

## A Reproducibility Study with Two New Echinocandins using the Sensititre® YeastOne® Susceptibility Plate

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### ABSTRACT

**Background:** A multi-site reproducibility study was performed to evaluate the Sensititre YeastOne susceptibility plate (TREK Diagnostic Systems, Cleveland, OH) for *Candida* spp. with two new echinocandins, anidulafungin (Pfizer Pharmaceuticals, Groton, CT) and micafungin (Astellas Pharmaceuticals US, Inc., Deerfield, IL). The YeastOne plate incorporates alamarBlue, a colorimetric agent which helps provide a less subjective, visual MIC endpoint determination. **Methods:** Anidulafungin (0.008-16 µg/ml) and micafungin (0.008-16 µg/ml) were tested against 25 isolates at 3 sites for reproducibility. Isolates were specifically selected to challenge each echinocandin. Assays were manually read after being incubated at 35°C for 24 hours. Modal MIC's and individual site reproducibility MIC's within +/- 2 two-fold dilutions were calculated for each antifungal/organism combination. YeastOne MIC results were compared with the reference CLSI broth microdilution method results (M27-A). The recommended CLSI M27-A quality control isolates were tested daily and performed within the specified manufacturer's ranges for both micafungin and anidulafungin. **Results:** YeastOne modal reproducibility MIC's +/- 2 two fold dilutions between sites for both echinocandins resulted in 99.3 % agreement at 24 hours. Three individual site reproducibility MIC's yielded results within +/- 2 two fold dilutions for both echinocandins (100%, 100%, and 98.6% at 24 hours, respectively). **Conclusion:** Echinocandin MIC determinations using the Sensititre YeastOne Susceptibility System demonstrated highly reproducible results between the three test sites.

### INTRODUCTION

Reproducibility of test results between laboratories is a major performance criterion used for evaluation of any susceptibility method. Sensititre YeastOne plates are designed for use in determining quantitative antifungal susceptibilities (MIC) of non-fastidious yeasts. Evaluating the overall reproducibility at all 3 sites determines the substantial equivalence of both anidulafungin and micafungin on the Sensititre YeastOne plate compared with the CLSI M27 frozen microbroth dilution reference method. The results need to be >95% (+/- 2 fold dilutions) agreement as compared to the mode.

### OBJECTIVE

To evaluate reproducibility results with anidulafungin and micafungin on the Sensititre YeastOne antifungal susceptibility plate.

To determine the substantial equivalence of both anidulafungin and micafungin on the Sensititre YeastOne plate compared with the CLSI M27 frozen microbroth dilution reference method.

### 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)

#### Reproducibility Isolates Tested

Organism	Number Tested
<i>Candida parapsilosis</i>	5
<i>Candida krusei</i>	5
<i>Candida lusitanae</i>	5
<i>Candida glabrata</i>	3
<i>Candida tropicalis</i>	2
<i>Candida albicans</i>	5
<b>Total</b>	<b>25</b>

### QUALITY CONTROL

The recommended CLSI M27-A 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* ATCC 6258

#### Antifungals Tested

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

### SUSCEPTIBILITY TESTING METHODS

- 25 isolates, provided by TREK, were tested and read at 24 hours on the Sensititre dried YeastOne Susceptibility System at each site.
- Each isolate was tested using the Sensititre YeastOne Susceptibility plate (TREK Diagnostic Systems) and were set up according to manufacturer's instruction.
- The CLSI frozen reference plate (TREK Diagnostic Systems Laboratory Services, Cleveland, OH) was tested according to the CLSI 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.
- Modal MIC's and individual site reproducibility MIC's within +/- 2 fold dilutions were calculated for anidulafungin and micafungin.



### RESULTS

Table 1. Anidulafungin MIC Response of Test and Reference Organisms at 24 Hours

Isolate Number	Site 1 Test/Reference		Site 2 Test/Reference		Site 3 Test/Reference		YeastOne MODE Between sites	YeastOne Modal MIC Response (+/- 2 fold dilutions)	Essential Agreement (+/- 2 fold dilutions)
	1	2	1	2	1	2			
1	1	1	0.25	0.5	1	1	1	3	3
2	0.25	1	0.06	0.12	0.5	1	0.25	3	3
3	0.5	2	0.25	0.25	0.5	0.5	0.5	3	3
4	0.5	2	0.25	0.12	0.5	1	0.5	3	3
5	0.12	0.06	0.12	0.06	0.12	0.06	0.12	3	3
6	0.12	0.12	0.12	0.12	0.12	0.25	0.12	3	3
7	0.12	0.06	0.12	0.06	0.25	0.25	0.12	3	3
8	0.12	0.06	0.12	0.06	0.25	0.12	0.12	3	3
9	0.12	0.5	0.06	0.06	0.12	0.5	0.12	3	3
10	0.25	0.06	0.0	0.12	0.25	0.5	0.25	3	3
11	0.25	0.5	0.12	0.12	0.25	0.06	0.25	3	3
12	0.12	0.5	0.12	0.12	0.25	0.25	0.12	3	3
13	0.12	0.5	0.06	0.12	0.5	1	0.12	3	3
14	0.06	0.03	0.12	0.03	0.12	0.06	0.12	3	3
15	0.12	0.5	0.12	0.06	0.12	0.06	0.12	3	3
16	0.12	0.06	0.12	0.06	0.12	0.06	0.12	3	3
17	0.12	0.06	0.06	0.06	0.12	0.12	0.12	3	3
18	0.12	0.06	0.03	0.015	0.12	0.06	0.12	3	3
19	0.06	0.015	0.03	<=0.008	0.06	0.03	0.06	3	3
20	0.03	0.015	0.03	0.015	0.03	0.015	0.03	3	3
21	0.06	0.03	0.03	0.15	0.06	0.03	0.06	3	3
22	<=0.008	0.015	0.015	0.015	0.03	0.015	0.015	3	3
23	0.015	0.015	0.015	0.015	0.12	0.015	0.015	2	2
24	1	1	0.25	0.5	1	1	1	3	3
25	0.06	0.12	0.06	0.06	0.12	0.06	0.06	3	3
<b>Total Essential Agreement</b>								<b>74/75</b>	
<b>Modal MIC Response</b>								<b>74/75</b>	
<b>% Essential Agreement</b>								<b>98.6%</b>	
<b>% Modal MIC Response</b>								<b>98.6%</b>	

Table 2. Micafungin MIC Response of Test and Reference Organisms at 24 Hours

Isolate Number	Site 1 Test/Reference		Site 2 Test/Reference		Site 3 Test/Reference		YeastOne MODE Between sites	YeastOne Modal MIC Response (+/- 2 fold dilutions)	Essential Agreement (+/- 2 fold dilutions)
	1	2	1	2	1	2			
1	0.5	1	0.25	0.12	0.5	1	0.5	3	3
2	0.25	1	0.12	0.06	0.5	1	0.25	3	3
3	0.25	0.5	0.25	0.5	0.5	0.5	0.25	3	3
4	0.25	1	0.25	0.25	0.5	1	0.25	3	3
5	0.12	0.12	0.12	0.12	0.12	0.12	0.12	3	3
6	0.12	0.12	0.12	0.25	0.12	0.12	0.12	3	3
7	0.12	0.12	0.12	0.25	0.12	0.25	0.12	3	3
8	0.12	0.12	0.12	0.25	0.12	0.12	0.12	3	3
9	0.03	0.12	0.015	0.06	0.03	0.06	0.03	3	3
10	0.06	0.12	0.03	0.12	0.06	0.25	0.06	3	3
11	0.12	0.12	0.06	0.06	0.06	0.03	0.06	3	3
12	0.03	0.12	0.06	0.06	0.06	0.12	0.06	3	3
13	0.03	0.12	0.015	0.015	0.03	0.06	0.03	3	3
14	<=0.008	0.015	<=0.008	<=0.008	<=0.008	0.015	<=0.008	3	3
15	<=0.008	<=0.008	0.015	0.015	<=0.008	0.015	<=0.008	3	3
16	<=0.008	0.015	0.015	0.015	<=0.008	0.015	<=0.008	3	3
17	0.015	0.015	<=0.008	0.015	0.015	0.06	0.015	3	3
18	0.03	0.03	0.015	0.03	0.015	0.06	0.03	3	3
19	<=0.008	0.015	<=0.008	0.015	<=0.008	<=0.008	<=0.008	3	3
20	<=0.008	0.015	<=0.008	0.015	<=0.008	0.015	<=0.008	3	3
21	<=0.008	0.015	<=0.008	0.015	0.015	0.015	<=0.008	3	3
22	<=0.008	0.015	0.015	0.06	<=0.008	0.015	<=0.008	3	3
23	0.03	0.03	0.015	0.03	0.03	0.015	0.03	3	3
24	1	1	0.5	1	1	1	1	3	3
25	0.06	0.12	0.12	0.12	0.12	0.12	0.12	3	3
<b>Total Essential Agreement</b>								<b>75/75</b>	
<b>Modal MIC Response</b>								<b>75/75</b>	
<b>% Essential Agreement</b>								<b>100%</b>	
<b>% Modal MIC Response</b>								<b>100%</b>	

Table 3. Overall % Essential Agreement +/- 2 fold dilutions for Each Site

Echinocandin	Site 1	Site 2	Site 3	Average
Anidulafungin	100	100	96	98.6
Micafungin	100	100	100	100
<b>Total</b>	<b>100</b>	<b>100</b>	<b>98</b>	<b>99.3</b>

Table 4. % Modal Response +/- 2 fold dilutions for Each Site

Echinocandin	Site 1	Site 2	Site 3	Average
Anidulafungin	100	100	96	98.6
Micafungin	100	100	100	100
<b>Total</b>	<b>100</b>	<b>100</b>	<b>98</b>	<b>99.3</b>

### CONCLUSION

This reproducibility study was designed to evaluate the YeastOne susceptibility plate's overall reproducibility with anidulafungin and micafungin, which is a major performance criteria used in the evaluation of any susceptibility test method. Anidulafungin and micafungin MIC determinations using the Sensititre YeastOne Susceptibility System demonstrates >95% reproducible results between the three test sites (+/- 2 fold dilutions). Agreement between sites was compared to the modal MIC.

The high level of agreement indicates the potential value of the clinical laboratory using YeastOne to determine MIC's for anidulafungin and micafungin.

### REFERENCES

1. Clinical and Laboratory Standards Institute. 2002. *Reference Method for broth dilution testing of yeasts*. Approved standard 2nd edition M27-A2. Clinical and Laboratory Standards Institute, Wayne, Pa.