

A Multi-Site Evaluation of Isavuconazole on the Sensititre YeastOne Test Plate with the Frozen Reference CLSI M27-A3 and ISO 16256 Micro Broth Dilution Methods for Antifungal Susceptibility Testing

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Abstract

Background: Isavuconazole (Astellas Pharmaceuticals, Northbrook, IL) is a triazole antifungal that is currently approved for use in the treatment of invasive mucormycosis and aspergillosis. A multi-site evaluation was undertaken to determine the performance of the Thermo Scientific™ Sensititre® YeastOne® susceptibility system (Thermo Fisher Scientific, Cleveland, OH) with Isavuconazole (ISA) compared to the CLSI M27 and ISO16256 (2012)(E) Broth Microdilution Methods (BMD). **Materials and Methods:** 400 clinical (100 per site) and 100 challenge strains along with 10 reproducibility isolates of *Candida* spp. were tested at 4 sites comparing the performance of ISA on the Sensititre YeastOne plate with CLSI and ISO BMD. The range tested for ISA was 0.004-8ug/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 and ISO BMD resulted in 99% and 92% essential agreement (EA) +/- 2 log₂ dilutions. Challenge isolates resulted in 99% and 89% EA +/- 2 log₂ dilutions. YeastOne modal reproducibility MICs +/- 2 fold dilutions between sites for ISA resulted in 98.6% agreement at 24 hours. **Conclusions:** This evaluation indicates that the Sensititre YeastOne plate with ISA is equivalent to the CLSI and ISO BMD and is a potential method for susceptibility testing of ISA.

Introduction and Objective

Isavuconazole (Astellas Pharma, Inc.) is a new broad spectrum triazole that has activity against yeasts, molds and dimorphic fungi. It is currently approved for use in treatment of invasive mucormycosis and aspergillosis.

This *in vitro* multi-site comparison study was done to validate the performance of **isavuconazole with *Candida* spp.** on the commercially manufactured Thermo Scientific Sensititre YeastOne Susceptibility plate. To establish equivalency performance, studies were conducted at 4 sites. MIC results obtained on the YeastOne plate were compared to the MIC results obtained from the reference CLSI M27 and ISO 16256 (2012) (E) broth microdilution method (BMD).

Methods

- Indications for use: Thermo Fisher Sensititre YeastOne susceptibility plates are designed for use in determining quantitative antifungal susceptibilities (MICs) of non fastidious yeasts.
- Each isolate was tested using a Sensititre YeastOne susceptibility plate containing **Isavuconazole** (0.004-8µg/ml).
- The dried colorimetric plates were set-up, tested and read according to the manufacturers' instructions.
- The reference BMD plates were prepared and tested on each isolate according to the Clinical Laboratory Standards Institute Method (CLSI M27-A3) and the ISO 16256 (2012) (E).
- Testing consisted of 400 fresh clinical isolates (combined 4 sites); 100 challenge isolates (one site) consisted of *Candida* spp. (**Tables 1 and 2**).
- Reproducibility consisted of 10 isolates tested in triplicate over 3 consecutive days at all 4 sites on the Sensititre YeastOne susceptibility plate only (**Table 1**).

Methods Cont.

- Quality control (QC) was assured by testing 20 replicates of each ATCC strain including *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, at each site (**Tables 1 and 3**).
- Colony counts were performed on the inoculums of all strains on each day of testing.

| Table 1. Organisms Tested | Number Tested |
|--|---------------|
| Clinical Isolates (4 sites) | 400 |
| CDC Challenge Isolates (one site) | 100 |
| Reproducibility Isolates (4 sites) | 40 |
| CLSI Quality Control Strains (20 replicates of each strain at 4 sites) | 160 |
| TOTAL | 700 |

| Table 2. Clinical and Challenge Isolates Tested | Number Tested |
|---|---------------|
| <i>Candida albicans</i> | 119 |
| <i>Candida krusei</i> | 87 |
| <i>Candida lusitanae</i> | 72 |
| <i>Candida parapsilosis</i> | 92 |
| <i>Candida tropicalis</i> | 56 |
| <i>Candida glabrata</i> | 74 |
| TOTAL | 500 |

| Table 3. Quality Control Strains | CLSI M27-A3 and ISO 16256 MIC Ranges (µg/ml) |
|--|--|
| <i>Candida parapsilosis</i> ATCC 22019 | 0.015-0.06 |
| <i>Candida krusei</i> ATCC 6258 | 0.06-0.5 |



Results

Essential agreement for **isavuconazole** on the Sensititre YeastOne susceptibility plate compared to the CLSI and ISO BMD plates were calculated for each method using the +/- two log₂ dilution standard. Essential agreement rates are shown for *Candida* spp. in **tables 4 and 5**.

Table 4. Summary Data and % Essential Agreement of *Candida* spp. Clinical and Challenge Isolates (CLSI Reference BMD vs. YeastOne)

| Organism Group | Number of Isolates | | Essential Agreement | | % Essential Agreement | |
|-----------------------------|--------------------|------------|---------------------|------------|-----------------------|--------------|
| | All | 'Evaluable | Total | 'Evaluable | Total | 'Evaluable |
| <i>Candida albicans</i> | 119 | 34 | 117 | 34 | 98.3% | 100% |
| <i>Candida krusei</i> | 87 | 86 | 86 | 85 | 98.9% | 98.8% |
| <i>Candida lusitanae</i> | 72 | 56 | 72 | 56 | 100% | 100% |
| <i>Candida parapsilosis</i> | 92 | 58 | 92 | 58 | 100% | 100% |
| <i>Candida tropicalis</i> | 56 | 54 | 54 | 52 | 96.4% | 96.3% |
| <i>Candida glabrata</i> | 74 | 74 | 74 | 74 | 100% | 100% |
| Total | 500 | 362 | 495 | 359 | 99.0% | 99.2% |

Table 5. Summary Data and % Essential Agreement of *Candida* spp. Clinical and Challenge Isolates (ISO BMD vs. YeastOne)

| Organism Group | Number of Isolates | | Essential Agreement | | % Essential Agreement | |
|------------------------------|--------------------|------------|---------------------|------------|-----------------------|--------------|
| | All | 'Evaluable | Total | 'Evaluable | Total | 'Evaluable |
| <i>Candida albicans</i> | 98 | 23 | 96 | 23 | 98.0% | 100% |
| <i>Candida krusei</i> | 67 | 66 | 66 | 65 | 98.5% | 98.5% |
| <i>Candida lusitanae</i> | 52 | 36 | 50 | 36 | 96.2% | 100% |
| <i>Candida parapsilosis</i> | 72 | 47 | 71 | 46 | 98.6% | 97.9% |
| <i>Candida tropicalis</i> ** | 46 | 35 | 20 | 18 | 43.5% | 51.4% |
| <i>Candida glabrata</i> | 64 | 64 | 61 | 61 | 95.3% | 95.3% |
| Total | 399 | 271 | 364 | 249 | 91.2% | 91.9% |

** *Candida tropicalis* results for the spectrophotometer had significant essential errors for both clinical and challenge organisms for all 3 trial sites that tested on the spec. Low colony counts with the spec plate were observed for this organism against isavuconazole and indicated poor growth resulting in lower MICs. This may be an indication that the MICs for *Candida tropicalis* are affected by the 2X RPMI with 2% Dextrose.

Footnotes

¹When the reference method result is on-scale and the new device result is also on scale.

Results

Table 6. Interlaboratory Reproducibility % Essential Agreement +/- two log₂ dilution from the Modal Value

| Isavuconazole | Difference in the number of wells between new test result and test mode | | | | | | | | | | OFF-Scale | Test Mode | |
|-------------------------------------|---|----------|----------|-----------|-----------|------------|-----------|----------|----------|----------|-----------|-----------|---------|
| | OFF-Scale | -4 | -3 | -2 | -1 | 0 | +1 | +2 | +3 | +4 | | | |
| | All Sites | | | | | | | | | | | | |
| *1 <i>Candida parapsilosis</i> | | | 2 | 7 | 10 | 15 | 2 | | | | | | |
| *2 <i>Candida parapsilosis</i> | | | | | | 19 | 17 | | | | | | <=0.004 |
| *3 <i>Candida krusei</i> | | | | 2 | 5 | 29 | | | | | | | 0.25 |
| *4 <i>Candida tropicalis</i> | | | | | 4 | 21 | 11 | | | | | | 0.06 |
| *5 <i>Candida glabrata</i> | | | | 1 | 17 | 18 | | | | | | | 1 |
| *6 <i>Candida glabrata</i> | | | 2 | 3 | 14 | 17 | | | | | | | 0.12 |
| *7 <i>Candida lusitanae</i> | | | | 7 | 14 | 15 | | | | | | | 0.03 |
| *8 <i>Candida albicans</i> | | | | | 1 | 22 | 13 | | | | | | 0.5 |
| *9 <i>Candida parapsilosis</i> | | | | | 10 | 11 | 10 | 4 | 1 | | | | 0.015 |
| *10 <i>Candida krusei</i> | | | | 3 | 11 | 22 | | | | | | | 0.25 |
| Total | 0 | 0 | 4 | 23 | 86 | 189 | 53 | 4 | 1 | 0 | 0 | | |
| Between-Site Reproducibility | 355/360=98.6% | | | | | | | | | | | | |

Conclusions

This study validated that the Sensititre YeastOne susceptibility plates demonstrates an equivalent level of performance compared to the **CLSI M27-A3** reference BMD when testing **isavuconazole** with *Candida* spp. clinical and challenge isolates.

This study also validated that the Sensititre YeastOne susceptibility plates demonstrates an equivalent level of performance compared to the **ISO 16256 (2012) (E)** reference BMD plate when testing **isavuconazole** with *Candida* spp. clinical and challenge isolates. As shown in table 5, *Candida tropicalis* results for the spectrophotometer had significant essential errors. This may be an indication that **isavuconazole** MICs for *Candida tropicalis* are affected by the 2X RPMI with 2% dextrose and would suggest that it is not an acceptable method for susceptibility testing of **isavuconazole** against this species.

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