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Instructions for Use

Thermo Scientific Sensititre YeastOne Susceptibility Plates

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Thermo Scientific Sensititre YeastOne Susceptibility Plates

For *in vitro* Diagnostic Use

For full plate information, including plate layout, QC information please refer to www.trekds.com/techinfo. The plate code and batch number will be required.

Sensititre YeastOne Susceptibility plates are designed for invitro use in determining quantitative antifungal susceptibilities (MIC) of Candida species (i.e. *C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis).*

SUMMARY AND PRINCIPLES

The Sensititre yeast susceptibility test is a micro-version of the broth dilution susceptibility test. Various antifungal agents are serially diluted to concentrations bridging the range of clinical interest in autoclaved diluent, which contains a colorimetric growth indicating compound (TABLE 1.). Each plate is individually packaged in foil. After inoculation with a standardized suspension of organisms in inoculum medium and incubation at 35˚C for 24 to 48 hours, the minimum inhibitory concentrations (MIC) for the test organism are determined by observing the lowest antifungal concentration showing inhibition of growth (as evidenced by no color change).

TABLE 1. Antifungal agents and dilutions**.**

Antifungal Agent	Abbreviation	Dilution Range (µg/ml)
Fluconazole	FZ	0.125 - 256
Itraconazole	IZ	0.008 - 16
5 - Flucytosine	FC	0.03 - 64
Voriconazole	VOR	0.008-16
Caspofungin	CAS	0.008-16
Micafungin	MF	0.008-16

PRECAUTIONS

1.Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, with special awareness that the inoculated plate contains potentially pathogenic organisms.

2. All materials should be disposed of according to local, state or federal regulations.

3. Use only with Sensititre yeast susceptibility inoculum broth. The use of other diluent or broth could result in error.

STORAGE AND SHELF LIFE

The Sensititre YeastOne Susceptibility plates should be stored at room temperature (15 - 25˚C) away from direct sunlight and direct heat. **Warning. Exposure to direct sunlight can affect color reaction.** Use the plates prior to the expiration date printed on the label. If the package has been damaged in any manner, the plate in that package should be discarded. A silica gel desiccant is included in each foil package. If it has not retained the color as stated on the carton label, the plate should be discarded.

PRODUCT DETERIORATION

Exposure to storage conditions other than those recommended may result in loss of potency of the antifungal agents and/or discoloration of indicator.

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected, transported, stored and then cultured on primary isolation media to ensure purity, according to procedures recommended in the Manual of Clinical Microbiology (Ref. 1).

Materials included:

- YeastOne™ susceptibility plate
- Adhesive seals

Materials not included**:**

* Available in the USA only

- Sensititre™ yeast susceptibility inoculum broth
- Sensititre ™ Demineralized water, 5ml
- Sensititre ™ AIM™ [V3020] /Sensititre™ AutoInoculator™ [V3010]
- Sensititre™ Vizion ™ [V2021]
- Sensititre™ Nephelometer™ [V3011]
- Sensititre™ doseheads (for automated inoculation only)
- 100µl pipette and tips for manual inoculation*
- Manual viewer [V4007]
- Plate configuration grid for manual viewer
- Sterile inoculum reservoir*
- 0.5 McFarland barium sulfate turbidity standard* (for preparation see Appendix 1) or 0.5 McFarland polymer turbidity standard
- Bacteriological loop*
- Quality control organisms (see QC section)
- Fungal growth medium agar plates e.g Sabauroud dextrose agar (SDA)
- Vortex mixer
- Incubator (Non-CO_2)

- 20µl pipette

INOCULATION PROCEDURE: *Candida* spp.

Allow all broths to come up to room temperature before use.

A final organism density of approximately 1.5 - 8 X 10^3 CFU/ml is recommended.

1. Pick several well-isolated colonies of >1mm diameter from a pure 24-hour culture of yeast isolates, and emulsify into sterile water. Vortex mix the suspension for 15 seconds, ensuring that the suspension is uniform. If clumping occurs, allow the clumps to settle before adjusting the density. Adjust to a 0.5 McFarland standard visually or using a Sensititre Nephelometer

2. Transfer 20 µl of the yeast suspension into 11 ml of YeastOne inoculum broth to give an inoculum of 1.5-8 x 103 viable CFU/ml. Steps and 1 and 2 should be completed in 15 minutes.
3. Transfer 100µl of the broth suspension into the plate by either:

a. **Sensititre AIM or AutoInoculator**. Replace the tube cap with a Sensititre single-use dosehead and inoculate the plate according to the AIM/ AutoInoculator instructions .Remove the rest tube/dosehead combination from the AIM/AutoInoculator within 30 seconds of dosing a plate and store inverted in a rack or discard.

b. Manual pipette. Pour the broth into a sterile seed trough and inoculate the plate or using an appropriate pipette.

4. A check of the colony count should be done to check inoculum density by removing 10µl from the positive control well and plating onto Sabauroud dextrose agar (SDA). A correct inoculum will produce 10-80 colonies.
5. Cover all wells with the adhesive seal. Avoid creases as these can lead to skips.

INCUBATION

1. Place plates in a stack of no more than three plates.

2. Minimally incubate the plates for 24-25 hours at 35˚C in a non-CO_2 incubator.

***Incubation at temperatures over 35°C* may affect the performance of these plates.**

READING TEST RESULTS

Plates may be read visually under normal laboratory lighting using a reading mirror, which displays the underside of the wells, alternatively, plates may be read on the Sensititre Vizion System. Refer to the Vizion User Manual for additional information. Yeast growth in the antifungal solutions will be evident as a change in the colorimetric growth indicator from blue (negative) to pink to purple (positive). Some organisms may not change the indicator completely to pink, but display more of a purpling of the indicator. Some yeast may show a slight purpling in Fluconazole and Itraconazole. See

Examples 1 & 2 in the Reading Notes below. Check purity test plates, results are invalid if a mixed culture is present.

Examine the positive growth well after 24 hours incubation. If the growth well is pink, the endpoints for the antifungals can be interpreted. If, after incubation, the well is still blue or only faintly purple, re-incubate for an additional 24 hours and examine for growth.

Reference strains of defined susceptibility may also help to train personnel.

DO NOT READ TURBIDITY IN THE SENSITITRE YEASTONE PLATES. READ ONLY COLOR CHANGE.

The MIC is the lowest concentration of an antifungal agent that substantially inhibits growth of the organism. This growth is detected visually by observing a color change in concentrations below the MIC. The amount of color change in the wells containing the agent is compared with the amount of color change in the growth-control well (no antifungal agent) used in each set of tests as follows:

1. "No growth" in the wells containing the antifungal agent is recorded when there is no change in the blue indicator.

2. For all antifungals: the MIC is recorded as the lowest concentration of antifungal agent preventing the development of a pink or purple (>50% of the growth control) color change, i.e. the first blue or faint purple well. (See Reading Notes below).

3. If all dilutions for an antifungal agent demonstrate good color change the endpoint is recorded as "greater than" (>) the highest concentration tested. If all dilutions for an antifungal show little to no color change, the endpoint for that antifungal is recorded as "less than or equal to" (≤) the lowest concentration tested.

4. If there is a blue well in a series of pink or purple wells, i.e. wells 1, 2 and 8 µg/ml are pink but well 4 µg/ml is blue, the "skip" should be ignored and the MIC reported as 16 µg/ml. If there are 2 skipped wells in a dilution series, either as a double skip or 2 single wells, the MIC should be invalidated and a repeat carried out. This skip well phenomenon is addressed in detail by Findell and Sheeris (Ref. 3). Refer to TABLE 2 for illustration.

5. If contamination is suspected, the test should be repeated for all antifungals.

TABLE 2.

Illustration of test results that may occur and the interpretation of each result

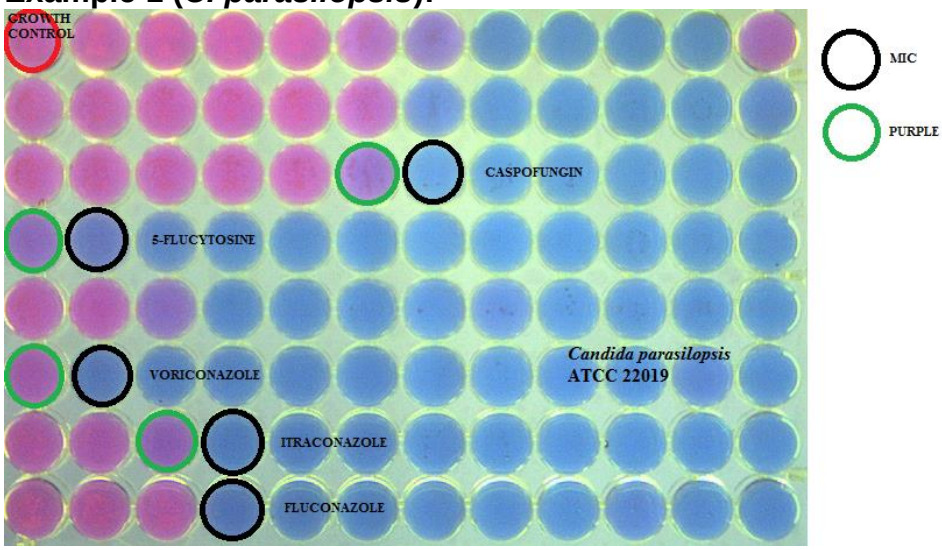
	Well Concentration µg/ml						**R = RED: Positive growth indication** **B = BLUE: Negative growth indication**
	1	2	4	8	16	32	
A.	R	R	R	B	B	B	Typical growth pattern; MIC endpoint is 8 µg/ml.
B.	R	R	R	R	R	R	Growth in all wells; MIC endpoint is >32 µg/ml.
C.	B	B	B	B	B	B	No growth in any well; MIC endpoint is ≤1 µg/ml.
D.	R	R	R	B	R	R	"Skipped Well". MIC endpoint is >32 µg/ml. Disregard "skip" when wells on either side have growth. If more than one "skip" should occur in a column, the test results are invalidated[1]
E.	R	R	B	B	R	R	Double "Skipped Well". The test should be repeated [1]

[1] With careful technique these occurrences are uncommon.

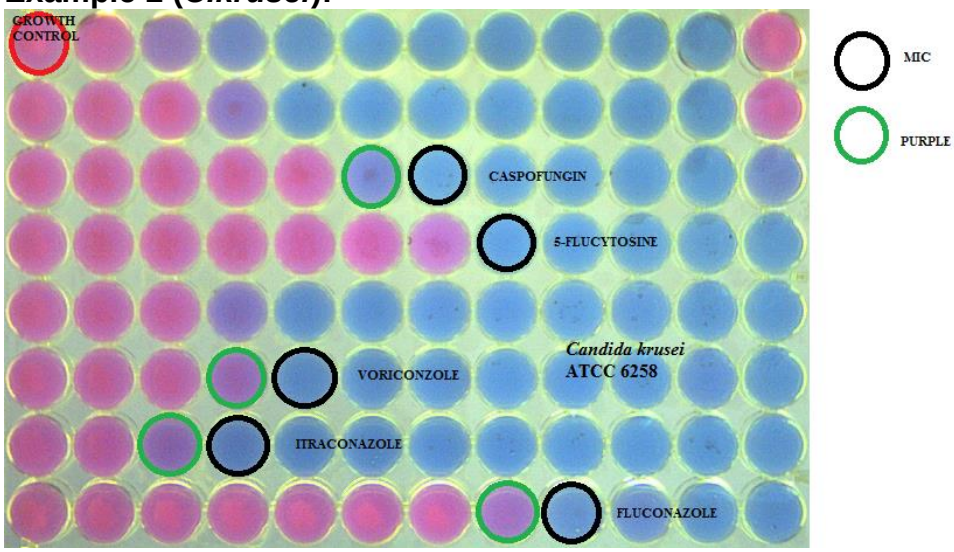
READING NOTES

Flucytosine, Caspofungin and Azole Antifungals.
Various strains with Flucytosine, Caspofungin, Itraconazole, Voriconazole may, at times, give endpoints that are typically less sharp. Therefore the MIC may be a well that shows weak/faint purple at a concentration above where pink or string purple wells are observed. See Examples 1 & 2 below.

Example 1 (*C. parasilopsis*):

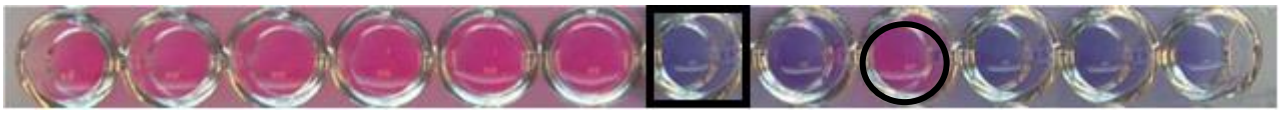


Example 2 (*C.krusei*):



Itraconazole:

Itraconazole can occasionally come out of solution at concentrations of ≥4 µg/ml. This can result in the affected well above the MIC exhibiting growth and turning pink.



Trailing Endpoints:

Trailing occurs when a slight color change persists above the MIC and it is often identical for several or all drug concentrations above the MIC. The MIC should be read as the first well showing a less intense color change compared to the more positive growth wells of the lower concentrations.

Isolates of *Candida krusei* are assumed to be intrinsically resistant to Fluconazole and their MICS should not be interpreted. (Ref. 2). A comment should accompany the test result reported.

Contamination/ Skips

Alternatively, a pink (growth) well between blue (no growth) wells could be indicative of contamination. Sub-culture well contents to ascertain the cause.

A blue well in a series of pink growth wells indicates a “skip” and should be ignored. The MIC should be read above any skip wells. If there is more than one skipped well, the antifungal should **not** be reported.

Echinocandins:

The MIC end-points should be determined after 24-hours of incubation at 35C. The MIC should be read as the first well showing a less intense color change as compared to the positive control well.

QUALITY CONTROL

1. A positive growth control well (A1) is provided on each plate to demonstrate growth typical of the test organism in the test medium without antifungal inhibition. This well must exhibit growth or the test must be repeated.

2. The potency of the antifungal agent dilutions should be checked by testing organisms with known endpoints. For user quality control of the MIC system, Sensititre recommends the following culture(s) from the American Type Culture Collection(ATCC):

*Candida krusei**	ATCC®	6258
Candida parapsilosis	ATCC®	22019

*ATCC now lists these organisms as *Issatchenkia orientalis.*

3. Yeast isolates should be maintained as described by the National Committee for Clinical Laboratory Standards (2). The inoculation, reading and Interpretation of Sensititre YeastOne susceptibility plates when tested for user quality control should be performed as described in the preceding section.
4.

TABLE 3. Acceptable quality control ranges of MIC's (µg/ml).

Ranges are as per CLSI M27 (2) except where underlined which are Sensititre QC ranges

Antifungal Agent	*Candida krusei* ATCC 6258		*Candida parapsilosis* ATCC 22019	
	24 hour	48 hour	24 hour	48 hour
Fluconazole	8 - 64	16-128	0.5-4	2-8
Itraconazole	0.12 - 1	0.25 - 1	0.06 - 0.5	0.06 - 0.5
5 - Flucytosine	4 - 16	8 - 32	0.12-0.5	0.12 - 0.5
Voriconazole	0.06-0.5	0.12-1	0.015-0.12	0.03-0.25
Caspofungin	0.12-1	-	0.25-1	-
Micafungin	0.12-0.5	-	0.5-2.0	-

4. Performance of quality control tests should be conducted on a regular basis in accordance with approved standard laboratory procedures. Trek recommends that laboratory procedures meet the minimum requirements outlined in the CLSI document M27 (Ref. 2) for quality control including frequency and corrective action when necessary.

TABLE 4. MIC Interpretative Criteria (µg/ml) for *Candida* Species as per FDA.

See CLSI M27 for further information on interpretive criteria.

Antifungal Agent	Susceptible	Susceptible dose dependent	Intermediate	Resistant
Fluconazole*	≤8	16 - 32		≥64
Itraconzole	≤0.125	0.25 - 0.5		≥1
Flucytosine	≤4		8 - 16	≥32
Caspofungin	≤2***			
Voriconazole**	≤1		2	≥4

**Candida krusei* recovered from super infections have not been found to be susceptible to Fluconazole and may require alternative antifungal therapy.

** In clinical studies, Voriconazole MIC_{90} for *C.glabrata* baseline was 4µg/ml; 13/50 (26%) *C.glabrata* baseline isolates were resistant (MIC ≥4µg/ml) to Voriconazole. However, based on 1054 isolates tested in surveillance studies the MIC_{90} was 1µg/ml.

*** For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any results categories other than “susceptible.” For strains yielding results suggestive of a “non-susceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

TABLE 5. MIC Interpretative Criteria for Micafungin

Organism	Susceptible	Intermediate	Resistant
Candida albicans	≤0.25	0.5	≥1
Candida tropicalis	≤0.25	0.5	≥1
Candida krusei	≤0.25	0.5	≥1
Candida parapsilosis	≤2	4	≥8
Candida glabrata	≤0.06	0.12	≥0.25

Note: Shown above are the breakpoints (µg/ml) for Micafungin against the five indicated for use Candida species conform to both FDA and CLSI.

LIMITATIONS

1. Sensititre YeastOne test plates are intended for use with *Candida* species. They are not intended for use with Cryptococcus species, miscellaneous rapid growing yeast species or slow growing yeast such as *Histoplasma* or *Blastomyces.* They are not intended for use with filamentous fungi.
2. Comparison between the Sensititre YeastOne at 24 hours and the CLSI reference method at 48 hours was evaluated. However due to the difficulty in correlating end points of trailing organisms (*C. albicans*) at 48 hours incubation, high error rates are observed. In addition to the *C.albicans,* high error rates with trailing endpoints were also observed with Voriconazole with *C.glabrata*. perform alternative method if this drug/bug combination result is required
3. Testing of fungi and antifungal agents is inherently less precise than testing bacteria.
4. Some investigators believe the 24-hour reading is more appropriate than the 48-hour reading because of the problem with trailing with certain isolates. The CLSI official standard indicates that readings should be accomplished at 48 hours. Until sufficient data is collected and analyzed, the question of most clinically relevant time of reading remains unanswered. Reporting of results should indicate clearly the times of reading.
5. For additional guidance, review of CLSI Antifungal Susceptibilities Standard M27 is encouraged.
6. In YeastOne , color change is the indicator of the end point, not turbidity. (This fact alleviates some major concerns with the interpretation of certain Candida species because of 'trailing'. Trailing is more commonly seen with isolates other than those of blood and other sterile body fluids.)
7. Do not read at 24 hours if the control well has not completely turned positive.
8. The ability of the Sensititre YeastOne to detect resistance to Voriconazole and Caspofungin is unknown because lack of resistant strains were available at the time of comparative testing. Any non-susceptible result should be confirmed by an alternate method.
9. As with any in-vitro susceptibility testing method, the results of testing should be correlated with the patient's clinical response to prescribed therapy.

10. Studies for Micafungin were evaluated using the AutoInoculator and manual pipette and manual read only. Performance has not been established for Sensititre AIM , or the Sensititre Vizion System.
11. The ability of the Sensititre YeastOne to detect resistance to Micafungin is unknown because resistant strains were not available at the time of comparative testing. For strains yielding results suggestive of a not susceptible category, organism identification and Micafungin should be retested and confirmed, and if the result is confirmed, the isolate should be submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

PERFORMANCE CHARACTERISTICS

A. Introduction -- The principal study used plates configured to the format of the Sensititre YeastOne MIC susceptibility plate. This study complies fully with the FDA Review Criteria for Assessment of Antimicrobial Susceptibility Devices (May 31, 1991). Frozen reference plates and Sensititre YeastOne test plates were utilized for testing procedures. The CLSI recommended QC organisms for the antimicrobials tested are Candida krusei ATCC 6258, and Candida parapsilosis ATCC 22019. The QC ranges obtained during the study at the 3 clinical trial sites were all within the ranges stated in the CLSI M27.

B. Clinical performance was established with a multi center 3 site study comparing YeastOne to the CLSI M27A, the reference standard. The plates were read at both 24 and 48 hours. Tables 6 and 8 summarize data from all Candida species read manually. Tables 7 and 9 summarize the performance of C.albicans only. % Category Agreements are particularly low for the azoles due to a high incidence of MIC's falling within the categorical break point range and also the difficulty in reading the end points with these drugs. These two factors cause the incidence of minor (mi) categorical disagreements to be high.

C. Performance of Sensititre Yeast One Panels read manually at 24 hours compared to CLSI M27 reference method at 24 hours. Clinical and Challenge isolates combined (Micafungin).

Total number of clinical yeast isolates used to evaluate the performance of the Sensititre YeastOne susceptibility system:

Yeast isolates	Total tested
Candida albicans	461
Candida krusei	61
Candida lusitaniae	74
Candida parapsilosis	89
Candida tropicalis	91
Candida torulopsis glabrata	93
Candida torulopsis species	26
Candida species	36
Grand Total	931

TABLE 6. *Candida species* including *C. albicans:* 24 hours within + or -2 well dilution for essential agreement.

ANTIFUNGAL AGENT	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT	% CATEGORY AGREEMENT*
Fluconazole	870	91.0	91.1
Itraconazole	931	92.4	68.2
5-Flucytosine	931	91.7	94.0

TABLE 7. *C. albicans* performance at 24 hours within + or -2 well dilution for essential agreement.

ANTIFUNGAL AGENT	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT	% CATEGORY AGREEMENT*
Fluconazole	461	87.4	92.0
Itraconazole	461	89.4	72.2

TABLE 8.***Candida species* including *C. albicans*: 24/48 hours within $\pm$2 well dilution for essential agreements.

ANTIFUNGAL AGENT	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT	% CATEGORY AGREEMENT*
Fluconazole	870	81.7	84.0
Itraconazole	931	81.6	51.6

TABLE 9.***C. albicans:* 24/48 hours with ±2 well dilution for essential agreement.

ANTIFUNGAL AGENT	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT	% CATEGORY AGREEMENT*
Fluconazole	461	70.4	74.4
Itraconazole	461	70.7	44.4

*% Category Agreements are particularly low for the azoles due to a high incidence of minor (mi) categorical disagreements.
** For explanation of performance of Table 7 and 8 the comparison between the Sensititre YeastOne at 24 hours and the CLSI reference method at 48 hours was evaluated. However due to the difficulty in correlating end points of trailing organisms (*C. albicans*) at 48 hours incubation, high error rates are observed.

Voriconazole

Performance characteristics of Sensititre YeastOne panels read manually at 24 hours compared to the reference method read at 48 hours. Clinical and challenge set combined

TABLE 10. AIM/AUTOINOCULATOR PERFORMANCE: *Candida* spp. Including *C.glabrata*: 24/48 hours:

Antifungal	Number of strains tested	% Essential Agreement	% Category Agreement
		24h vs. 48h	24h vs. 48h
Voriconazole	367	96.7	94.5
Voriconazole *	298	97.0	98.0

* With *C.glabrata* performance excluded

TABLE 11. MANUAL PIPETTE PERFORMANCE: *Candida* spp. Including *C.glabrata*: 24/48 hours:

Antifungal	Number of strains tested	% Essential Agreement	% Category Agreement
		24h vs. 48h	24h vs. 48h
Voriconazole	367	97.2	94.3
Voriconazole *	318	97.8	96.5

* With *C.glabrata* performance excluded

TABLE 12. AIM /AUTOINOCULATOR PERFORMANCE: *C.glabrata* *: 24/48 hours:

Antifungal	Number of strains tested	MI	% Essential Agreement	% Category Agreement
			24h vs. 48h	24h vs. 48h
Voriconazole	69	14	97.7	79.7

TABLE 13. MANUAL PIPETTE PERFORMANCE: *C.glabrata**: 24/48 hours:

Antifungal	Number of strains tested	MI	% Essential Agreement	% Category Agreement
			24h vs. 48h	24h vs. 48h
Voriconazole	51	10	94.1	80.4

*% Category agreements are particularly low for voriconazole due to a high incidence of minor categorical disagreements. This is due to the difficulty in correlating end points of trailing organisms (*Candida glabarata*)) at 48 hours of incubation, high errors are observed

Caspofungin

Performance characteristics of Sensititre YeastOne panels read manually at 24 hours compared to the reference method read at 24 hours. Clinical and challenge set combined

TABLE 14. AIM /AUTOINOCULATOR PERFORMANCE: *Candida* spp. 24 hours:

Antifungal	Number of strains tested	% Essential Agreement	% Category Agreement
		24h	24h
Caspofungin	473	98	100

TABLE 15. MANUAL PIPETTE PERFORMANCE: *Candida* spp: 24 hours:

Antifungal	Number of strains tested	% Essential Agreement	% Category Agreement
		24h	24h
Caspofungin	573	99.8	99.8

Micafungin

TABLE 16. *Candida* spp: 24 hours

MICAFUNGIN vs CANDIDA SPP	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT 24h	% CATEGORY AGREEMENT 24h
Candida albicans	98	100	100
Candida tropicalis	41	100	100
Candida krusei	67	100	100
Candida parapsilosis	70	100	100
Candida glabrata	39	100	97.4

The Distribution of 50 Challenge Strains tested on the AutoInoculator

MICAFUNGIN vs CANDIDA SPP	NUMBER OF STRAINS TESTED	% ESSENTIAL AGREEMENT 24h	% CATEGORY AGREEMENT 24h
Candida albicans	12	100	100
Candida tropicalis	9	100	100
Candida krusei	8	100	100
Candida parapsilosis	12	100	100
Candida glabrata	9	100	100

Reproducibility Data

The reproducibility data of MIC tests was measured against ten organisms tested three times on 3 separate days at three sites. Testing was performed using Sensititre YeastOne susceptibility plates read manually at 24 and 48 hours. The frozen reference panels were also evaluated in the same manner. Reproducibility was calculated as the percent of results for the combined sites, which were within plus or minus one dilution of the combined site mode. This data is summarized in Table 17.

TABLE 17. Reproducibility Results

Antifungal Agents	Overall agreement +/- 1 dilution of 3 site mode with *Candida* species	
Test Panel	24 hours	48 hours
Fluconazole	100	99
Itraconazole	100	99.6
5-Flucytosine	96	99
Reference Panel		
Fluconazole	99.6	99.6
Itraconazole	95.5	97
5-Flucytosine	95	95.5

Voriconazole and Caspofungin

The reproducibility data of MIC tests was measured against 10 *Candida* spp. tested three times on 3 separate days at three sites. Testing was performed using Sensititre YeastOne susceptibility plates read manually at 24 hours. Frozen reference panels were prepared according to CLSI M27 for reference read after 48 hours (Voriconazole) and 24 hours (Caspofungin) incubation. Results shown in Table 18.

TABLE 18: SENSITITRE YEASTONE MANUAL

Antifungal	Between site reproducibility
Voriconazole	24h vs. 48h
	100%
Caspofungin	24h
	100%
Micafungin*	24h
	100%

The reproducibility of MIC tests was measured against 25 *Candida* spp. Tested at 2 sites. Testing was performed using Sensititre YeastOne plates read manually (24hrs.); frozen plates were prepared according to CLSI M27 for reference read after 48 hours (Voriconazole) and 24 hours (Caspofungin) incubation for essential agreement comparisons. YeastOne plates were set up using both the Autoinoculator /AIM and a manual pipette to show between site reproducibility. Results shown in Table 19 and 20.

*25 yeast isolates provided by TREK were tested on the Sensititre dried YeastOne Susceptibility Plate and inoculated with a manual pipette.

TABLE 19: AIM / AUTOINOCULATOR PERFORMANCE

Antifungal	Between site reproducibility
Voriconazole	24h vs. 48h
	100%/100%
Micafungin**	24h
	100%

**25 yeast isolates provided by TREK were tested on the Sensititre dried YeastOne Susceptibility Plate and inoculated with the AutoInoculator.

Antifungal	Essential agreement/Between site reproducibility
Caspofungin	24h
	100%/100%

TABLE 20: MANUAL PIPETTE PERFORMANCE

Antifungal	Between site reproducibility
Voriconazole	24h vs. 48h
	100%/100%

Antifungal	Essential agreement/Between site reproducibility
Caspofungin	24h
	100%/100%

Performance Characteristics with the Vizion

The Vizion was validated in the following manner: A total of 528 clinical and challenge isolates representing a wide variety of isolates including some of the current resistance patterns were tested on the Sensititre system comparing the Vizion read to the mirror read.

For results representing these yeast isolates, the overall essential agreement rate (+/- 2 log2 dilution of the VIZION read compared to the mirror read) was 99.9% and the overall categorical agreement (qualitative (S,I,R) according to the FDA MIC interpretive categories comparing the VIZION to the mirror read) was 99.6%. See table 1 and 2 below:

TABLE 1. Clinical and Challenge isolate results VIZION vs. Manual Mirror Read

Organism	Total Isolates Tested	% Essential agreement	% Categorical agreement
**Candida* spp.	528	99.9	99.6

**Candida* spp. isolates include *C. albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii* (three isolates), *C. lusitaniae*, *C. parapsilosis*, and *C.tropicalis*

TABLE 2. A combination of all MIC/SIR values for Yeast organisms per antifungal drug using the VIZION

Organisms	Overall % MIC/SIR Comparative Agreement with the VIZION				
	Drug				
	Fluconazole	Itraconazole	5-Flucytosine	Voriconazole	Caspofungin
Candida albicans	100/100	100/96.7	100/100	100/100	100/100
Candida glabrata	100/95.1	100/N/A	100/100	100/97.6	100/100
Candida krusei	98.4/N/A	98.4/N/A	98.4/93.8	98.4/100	100/100
Candida lusitaniae	100/100	100/N/A	100/100	100/100	NT
Candida parapsilosis	100/100	100/N/A	100/100	100/100	100/100
Candida tropicalis	100/100	100/N/A	100/100	100/100	100/100

N/A=Not applicable. No categorical and/or essential agreement was calculated for these drug/organism combinations since they are considered resistant irrespective of the MIC.

*NT indicates that Candida lusitaniae isolates were Not Tested against Caspofungin.

Summary of Reproducibility Data:

The reproducibility of MIC tests at 3 trial sites was measured against: 25 Candida spp. Testing was performed using Sensititre plates read on the VIZION. Reproducibility was calculated as occurrences of the difference in number of wells between the test result and test mode. Percentages of results were calculated for the combined sites plus or minus two dilutions of the modal value (best and worst case scenarios assuming the off scale result is within two wells from the mode and assuming the off-scale result is greater than two wells from the mode) for all sites.
The MIC values were off-scale for some isolates, which has resulted in low performance based on “worst-case” calculations, the overall reproducibility was considered acceptable, since very high essential agreement was observed based on “best-case” calculations and when calculations were made using on-scale MIC values only. Please refer to Table 3 for results.

TABLE 3. Yeast Reproducibility of the VIZION

	% Reproducibility VIZION		
	Best Case (Assuming off scale result is within 2 wells from the mode)	On scale (On scale values only)	*Worst case (Assuming off scale result is greater than 2 wells from the mode)
VIZION	99.9	99.8	90.9

* Off-scale isolates were used due to unavailability or insufficient number of isolates with on-scale MIC values for some drug/organism combinations.

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