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Clinical Evaluation of the Sensititre YeastOne Plate for Testing Susceptibility of Filamentous Fungi to Posaconazole[▽]

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Sensititre YeastOne colorimetric antifungal panels were compared with the CLSI (formerly NCCLS) M38-A reference method for testing the susceptibility of filamentous fungi to posaconazole; agreement (±2 $\log_2$ dilutions) between the two methods was 97%. These data confirm the utility of YeastOne panels for measuring the susceptibility of filamentous fungi to posaconazole.

The Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) M38-A reference method is approved for testing the susceptibility of filamentous molds that cause invasive fungal infections, including *Aspergillus* spp., *Fusarium* spp., *Rhizopus arrhizus*, *Pseudallescheria boydii* (anamorph, *Scedosporium apiospermum*), *Sporothrix schenckii*, and other opportunistic pathogenic molds, to antifungal agents (1). However, the method is labor-intensive and requires 48 h of incubation (*R. arrhizus* requires 24 h) before MIC results can be read. In contrast, the Sensititre YeastOne colorimetric antifungal panel (TREK Diagnostic Systems, Cleveland, OH) is a commercially prepared plate that requires only the addition of a medium containing the fungal inoculum, and MICs can be read after 24 h. Furthermore, reading of the end point is facilitated by the inclusion of a metabolic dye, alamarBlue.

Posaconazole is a new azole antifungal with a broad spectrum of activity, including activity against filamentous molds, such as the zygomycetes, that were not previously regarded as being susceptible to azoles (4). The goal of this study was to compare the reproducibility of the Sensititre YeastOne method with that of the CLSI reference method for testing the susceptibility of a broad spectrum of filamentous fungi to posaconazole.

In vitro susceptibility testing methods. Two hundred forty-nine filamentous fungi from the Schering-Plough Research Institute fungal collection were tested: 96 were *Aspergillus fumigatus*, 32 *Aspergillus flavus*, 20 *Aspergillus niger*, 19 *Aspergillus terreus*, 3 *Aspergillus nidulans*, 3 *Aspergillus ustus*, 3 *Aspergillus versicolor*, 3 *Aspergillus oryzae*, 14 *Fusarium solani*, 7 *Fusarium oxysporum*, 6 *Fusarium moniliforme*, 6 *Fusarium proliferatum*, 8 *Fusarium* spp., 3 *Mucor* spp., 3 *Absidia* spp., 17 *Rhizopus* spp., 4 *Rhizopus arrhizus*, and 2 *Rhizopus microsporus*.

Reference MICs were determined using the broth microdilution technique as described in the CLSI M38-A document (1). Posaconazole concentrations ranged from 0.08 to 8 μg/ml; the last well was a positive control. Plates were incubated for either 24 h (*Rhizopus* spp. only) or 48 h at 35°C. The end point was the first well showing complete growth inhibition.

In the Sensititre YeastOne colorimetric antifungal susceptibility panel, the posaconazole concentrations ranged from 0.08 to 8 μg/ml; the last well was a positive control. The panels were inoculated according to the manufacturer's instructions. The colorimetric MIC for each isolate was determined after a 24-h incubation at 35°C and was the lowest antifungal concentration showing inhibition of growth. Fungal growth in the wells was evident as a change in the colorimetric growth indicator, alamarBlue, from blue (negative) to pink (positive); therefore, the MIC was interpreted as the well with the lowest drug concentration in which the growth indicator remained blue. The following CLSI-approved quality control (QC) strains were run on each test occasion: *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, and *Paecilomyces variotii* ATCC MYA-3630.

In vitro susceptibility testing results. The methods were considered to be in agreement if the MICs determined using Sensititre YeastOne were within 2 doubling dilutions of those obtained using the CLSI reference method. Isolates for which the values were outside this range were retested. The MICs for all the QC isolates, including the recently approved mold QC isolate *P. variotii* ATCC MYA-3630, were all within range. The overall agreement (range, ±2 $\log_2$ dilutions) between the Sensititre YeastOne MICs and the corresponding CLSI reference method was 97% (Table 1). There were seven isolates (four *A. fumigatus*, two *R. arrhizus*, and one *A. ustus* isolate) for which the MICs did not agree within the prescribed dilution range; for all seven isolates, the Sensititre YeastOne MICs were higher than the reference values, which ranged from 0.03 to 2 μg/ml. Both tests were repeated, and all seven isolates gave concordant results; for six of the seven, the results were identical for the two methods, while for the remaining isolate, agreement was within 1 doubling dilution.

Laboratory-generated *A. fumigatus* mutants (2, 3) with reduced susceptibility to posaconazole were included in this study; for 2 isolates, MICs were >8 μg/ml, and for 11, MICs were ≥1 μg/ml. When these isolates were excluded from the analysis, the MIC at which 90% of isolates were inhibited (MIC_{90}) was 0.5 μg/ml for *A. fumigatus*, in agreement with the findings of a previous survey (4). The Sensititre YeastOne method identified the two resistant *A. fumigatus* isolates, with MICs of >8 μg/ml. For 10 of the remaining 11 isolates, the Sensititre YeastOne MICs were within the prescribed agreement range. For the final isolate (CLSI MIC, 2 μg/ml), the

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TABLE 1. Agreement between CLSI reference method M38-A and the Sensititre YeastOne method for testing the susceptibility of filamentous molds

Test organism(s)	No. of strains	MIC[a] by CLSI/MIC by YO		% Agreement within the following $\log_2$ dilution		
		MIC_{90}	Range	±2	±1	0
All isolates	249			97	94	56
Aspergillus	179			97	94	50
A. fumigatus	96	1/1	0.015–>8/0.015–>8	96	94	54
A. flavus	32	0.5/0.25	0.12–1/0.06–0.25	100	91	34
A. niger	20	0.25/0.25	0.03–0.25/0.03–0.25	100	100	70
A. terreus	19	0.25/0.25	0.015–0.5/0.03–0.5	100	95	32
A. nidulans	3	—[b]	0.015–0.06/0.015–0.06	100	100	33
A. oryzae	3	—	0.12–0.5/0.12–0.25	100	100	33
A. ustus	3	—	2–>8/>8	67	67	67
A. versicolor	3	—	0.06–>8/0.03–>8	100	100	67
Fusarium	41			100	98	90
F. solani	14	>8/>8	>8/>8	100	100	100
Fusarium spp.	8	—	0.5–>8/0.5–>8	100	100	88
F. oxysporum	7	—	2–>8/2–>8	100	100	100
F. moniliforme	6	—	1–2/1–2	100	100	67
F. proliferatum	6	—	4–>8/1–>8	100	83	83
Zygomycetes	29			93	86	45
Absidia						
Absidia spp.	2	—	1–2/0.5	100	50	0
A. corymbifera	1	—	2/1	100	100	0
Mucor						
M. circinelloides	1	—	2/0.5	100	0	0
M. ramosissimus	1	—	2/1	100	100	0
Mucor spp.	1	—	2/2	100	100	100
Rhizopus[c]				96	96	57
Rhizopus spp.	17	1/1	0.06–>8/0.12–>8	100	100	47
R. arrhizus	4	—	0.03–2/0.25–>8	50	50	50
R. microsporus	2	—	0.5/0.5	100	100	100

[a] In micrograms per milliliter. YO, Sensititre YeastOne.
[b] —, not determined. MIC_{90}s were calculated only when >10 isolates were tested.
[c] CLSI MICs were read after 24 h.

Sensititre YeastOne MIC was >8 μg/ml; upon retesting, both methods returned values of 1 μg/ml. Similarly, the *Fusarium* isolates exhibiting reduced susceptibility to posaconazole were accurately identified using the Sensititre YeastOne method.

The high levels of agreement between the MICs obtained using Sensititre YeastOne and the CLSI reference method suggest the potential value of Sensititre YeastOne for use in the clinical laboratory to determine posaconazole MICs for filamentous mold isolates.

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