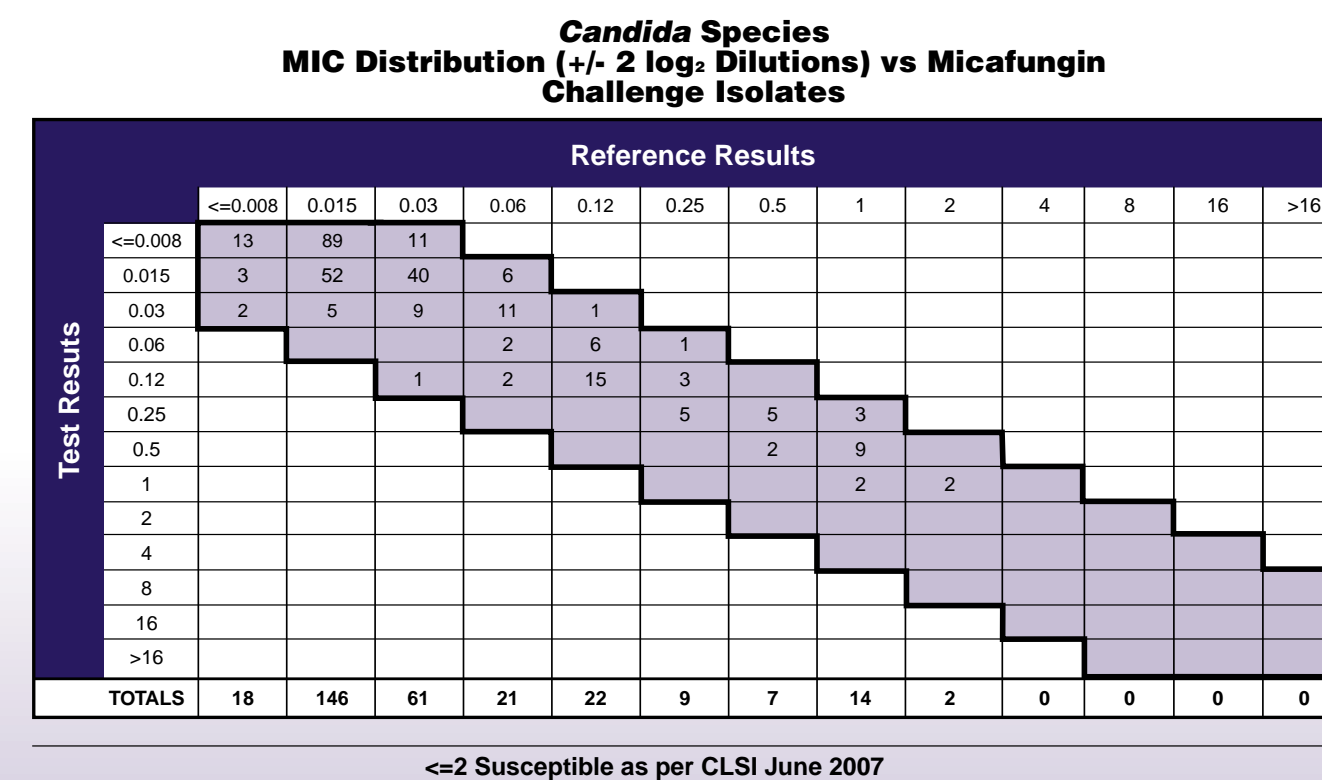
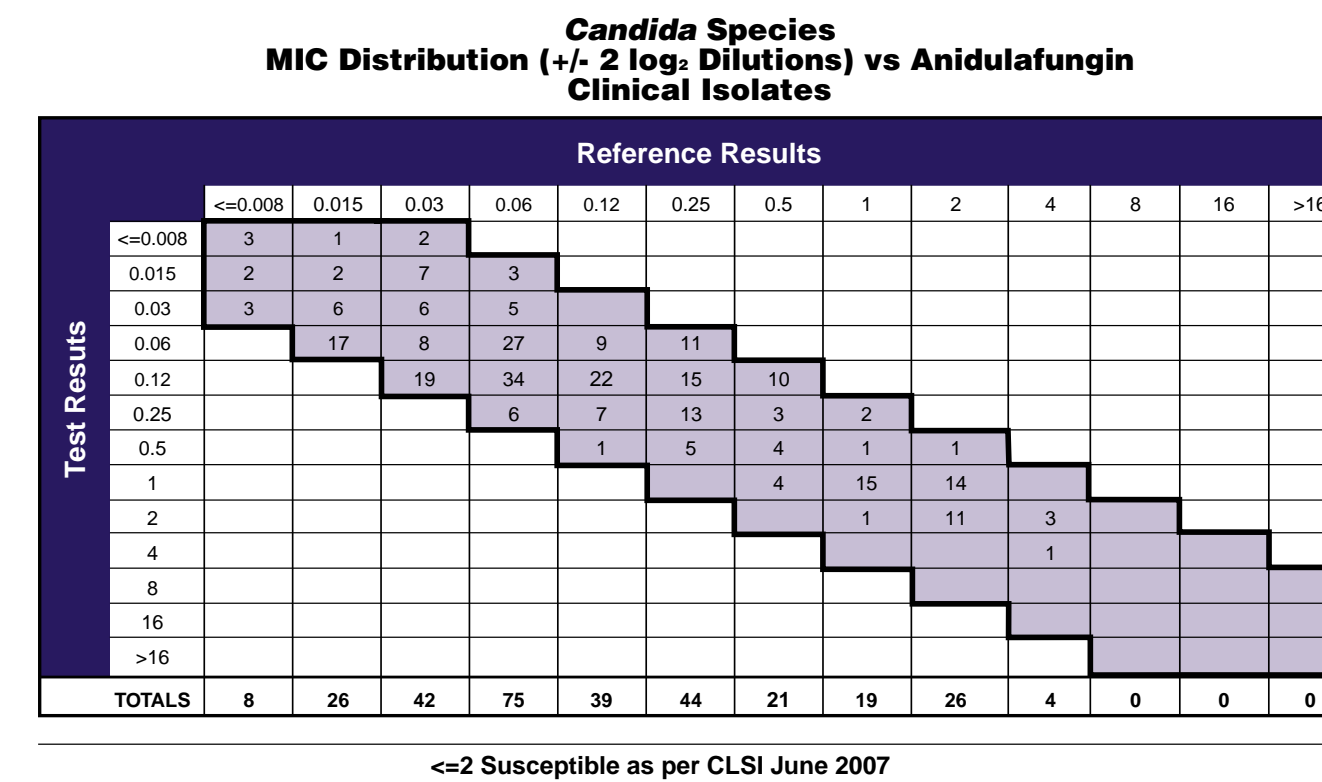
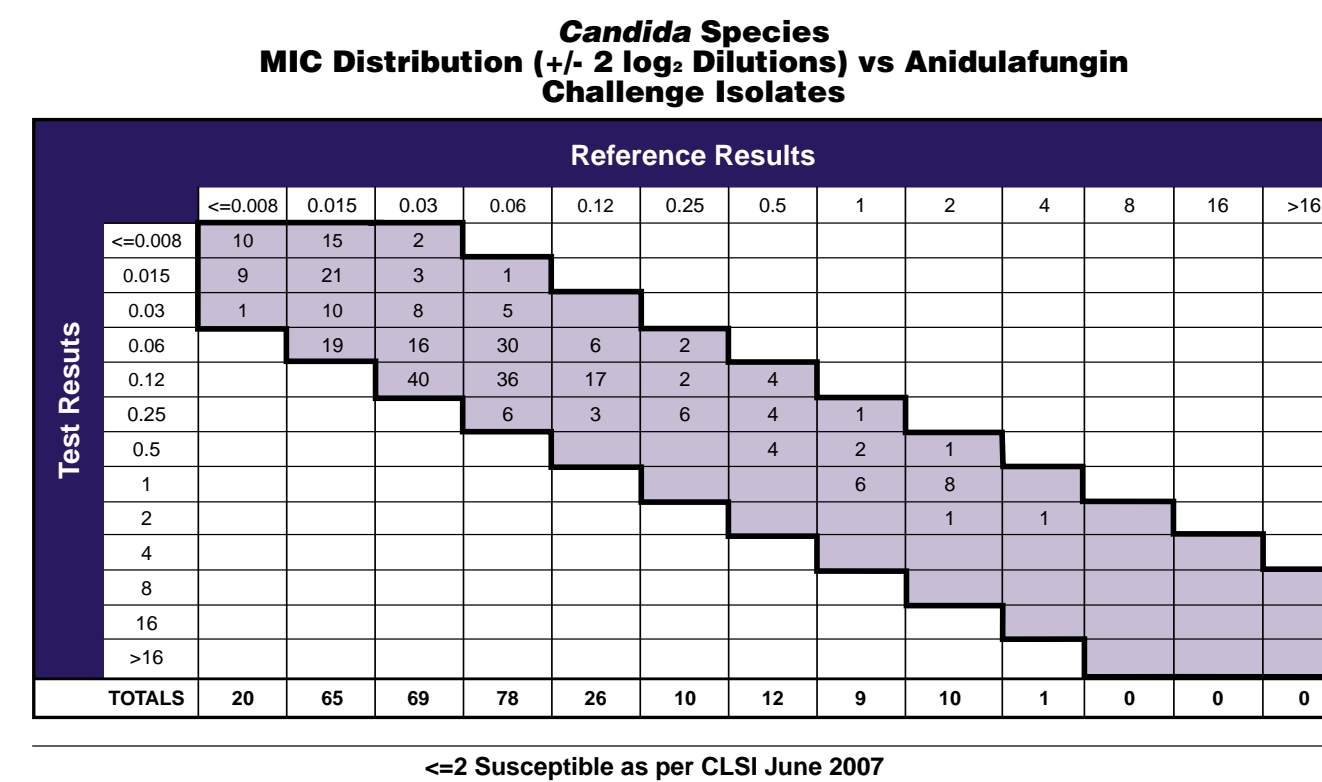
- Each isolate was tested using the Sensititre YeastOne Susceptibility System (TREK Diagnostic Systems, Cleveland, OH). The plates were set up according to manufacturer's instruction.
- The CLSI frozen reference plate (TREK Diagnostic Systems, Cleveland, OH) was tested according to the M27 standard.
- The MIC for the YeastOne plate was read after 24 hours of incubation at 35°C and was determined by observing the lowest antifungal concentration showing inhibition of growth (as evidenced by no color change). Growth in the wells was evident as a change in the colorimetric growth indicator from blue (negative) to pink (positive). MIC's are interpreted as the lowest concentration of antifungal solution remaining blue in color.
- For the CLSI reference methodology endpoint determination, MIC's were read after 24 hours of incubation at 35°C and as complete inhibition of growth or prominent growth inhibition (50% inhibition of growth M27) compared to the growth control well.
- Data Analysis
 • Essential agreement statistics of observed MIC results for the 3 sites, within +/- 2 log₂ dilutions of the reference, were calculated for anidulafungin and micafungin.

RESULTS cont.

CLINICAL Overall % Essential Agreement +/- 2 log ₂ Dilutions for Each Site (24h Test vs. 24h Reference)				
Antifungal	Site 1	Site 2	Site 3	Average
Anidulafungin	100	100	100	100
Micafungin	100	100	100	100
Total	100	100	100	100

CHALLENGE Overall % Essential Agreement +/- 2 log ₂ Dilutions for Each Site (24h Test vs. 24h Reference)				
Antifungal	Site 1	Site 2	Site 3	Average
Anidulafungin	100	100	100	100
Micafungin	100	100	100	100
Total	100	100	100	100

RESULTS



CONCLUSION

This evaluation indicates that the Sensititre YeastOne colorimetric dried susceptibility system with micafungin and anidulafungin is equivalent to the CLSI M27 BMD, and is a potential method for susceptibility testing of micafungin and anidulafungin. The high level of agreement indicates the potential value of the clinical laboratory using YeastOne to determine MIC's for anidulafungin and micafungin.

A <=2 MIC value is the susceptible breakpoint for both anidulafungin and micafungin as per CLSI June 2007. Categorical agreement for anidulafungin for both challenge and clinical isolates was 99.3%. Categorical agreement for micafungin for both challenge and clinical isolates was 100%.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2006. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*. Approved standard 2nd edition M27-A2. Clinical and Laboratory Standards Institute, Wayne, Pa.
2. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints (M27-S2) For use with: Approved standard 2nd edition M27-A2. Clinical and Laboratory Standards Institute, Wayne, Pa.