

# A Multi-Site Evaluation of Caspofungin (CAS) on Sensititre® YeastOne® Susceptibility System Compared with the CLSI M27 Reference Broth Micro Dilution (BMD) Plate for Antifungal Susceptibility Testing

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## ABSTRACT

**Background:** Caspofungin (Merck & Co. Rahway, New Jersey) is an echinocandin that is currently approved for use in the treatment of fungal infections and invasive aspergillosis. A multi-site evaluation was undertaken to determine the performance of the Sensititre YeastOne susceptibility system (TREK Diagnostic Systems, Cleveland, OH) with Caspofungin (CAS) compared to the CLSI Broth Microdilution Method BMD.  
**Materials and Methods:** 300 clinical (100 per site) and 100 challenge strains along with 25 reproducibility isolates of *Candida* spp. were tested at 3 sites comparing the performance of CAS on the Sensititre YeastOne plate with CLSI BMD. The range tested for CAS was (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 100% essential agreement (EA) +/- 2 log<sub>2</sub> dilutions. Challenge isolates resulted in 100% EA +/- 2 log<sub>2</sub> dilutions. YeastOne modal reproducibility MICs +/- 2 fold dilutions between sites for CAS resulted in 97.3% agreement at 24 hours. Three individual site reproducibility MICs yielded results within +/- 2 fold dilutions for CAS (100%, 96% and 96%) respectively. All isolates were susceptible (≤2) and resulted in 100% categorical agreement.  
**Conclusions:** This evaluation indicates that the Sensititre YeastOne plate with CAS is equivalent to the CLSI BMD and is a potential method for susceptibility testing of CAS.

## INTRODUCTION / OBJECTIVES

- To evaluate MIC results with Caspofungin, using the Sensititre® YeastOne® colorimetric Antifungal Susceptibility Plate compared with the CLSI M27 frozen reference BMD method at three different test sites.
- Sensititre YeastOne test plates are designed for use in determining quantitative antifungal susceptibilities (MIC's) of *Candida* spp.
- MIC results for Caspofungin were determined with the YeastOne plate at 24 hours using a 2-fold serial dilution range of 0.008-16µg/mL with alamarBlue® included 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)

Antifungal Tested		
Antifungal	Range Tested ug/ml	Supplied By
Caspofungin	0.008-16 µg/mL	Merck & Co., Rahway, NJ

### Organisms Tested:

- 300 clinical (100 per site) and 100 challenge isolates were tested at three sites and consisted of the following:

Isolates Tested		
Organism	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</i> species	5	N/A
<b>Total</b>	<b>100</b>	<b>100</b>

## MATERIALS & METHODS cont.

### Reproducibility Isolates Tested

Organism	# 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 quality control isolates were tested daily and performed within the CLSI expected QC ranges for Caspofungin.

Quality Control Strains	Caspofungin MIC (µg/mL) QC Ranges 24h
<i>Candida parapsilosis</i> ATCC 22019	0.25-1
<i>Candida krusei</i> ATCC 6258	0.12-1

## 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 BMD reference plate (TREK Diagnostic Systems, Lab Services, 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 (M27) MIC endpoint determinations were read after 24 hours of incubation at 35°C as complete inhibition of growth or prominent growth inhibition (50% inhibition of growth) compared to the growth control well.
- 25 reproducibility isolates, provided by TREK, were tested and read at 24 hours on the Sensititre dried YeastOne susceptibility system at each site.

### Data Analysis

- Caspofungin essential agreement statistics of MIC results comparing the YeastOne to the CLSI reference plate at the 3 sites were calculated using +/- 2 log<sub>2</sub> dilutions.

## RESULTS

### Candida Species MIC Distribution (+/- 2 log<sub>2</sub> Dilutions) Caspofungin Clinical Isolates

		Reference Results												
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Test Results	≤0.008	1	1	1										
	0.015	3	6	1										
	0.03	1	23	37	4									
	0.06		9	25	6	4								
	0.12			9	16	9	14							
	0.25				3	15	40	31	3					
	0.5					3	6	22	6					
	1							2	2					
	2													
	4													
	8													
	16													
	>16													
TOTALS	1	5	39	75	44	59	69	11	0	0	0	0	0	

≤2 Susceptible as per CLSI June 2007

## RESULTS cont.

### Candida Species MIC Distribution (+/- 2 log<sub>2</sub> Dilutions) Caspofungin Challenge Isolates

		Reference Results												
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Test Results	≤0.008	1	1	10	1									
	0.015	4	34	25										
	0.03	2	25	72	7	3								
	0.06		8	18	14	5	3							
	0.12			9	10	14	14	2						
	0.25				3	3	9	2						
	0.5							1						
	1													
	2													
	4													
	8													
	16													
	>16													
TOTALS	1	7	77	125	34	25	26	5	0	0	0	0	0	

≤2 Susceptible as per CLSI June 2007

### Caspofungin Reproducibility MIC Response of Test and Reference Organisms 24h

Isolate Number	Site 1 Test/Reference	Site 2 Test/Reference	Site 3 Test/Reference	YeastOne MODE Between sites	YeastOne Modal Response (+/-2 fold dilutions)	Essential Agreement +/-2 fold dilutions			
1	0.25	0.25	0.25	0.12	0.25	0.5	0.25	3	3
2	0.12	0.25	0.03	0.03	0.12	0.5	0.12	3	3
3	0.25	0.5	0.12	0.12	0.25	0.25	0.25	3	3
4	0.2	0.5	0.12	0.06	0.12	0.5	0.12	3	3
5	0.25	0.25	0.25	0.25	0.5	0.25	0.25	3	3
6	0.25	0.12	0.25	0.5	0.5	0.5	0.25	3	3
7	0.25	0.12	0.25	0.25	0.5	0.5	0.25	3	3
8	0.25	0.25	0.25	0.25	0.5	1	0.25	3	3
9	0.12	0.5	0.015	0.03	0.25	0.25	0.12	2	3
10	0.25	1	0.12	0.06	0.5	0.5	0.25	3	3
11	0.12	0.5	0.06	0.06	0.5	0.5	0.12	2	3
12	0.12	0.25	0.06	0.06	0.25	0.5	0.12	3	3
13	0.06	0.25	0.06	0.12	0.12	0.25	0.06	3	3
14	0.06	0.03	0.12	0.06	0.06	0.06	0.06	3	3
15	0.06	0.12	0.12	0.06	0.12	0.12	0.12	3	3
16	0.06	0.06	0.06	0.06	0.06	0.06	0.06	3	3
17	0.03	0.03	0.06	0.03	0.06	0.06	0.06	3	3
18	0.03	0.06	0.06	0.06	0.06	0.06	0.06	3	3
19	0.015	0.03	0.03	0.015	0.015	0.03	0.015	3	3
20	0.03	0.03	0.06	0.03	0.03	0.03	0.03	3	3
21	0.06	0.06	0.015	0.03	0.06	0.03	0.06	3	3
22	0.06	0.06	0.06	0.03	0.12	0.06	0.06	3	3
23	0.06	0.06	0.06	0.06	0.12	0.03	0.06	3	3
24	0.25	0.25	0.25	0.25	0.5	0.5	0.25	3	3
25	0.12	0.12	0.25	0.25	0.25	0.25	0.25	3	3

Total Essential Agreement 75/75  
 Modal MIC Response 73/75  
 Essential Agreement % 100%  
 % Modal MIC Response 97.3%

## RESULTS cont.

### Clinical Overall % Essential Agreement +/- 2 log<sub>2</sub> dilutions for Each Site (24h Test vs. 24h Reference)

Antifungal	Site 1	Site 2	Site 3	Average
Caspofungin	100	100	100	100

### Challenge Overall % Essential Agreement +/- 2 log<sub>2</sub> dilutions for Each Site (24h Test vs. 24h Reference)

Antifungal	Site 1	Site 2	Site 3	Average
Caspofungin	100	100	100	100

### Reproducibility % Modal MIC Response +/- 2 log<sub>2</sub> dilutions for Each Site

Antifungal	Site 1	Site 2	Site 3	Average
Caspofungin	100	96	96	97.3

## CONCLUSION

The reproducibility of the YeastOne susceptibility system with caspofungin, a major performance criteria used in the evaluation of any susceptibility test method, demonstrated >95% reproducible results among the three test sites (+/- 2 log<sub>2</sub> dilutions). Agreement between sites was compared to the mode.

The categorical agreement for caspofungin for both challenge and clinical isolates was 100%. A ≤2 MIC value is the susceptible breakpoint for caspofungin as per CLSI June 2007.

Overall the results indicated that the Sensititre YeastOne colorimetric dried susceptibility system with caspofungin was equivalent to the CLSI M27 BMD.

The high level of inter-laboratory agreement observed suggested the potential value of YeastOne to determine Caspofungin MICs in the clinical laboratories.

## REFERENCES

- 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.
- 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.