Paper # **F-44**

Multicenter Evaluation of the Sensititre® YeastOne® Colorimetric Antifungal Panel with the NCCLS M27-A Reference Microdilution Method for Testing *Candida* species

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ABSTRACT

Background: National Committee for Clinical Laboratory Standards (NCCLS) microdilution method for antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* (M27-A document) may not be the most efficient and convenient procedure for use in the clinical laboratory. Commercially prepared Sensititre YeastOne Colorimetric Antifungal Panel provides similar MICs to those obtained by the NCCLS method for reference agents against yeasts.
Methods: This multicenter (3 centers) study compared MIC values obtained simultaneously by Sensititre YeastOne and NCCLS M27-A broth microdilution methods after 24- and 48-h of incubation for reference agents amphotericin B, fluconazole, flucytosine, itraconazole, and the novel triazoles voriconazole and posaconazole. The 100 clinical isolates evaluated included 38 *Candida albicans*, 24 C. *glabrata*, 7 C. *krusei*, 5 C. *lusitaniae*, 10 C. *parapsilosis*, and 16 C. *tropicalis*. Colorimetric MIC endpoints for amphotericin B corresponded to the first blue well (no growth) and MICs for the other agents to the first purple or blue well.
Results: Three comparisons of MIC pairs by the two methods were evaluated to obtain percentages of agreement (+ 2 dilution range): 24- and 48-h colorimetric MICs versus corresponding NCCLS values and 24-h colorimetric versus 48-h reference MICs. The best agreements between the methods were for 24-and 48 h YeastOne and 24- and 48 h NCCLS MICs (90.1 to 99.7% & 82.9 to 99.3%, respectively) for all agents followed by 24-h colorimetric versus 48-h NCCLS values (79.5 to 97.3%). Interlaboratory agreement for YeastOne panel results ranged from 97.6 to 100% at 24 h (all agents) and from 90.1 to 99.3% at 48-h; agreement was lower for itraconazole and posaconazole (87.7% at 48 h).
Conclusion: These data suggest the potential value of the YeastOne panel for use in the clinical laboratory to determine MIC values of reference agents as well as the new triazoles voriconazole and posaconazole for isolates of *Candida* spp

INTRODUCTION / OBJECTIVES

- The commercially prepared Sensititre YeastOne Colorimetric Antifungal Panel is the first antifungal colorimetric panel to receive FDA clearance for the antifungal susceptibility testing of fluconazole, itraconazole and flucytosine against isolates of *Candida* spp. The purpose of this study was dual:
- To compare MIC results of two new itriazoles voriconazole and posaconazole obtained by the Sensititre YeastOne Colorimetric Antifungal Susceptibility Panel for 100 clinical isolates of *Candida* spp. to those obtained by the NCCLS M27-A method.
- To evaluate the interlaboratory reproducibility among three independent laboratories of YeastOne MIC results of two new triazoles voriconazole and posaconazole for 100 clinical isolates of *Candida* spp.

MATERIALS & METHODS

Materials

Organisms: The testing at 3 sites consisted of the following:

- 100 isolates provided by Dr. Michael Pfaller, University of Iowa

Table 1. Isolates Tested

Species	No. of isolates tested	Species	No. of isolates tested
1. Candida albicans	38	*5. C.parapsilosis*	10
2. C. glabrata	24	*6. C. tropicalis*	16
3. C.krusei	7	*QC C. parapsilosis 22019*	
4. C.luistaniae	5	*QC C.krusei 6258*	

Table 2. Antifungal Agents Tested and their Ranges

Antifungals	Concentration Range Tested (µg/m/l)	MFG
Posaconazole (New)	0.004-8	Schering Plough
Itraconazole (Comparator)	0.004-8	Janssen
Voriconazole (New)	0.008-16	Pfizer
Amphotericin B (Comparator)	0.016-16	Sigma
5-Flucytosine (Comparator)	0.03-64	Sigma
Fluconazole (Comparator)	0.12-256	Pfizer

Methods

Each isolate was tested by the two methods in each of the three sites.

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Susceptibility Testing Methods

- Each isolate was tested using a Sensititre YeastOne Colormetric Antifungal Susceptibility Panel. The panels were set-up according to the manufacturer's instructions.
- Reference MICs were determined according to the guidelines described in the NCCLS M27-A document.

MATERIALS & METHODS con't

- The colorimetric MIC for each isolate was determined by observing the lowest antifungal concentration showing inhibition of growth (as evidenced by no color change). Yeast growth in the antifungal solutions was evident as a change in the colormetric growth indicator from blue (negative) to pink (positive). MIC's are interpreted as the lowest drug concentration remaining blue in color. In the case of isolates that exhibited heavy trailing, the MIC was determined as being the first well exhibiting a significant color difference when compared to the positive growth control well.
- Endpoint determinations of the reference plates were performed according to the guidelines published in the NCCLS Document M27-A, reference method for broth dilution antifungal susceptibility testing of yeasts.

Data Analysis

- The agreement of MIC results from the three sites (within three- well dilutions) obtained by the two methods was charted out for each drug and isolate combination at both 24 and 48 hours of incubation.
- The agreement among the three centers of MICs (within three- well dilutions) obtained by the YeastOne Panel was also calculated at both 24 and 48 hours of incubation.
- The percentages of MIC endpoints between the two methods as well as among the centers were then determined for each combination of isolate, drug, and incubation time.

RESULTS

Table 3. MIC ranges for two new antifungals (µ/ml) for 100 *Candida* isolates by YeastOne and NCCLS M27-A methods 24 Hours

Species	PZ -Reference	PZ-YeastOne	VZ -Reference	VZ-YeastOne
C. albicans	0.004-2	0.016-1	0.008-4	0.008-4
C. glabrata	0.12-2	0.03-8	0.03-4	0.016-2
C. krusei	0.12-0.5	0.25-0.5	0.06-0.5	0.25-0.5
C. lusitania	0.06-0.12	0.016-0.06	0.008-0.016	0.008-0.03
C. parapsilosis	0.03-0.25	0.03-0.25	0.008-0.12	0.016-0.25
C. tropicalis	0.03-0.5	0.03-0.5	0.008-0.25	0.008-1

RESULTS con't

Table 4. Overall % Agreement (Within a Three-well Range) Between the Methods for 100 Isolates of *Candida* spp.

Methods	AB	VZ	PZ	FZ	IZ	FC
24 vs. 48h	97.3	88.4	79.5	92.2	88.7	83.3
24 vs. 24h	99.7	94.5	90.1	97.3	95.9	98
48 vs. 48h	99.3	82.9	82.9	84.3	84	97.3

Table 5. % Agreement (Within a Three- Well Range) Between the Methods for Five *Candida* Species
(24 vs. 24 hrs)

Antifungals	*C. albi*	*C. glab*	*C. krusei*	*C. lus*	*C. para*	*C. tropicalis*
Posaconazole	95	97	100	87	100	98
Voriconazole	99	99	100	100	100	98

Table 6. Overall % Agreement (Within a Three-well Range) Among Three Test Sites of YeastOne MICs for 100 *Candida* isolates

Incubation times	AB	VZ	PZ	FZ	IZ	FC
24h	100	97.6	97.6	97.6	98.3	99
48h	99.3	90.1	88.7	93.5	87.7	98

CONCLUSIONS

This study was designed to evaluate the YeastOne antifungal panel for susceptibility testing of *Candida* spp. isolates to the two new triazoles, voriconazole and posaconazole. These data suggest the potential value of the YeastOne panel for use in the clinical laboratory for determination of MIC values with the newer triazoles. The data presented here indicate this system is highly reproducible within each laboratory as well as between the laboratories.