



YEASTONE®

**For Research Use Only.
Not for use in diagnostic procedures.**

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 **Research Use Only** in determining quantitative antifungal susceptibilities (MIC) of non-fastidious yeast, including *Candida* species, *Cryptococcus* species, and miscellaneous other rapid growing yeast species.

There have been reports in the literature of the use of Yeastone® plates with filamentous fungi including *Aspergillus* species (10-14), *Prototheca wickerhamii* (16), *Madurella mycetomatis* (17). Please refer to these references for further information.

SUMMARY AND PRINCIPLES

The Sensititre® yeast susceptibility test is a colorimetric microdilution susceptibility test. Each plate is dosed with appropriate dilutions of antifungal agents and a colorimetric indicator. 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)
Amphotericin B	AB	0.12 -8
Anidulafungin	AND	0.015- 8
Caspofungin	CAS	0.008 - 8
Fluconazole	FZ	0.12 - 256
5 - Flucytosine	FC	0.06 - 64
Itraconazole	IZ	0.015 - 16
Micafungin	MF	0.008 - 8
Posaconazole	PZ	0.008 - 8
Voriconazole	VOR	0.008 -8

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 broths 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.** Exposure to storage conditions other than those recommended may result in loss of potency of the antifungal agents and/or discoloration of indicator. Each plate is individually packaged in foil and a silica gel desiccant included. If the silica gel has not retained the color as stated on the carton label, or the package has been damaged in any manner the plate should be discarded. The plates should be used prior to the expiration date printed on the label.

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 YeastOne[®] inoculum broth

Demineralized water, 5ml

Sensititre Autoinoculator[®]

Sensititre[®] doseheads (for automated inoculation only)

100 µl pipette and tips for manual inoculation*

Manual viewer

Plate configuration grid for manual viewer

0.5 McFarland turbidity standard*

Tips for manual inoculation*

Bacteriological loop*

Sterile inoculum reservoir*

Quality control organisms (see QC section)

Fungal growth medium agar plates e.g. Sabouroud dextrose agar (SDA) Vortex mixer

Incubator (Non-CO₂)

20 µl pipette

INOCULATION PROCEDURE

A final organism density of approximately $1.5-8 \times 10^3$ CFU/ml is recommended. As with all susceptibility tests the inoculum density is important and must be standardized to a 0.5 McFarland turbidity standard. The time period from inoculation of the water is critical and this transfer should be done within a maximum of 15 minutes. The final inoculum in the broth should be inoculated into the YeastOne[®] plates within 15 minutes.

1. Using a sterile wooden applicator swab or bacteriological loop, touch several well-isolated colonies of >1 mm diameter from a pure 24 hour culture of the yeast isolate. Transfer to 5ml of sterile demineralized water.

2. The resulting suspension should be vortexed for 15 seconds. Ensure that the suspension is uniform without large clumps of organisms. If clumping occurs, allow the clumps to settle before adjusting the density. The turbidity can be adjusted to be equivalent to a 0.5 McFarland standard using a Sensititre Nephelometer[®] or visually by comparison to a 0.5 McFarland standard.

3. Transfer 20 μ l of the yeast suspension into 11 ml of YeastOne[®] inoculum broth. Vortex or mix vigorously. This results in $1.5-8 \times 10^3$ viable CFU/ml.

4. A confirmatory plate count should be done to check inoculum density by removing 10 μ l from the growth control well and plating onto Sabauroud dextrose agar (SDA). A correct inoculum will produce 15-80 colonies

a. Manual Inoculation

1. Pour the inoculum into a sterile inoculum reservoir.

2. Using an appropriate pipette (e.g. 8-channel multi-pipettor), carefully inoculate 100 μ l into each well of the Sensititre[®] plate. Avoid drawing up any air bubbles as they will interfere with the accurate delivery of the inoculum. Since the tip will be used to inoculate a series of wells, avoid touching the tip to the bottom of any well.

3. It is recommended to use the excess yeast suspension to inoculate an SDA plate as a purity check. Incubate the plate for 24-72 hours at 35°C.

4. Place contaminated tips and inoculum reservoir into an approved safety biohazard container.

5. Cover the plates with the adhesive seal, ensuring that all wells are covered.

b. Automated Inoculation

1. Replace the tube cap with a Sensititre[®] dosehead and inoculate 100 μ l into each well of the Sensititre YeastOne[®] plate according to the Autoinoculator[®] instructions.

2. Cover the plate with the adhesive seal, ensuring that all wells are covered.

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-CO₂ incubator. *Cryptococcus* species should be incubated for 48-72 hours.

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. Yeast growth in the antifungal solutions will be evident as a change in the colorimetric growth indicator from blue (negative) to red (positive). Some yeast may not change the indicator completely to red, but display more of a purpling of the indicator. Some organisms may show a slight purpling in fluconazole and itraconazole. (See details for reading below).

1. Examine the positive growth well after 24 hours incubation. If the growth well is red, the endpoints for the antifungals can be interpreted. If, after incubation, the well is still blue or only faintly purple, reincubate for an additional 24 hours and examine for growth.
2. With modifications made for colorimetric reading endpoint determinations should be done similarly to the descriptions provided in CLSI (NCCLS) Document M27, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; (Ref. 2).

DO NOT READ TURBIDITY IN THE SENSITRE YEASTONE[®] PLATES. Read Only Color Change.

3. The MIC is the lowest concentration of an antifungal agent that substantially inhibits growth of the organism as detected visually by observing a color change. The amount of color change in the wells containing the agent is compared with the amount of color change in the growth-control wells (no antifungal agent) used in each set of tests as follows:
4. "No growth" in the antifungal solutions is recorded when there is no change in the blue indicator in the well.
5. The MIC is recorded as the lowest concentration of antifungal agent preventing the development of a red or purple growth well, i.e. first blue. When reading from the lowest concentration dilution to the highest concentration, the MIC is the lowest concentration in which no growth appears. If all dilutions for antifungal agent demonstrate growth the endpoint is recorded as "greater than" (>) the highest concentration tested. If all dilutions for an antifungal show inhibition of growth, the endpoint for that antifungal is recorded as "less than or equal to" (≤) the lowest concentration tested.
6. If there is a blue well in a series of red growth wells, i.e. wells 1, 2 and 8 µg/ml are red but well 4 µg/ml is blue, the "skip" should be ignored and the MIC reported as 16 µg/ml. This skip well phenomenon is addressed in detail by Findell and Sheeris (Ref. 3) and described in TABLE 2 example D and E. If contamination is suspected, the test should be repeated for all antifungals.

TABLE 2. Illustration and the interpretation of test results that may occur

Well Concentration $\mu\text{g/ml}$	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 $\mu\text{g/ml}$.
B.	R	R	R	R	R	R	Growth in all wells; MIC endpoint is >32 $\mu\text{g/ml}$.
C.	B	B	B	B	B	B	No growth in any well; MIC endpoint is <1 $\mu\text{g/ml}$.
D.	R	R	R	B	R	R	"Skipped Well". MIC endpoint is >32 $\mu\text{g/ml}$. Disregard "skip" when wells on either side have growth. If more than one "skip" should occur in a column, the test results are invalidated)
E.	R	R	B	B	R	R	Double "Skipped Well". The test should be repeated)

¹ With careful technique these occurrences are uncommon.

READING NOTES

Amphotericin B. For Amphotericin B at 24 hours, the endpoints are typically easily defined and the MIC is read as the lowest drug concentration that prevents any discernible color change. Trailing endpoints with Amphotericin B are not usually encountered.

Flucytosine, Caspofungin and Azole Antifungals. *Candida albicans*, *C. glabrata* and *C. tropicalis* with flucytosine and azoles, such as fluconazole, itraconazole, posaconazole and voriconazole may give endpoints that are typically less sharp because of trailing growth, and may be a significant source of variability. Trailing occurs when a slight color change persists and it is often identical for all drug concentrations above the MIC. The MIC should be read as the first well showing a less intense color change compared to the positive growth control well. Reference strains of defined susceptibility may also help to train personnel. 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.

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):

<i>Candida krusei</i> *	ATCC [®] 6258
<i>Candida parapsilosis</i>	ATCC [®] 22019

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

3. Yeast isolates should be maintained as described by The Clinical and Laboratory Standards Institute (NCCLS) (Ref. 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. 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 outlines in the CLSI (NCCLS) document M27 (Ref. 2) for quality control including frequency and corrective action.

TABLE 3. Recommended 24 and 48 hour MIC limits for two quality control strains as per Broth Microdilution CLSI (NCCLS) M27 (Ref. 2) Ranges that are different or additional to published quality control ranges are underlined.

Antifungal Agent	Candida krusei		Candida parapsilosis	
	ATCC 6258		ATCC 22019	
	24 hour	48 hour	24 hour	48 hour
5 – Flucytosine	4-16	8-32	<u>0.12-0.5</u>	0.12-0.5
Amphotericin B	0.5-2	1-4	<u>0.25-2</u>	0.5-4
Anidulafungin	0.03-0.12	-	0.25-2	-
Caspofungin	0.12-1	0.25-1	0.25-1	0.5-4
Fluconazole	8-64	16-128	<u>2-8</u>	<u>2-8</u>
Itraconazole	0.12-1	0.25-1	0.12-0.5	0.12-0.5
Micafungin	<u>0.06-0.25</u>	0.12 – 0.5	0.5-2	0.5-4
Posaconazole	0.06-0.5	0.12-1	0.06-0.25	0.06-0.25
Voriconazole	0.06-0.5	0.12-1	0.016-0.12	0.03-0.25

TABLE 4. MIC Interpretative Criteria ($\mu\text{g/ml}$) for *Candida* Species as per CLSI M27

Antifungal Agent	Susceptible	Susceptible ³ Dose Dependent	Intermediate ⁴	Resistant	Non-susceptible
Anidulafungin⁵	≤ 2				> 2
Caspofungin⁵	≤ 2				> 2
Fluconazole ¹	≤ 8	16-32		≥ 64	
5 – Flucytosine ⁶	≤ 4		8-16	≥ 32	
Itraconazole ²	≤ 0.125	0.25-0.5		≥ 1	
Micafungin⁵	≤ 2				> 2
Voriconazole ⁵	≤ 1	2		≥ 4	

NOTE 1 Shown are the breakpoints ($\mu\text{g/ml}$) for *Candida* species against the indicated agents. If MICs are measured using a scale that yields results falling between categories, the next higher category is implied. Thus, an isolate with a fluconazole MIC of 12.5 $\mu\text{g/mL}$ would be placed in the S-DD category.

NOTE 2 The MIC breakpoints in boldface type were adopted at a meeting of the subcommittee held on 9th June 2007 in Boston, MA. These breakpoints are considered

tentative for one year and are open for comments. There is no resistant category assigned for the echinocandin agents; isolates with higher MICs may be described as nonsusceptible.

¹ For fluconazole, these guidelines are based on extensive experience with mucosal and invasive infections due to *Candida* spp. . It is also pertinent that the 8- μ g/mL upper boundary for the susceptible range of fluconazole is not known with certainty – the data would permit selection of either 4 or 8 μ g/mL for this cut-off.

When an isolate is indentified as *Candida glabrata* and the MIC is \leq 32, patients should receive the maximum dosage regimen of fluconazole. Expert consultation on selection of maximum dosage regime may be useful. Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole, and their MIC's should not be interpreted using this scale.

² For itraconazole, the data are based entirely on experience with mucosal infections, and data supporting breakpoints for invasive infections due to *Candida* spp are not available.

³ Susceptibility is dependent on achieving the maximal possible blood level. For fluconazole, doses of 400 mg/day or more may be required in adults with normal renal function and body habitus. For itraconazole, measures to assure adequate drug absorption and plasma itraconazole concentrations of >0.5 μ g/mL may be required for optimal response.

⁴ The susceptibility of these isolated is not certain, and the available data do not permit them to be clearly categorised as either “susceptible” or “resistant”.

⁵.For these drugs, the data are based substantially on experience with non-neutropenic patients with candidemia, and their clinical relevance in other settings is uncertain.

⁶ Flucytosine MIC breakpoints are based largely on historical data and partially on the drug's pharmacokinetics.

LIMITATIONS

Intended Use: For "Research Use Only". Not for use in diagnostic procedures.

1. Testing of fungi and antifungal agents is inherently less precise than testing bacteria.
2. For additional guidance, review of CLSI (NCCLS) Antifungal Susceptibilities Standard M27 (Ref. 2) is encouraged.
3. 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.)
4. Do not read at 24 hours if the control well has not completely turned positive.
5. Correlation of the MIC for caspofungin to the treatment outcome following caspofungin use has not been fully established (14)

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DISCLAIMER

The information provided in this technical insert is current at the time of printing and may change without notice.

The latest information can be downloaded from www.trekds.com/techinfo or by Contacting TREK Technical services.



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YEASTONE®

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There have been reports in the literature of the use of yeastone plates with filamentous fungi including *Aspergillus* species (10-14), *Prototheca wickerhamii* (16), *Madurella mycetomatis* (17). Please refer to these references for further information.

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The Sensititre yeast susceptibility test is a colorimetric microdilution susceptibility test. Each plate is dosed with appropriate dilutions of antifungal agents and a colorimetric indicator. 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).

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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 broths 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 colour reaction.** Exposure to storage conditions other than those recommended may result in loss of potency of the antifungal agents and/or discoloration of indicator. Each plate is individually packaged in foil and a silica gel desiccant included. If the silica gel has not retained the color as stated on the carton label, or the package has been damaged in any manner the plate should be discarded. The plates should be used prior to the expiration date printed on the label.

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 provided:

- YeastOne susceptibility plate
- Adhesive seals

Materials available from TREK:

- (*Available in the USA only)
- Sensititre YeastOne inoculum broth
- Demineralized water, 5ml
- Sensititre AutoInoculator™
- Sensititre doseheads (for automated inoculation only)
- 100 µl pipette and tips for manual inoculation*
- Manual viewer
- Plate configuration grid for manual viewer
- 0.5 McFarland turbidity standard*
- Tips for manual inoculation*
- Bacteriological loop*
- Sterile inoculum reservoir*

Materials not provided:

- Quality control organisms (see QC section)
- Fungal growth medium agar plates e.g. Sabouroud dextrose agar (SDA)
- Vortex mixer
- Incubator (Non-CO₂)
- 20 µl pipette

INOCULATION PROCEDURE

A final organism density of approximately 1.5-8x10³ CFU/ml is recommended. As with all susceptibility tests the inoculum density is important and must be standardized to a 0.5 McFarland turbidity standard. The time period from inoculation of the water is critical and this transfer should be done within a maximum of 15 minutes. The final inoculum in the broth should be inoculated into the YeastOne plates within 15 minutes.

1. Using a sterile wooden applicator swab or bacteriological loop, touch several well-isolated colonies of >1 mm diameter from a pure 24 hour culture of the yeast isolate. Transfer to 5ml of sterile demineralized water.
2. The resulting suspension should be vortexed for 15 seconds. Ensure that the suspension is uniform without large clumps of organisms. If clumping occurs, allow the clumps to settle before adjusting the density. The turbidity can be adjusted to be equivalent to a 0.5 McFarland standard using a Sensititre nephelometer or visually by comparison to a 0.5 McFarland standard.
3. Transfer 20 µl of the yeast suspension into 11 ml of YeastOne inoculum broth. Vortex or mix vigorously. This results in 1.5 - 8 x10³ viable CFU/ml.
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a. Manual Inoculation

1. Pour the inoculum into a sterile inoculum reservoir.
2. Using an appropriate pipette (e.g. 8-channel multi-pipettor), carefully inoculate 100 µl into each well of the Sensititre plate. Avoid drawing up any air bubbles as they will interfere with the accurate delivery of the inoculum. Since the tip will be used to inoculate a series of wells, avoid touching the tip to the bottom of any well.
3. It is recommended to use the excess yeast suspension to inoculate an SDA plate as a purity check. Incubate the plate for 24-72 hours at 35°C.
4. Place contaminated tips and inoculum reservoir into an approved safety biohazard container.
5. Cover the plates with the adhesive seal, ensuring that all wells are covered.

b. Automated Inoculation

1. Replace the tube cap with a Sensititre dosehead and inoculate 100 µl into each well of the Sensititre YeastOne plate according to the AutoInoculator instructions.
2. Cover the plate with the adhesive seal, ensuring that all wells are covered.

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-CO₂ incubator. *Cryptococcus* species should be incubated for 48-72 hours.

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1. Examine the positive growth well after 24 hours incubation. If the growth well is red, the endpoints for the antifungals can be interpreted. If, after incubation, the well is still blue or only faintly purple, reincubate for an additional 24 hours and examine for growth.
2. With modifications made for colorimetric reading endpoint determinations should be done similarly to the descriptions provided in CLSI (NCCLS) Document M27, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; (Ref. 2).

DO NOT READ TURBIDITY IN THE SENSITITRE YEASTONE PLATES. Read Only Color Change.

3. The MIC is the lowest concentration of an antifungal agent that substantially inhibits growth of the organism as detected visually by observing a color change. The amount of color change in the wells containing the agent is compared with the amount of color change in the growth-control wells (no antifungal agent) used in each set of tests as follows:
4. "No growth" in the antifungal solutions is recorded when there is no change in the blue indicator in the well.

5. The MIC is recorded as the lowest concentration of antifungal agent preventing the development of a red or purple growth well, i.e. first blue. When reading from the lowest concentration dilution to the highest concentration, the MIC is the lowest concentration in which no growth appears. If all dilutions for antifungal agent demonstrate growth the endpoint is recorded as "greater than" (>) the highest concentration tested. If all dilutions for an antifungal show inhibition of growth, the endpoint for that antifungal is recorded as "less than or equal to" (≤) the lowest concentration tested.

6. If there is a blue well in a series of red growth wells, i.e. wells 1, 2 and 8 µg/ml are red but well 4 µg/ml is blue, the "skip" should be ignored and the MIC reported as 16 µg/ml. This skip well phenomenon is addressed in detail by Findell and Sheeris (Ref. 3) and described in TABLE 2 example D and E. If contamination is suspected, the test should be repeated for all antifungals.

TABLE 2. Illustration and the interpretation of test results that may occur

Well	Concentration µg/ml	1	2	4	8	16	32	Interpretation
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

¹With careful technique these occurrences are uncommon.

READING NOTES

Amphotericin B. For Amphotericin B at 24 hours, the endpoints are typically easily defined and the MIC is read as the lowest drug concentration that prevents any discernible color change. Trailing endpoints with Amphotericin B are not usually encountered.

Flucytosine, Caspofungin and Azole Antifungals. *Candida albicans*, *C. glabrata* and *C. tropicalis* with flucytosine and azoles, such as fluconazole, itraconazole, posaconazole and voriconazole may give endpoints that are typically less sharp because of trailing growth, and may be a significant source of variability. Trailing occurs when a slight color change persists and it is often identical for all drug concentrations above the MIC. The MIC should be read as the first well showing a less intense color change compared to the positive growth control well. Reference strains of defined susceptibility may also help to train personnel. 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.

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):

<i>Candida krusei</i> *	ATCC®	6258
<i>Candida parapsilosis</i>	ATCC	22019

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

3. Yeast isolates should be maintained as described by The Clinical and Laboratory Standards Institute (NCCLS) (Ref. 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. 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 outlines in the CLSI (NCCLS) document M27 (Ref. 2) for quality control including frequency and corrective action.

TABLE 3. Recommended 24 and 48 hour MIC limits for two quality control strains as per Broth Microdilution CLSI (NCCLS) M27 (Ref. 2) Ranges that are different or additional to published quality control ranges are underlined.

Antifungal Agent	<i>Candida krusei</i>		<i>Candida parapsilosis</i>	
	ATCC 6258		ATCC 22019	
	24 hour	48 hour	24 hour	48 hour
5 – Flucytosine	4-16	8-32	<u>0.12-0.5</u>	0.12-0.5
Amphotericin B	0.5-2	1-4	0.25-2	0.5-4
Anidulafungin	0.03-0.12	-	0.25-2	-
Caspofungin	0.12-1	0.25-1	0.25-1	0.5-4
Fluconazole	8-64	16-128	<u>2-8</u>	<u>2-8</u>
Itraconazole	0.12-1	0.25-1	0.12-0.5	0.12-0.5
Micafungin	<u>0.06-0.25</u>	<u>0.12 – 0.5</u>	<u>0.5-2</u>	<u>0.5-4</u>
Posaconazole	0.06-0.5	0.12-1	0.06-0.25	0.06-0.25
Voriconazole	0.06-0.5	0.12-1	0.016-0.12	0.03-0.25

TABLE 4. MIC Interpretative Criteria (µg/ml) for *Candida* Species as per CLSI M27

Antifungal Agent	Susceptible	Susceptible ³ Dose Dependent	Intermediate ⁴	Resistant	Non-susceptible
Anidulafungin	≤2				≥2
Caspofungin	≤2				≥2
Fluconazole ¹	≤8	16-32		≥64	≥32
Flucytosine	≤4		8-16	≥1	
Itraconazole ²	≤0.125	0.25-0.5		≥1	
Micafungin	≤2				≥2
Voriconazole	≤1	2		≥4	

Ranges underlined are M27-A3 in press

Shown are the breakpoints (µg/ml) for *Candida* species against the indicated agents. If MICs are measured using a scale that yields results falling between categories, the next higher category is implied. Thus, an isolate with a fluconazole MIC of 12.5 µg/ml would be placed in the S-DD category.

¹ For fluconazole, these guidelines are based substantially on experience with mucosal infections, but are consistent with the limited information for invasive infections due to *Candida* spp. Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole, and their MIC's should not be interpreted using this scale. It is also pertinent that the 8-µg/ml upper boundary for the susceptible range of fluconazole is not known with certainty – the data would permit selection of either 4 or 8 µg/ml for this cut-off.

² For itraconazole, the data are based entirely on experience with mucosal infections, and data supporting breakpoints for invasive infections due to *Candida* spp are not available.

³ Susceptibility is dependent on achieving the maximal possible blood level. For fluconazole, doses of 400 mg/day or more may be required in adults with normal renal function and body habitus. For itraconazole, measures to assure adequate drug absorption and plasma itraconazole concentrations of >0.5 µg/ml may be required for optimal response.

⁴ The susceptibility of these isolated is not certain, and the available data do not permit them to be clearly categorised as either "susceptible" or "resistant".

LIMITATIONS

Intended Use: For "Research Use Only". Not for use in diagnostic procedures.

1. Testing of fungi and antifungal agents is inherently less precise than testing bacteria.
2. For additional guidance, review of CLSI (NCCLS) Antifungal Susceptibilities Standard M27 (Ref. 2) is encouraged.
3. 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.)
4. Do not read at 24 hours if the control well has not completely turned positive.
5. Correlation of the MIC for caspofungin to the treatment outcome following caspofungin use has not been fully established (14)

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U.S. Patent #5,501,959

YSTR9_US_GB_V1.2

SENSITITRE® YeastOne® w/ Micafungin & Anidulafungin (YO-9)*



Date:	Patient Name:	Lot #:
Isolate #:	Technician:	Signature:

Interpretive Criteria for Candida spp. only

	1	2	3	4	5	6	7	8	9	10	11	12
POS	AND 0.015	AND 0.03	AND 0.06	AND 0.12	AND 0.25	AND 0.5	AND 1	AND 2	AND 4	AND 8	AB 0.12	
MF 0.008	MF 0.015	MF 0.03	MF 0.06	MF 0.12	MF 0.25	MF 0.5	MF 1	MF 2	MF 4	MF 8	AB 0.25	
CAS 0.008	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.12	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	AB 0.5	
FC 0.06	FC 0.12	FC 0.25	FC 0.5	FC 1	FC 2	FC 4	FC 8	FC 16	FC 32	FC 64	AB 1	
PZ 0.008	PZ 0.015	PZ 0.03	PZ 0.06	PZ 0.12	PZ 0.25	PZ 0.5	PZ 1	PZ 2	PZ 4	PZ 8	AB 2	
VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.12	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	VOR 8	AB 4	
IZ 0.015	IZ 0.03	IZ 0.06	IZ 0.12	IZ 0.25	IZ 0.5	IZ 1	IZ 2	IZ 4	IZ 8	IZ 16	AB 8	
FZ 0.12	FZ 0.25	FZ 0.5	FZ 1	FZ 2	FZ 4	FZ 8	FZ 16	FZ 32	FZ 64	FZ 128	FZ 256	

- POS -- Positive Control
- AND -- Anidulafungin**
- MF -- Micafungin**
- CAS -- Caspofungin**
- FC -- 5-Flucytosine
- PZ -- Posaconazole
- VOR -- Voriconazole
- IZ -- Itraconazole
- FZ -- Fluconazole
- AB -- Amphotericin B

*For research use only. Not for use in diagnostic procedures.
 **Interpretations based on Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts- M27-A3, National Committee for Clinical Laboratory Standards, Villanova, PA, USA. Currently IN PRESS.

	Susceptible		Intermediate		Resistant		Susceptible-Dose Dependent		Non-Susceptible		No Interpretation
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SENSITITRE® YeastOne® w/ Micafungin & Anidulafungin (YO-9)*



Date:	Patient Name:	Lot #:
Isolate #:	Technician:	Signature:

24-Hour Quality Control Ranges Candida parapsilosis ATCC 22019

	1	2	3	4	5	6	7	8	9	10	11	12
POS	AND 0.015	AND 0.03	AND 0.06	AND 0.12	AND 0.25	AND 0.5	AND 1	AND 2	AND 4	AND 8	AB 0.12	
MF 0.008	MF 0.015	MF 0.03	MF 0.06	MF 0.12	MF 0.25	MF 0.5	MF 1	MF 2	MF 4	MF 8	AB 0.25	
CAS 0.008	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.12	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	AB 0.5	
FC 0.06	<u>EQ 0.12</u>	<u>EQ 0.25</u>	<u>EQ 0.5</u>	FC 1	FC 2	FC 4	FC 8	FC 16	FC 32	FC 64	AB 1	
PZ 0.008	PZ 0.015	PZ 0.03	PZ 0.06	PZ 0.12	PZ 0.25	PZ 0.5	PZ 1	PZ 2	PZ 4	PZ 8	AB 2	
VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.12	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	VOR 8	AB 4	
IZ 0.015	IZ 0.03	IZ 0.06	IZ 0.12	IZ 0.25	IZ 0.5	IZ 1	IZ 2	IZ 4	IZ 8	IZ 16	AB 8	
FZ 0.12	FZ 0.25	FZ 0.5	FZ 1	<u>EZ 2</u>	<u>EZ 4</u>	<u>EZ 8</u>	FZ 16	FZ 32	FZ 64	FZ 128	FZ 256	

- POS -- Positive Control
- AND -- Anidulafungin
- MF -- Micafungin**
- CAS -- Caspofungin
- FC -- 5-Flucytosine
- PZ -- Posaconazole
- VOR -- Voriconazole
- IZ -- Itraconazole
- FZ -- Fluconazole
- AB -- Amphotericin B

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 **QC ranges based on CLSI M27-A3, currently IN PRESS.

Sensititre in-house ranges different or in addition to CLSI M27 are underlined.

	Quality Control Range
--	-----------------------

SENSITITRE® YeastOne® w/ Micafungin & Anidulafungin (YO-9)*



Date:	Patient Name:	Lot #:
Isolate #:	Technician:	Signature:

24-Hour Quality Control Ranges *Issatchenkia orientalis* (Candida krusei) ATCC 6258

- POS -- Positive Control
- AND -- Anidulafungin
- MF -- Micafungin**
- CAS -- Caspofungin
- FC -- 5-Flucytosine
- PZ -- Posaconazole
- VOR -- Voriconazole
- IZ -- Itraconazole
- FZ -- Fluconazole
- AB -- Amphotericin B

	1	2	3	4	5	6	7	8	9	10	11	12
POS	AND 0.015	AND 0.03	AND 0.06	AND 0.12	AND 0.25	AND 0.5	AND 1	AND 2	AND 4	AND 8	AB 0.12	
MF 0.008	MF 0.015	MF 0.03	<u>ME</u> 0.06	<u>ME</u> 0.12	<u>ME</u> 0.25	MF 0.5	MF 1	MF 2	MF 4	MF 8	AB 0.25	
CAS 0.008	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.12	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	AB 0.5	
FC 0.06	FC 0.12	FC 0.25	FC 0.5	FC 1	FC 2	FC 4	FC 8	FC 16	FC 32	FC 64	AB 1	
PZ 0.008	PZ 0.015	PZ 0.03	<u>PZ</u> 0.06	<u>PZ</u> 0.12	<u>PZ</u> 0.25	PZ 0.5	PZ 1	PZ 2	PZ 4	PZ 8	AB 2	
VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.12	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	VOR 8	AB 4	
IZ 0.015	IZ 0.03	IZ 0.06	<u>IZ</u> 0.12	<u>IZ</u> 0.25	IZ 0.5	IZ 1	IZ 2	IZ 4	IZ 8	IZ 16	AB 8	
FZ 0.12	FZ 0.25	FZ 0.5	FZ 1	FZ 2	FZ 4	FZ 8	FZ 16	FZ 32	FZ 64	FZ 128	FZ 256	

Sensititre in-house ranges different or in addition to Table 5 of CLSI M27 are underlined.

Quality Control Range

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**QC ranges based on CLSI M27-A3, currently IN PRESS.