

PROCEDURE: FUNGAL MICS

I. PRINCIPLE

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 lists agents available.

TABLE 1. Antifungal agents and dilutions available on YEASTONE YO11

Antifungal Agent	Abbreviation	Dilution Range (µg/ml)
Amphotericin B	AB	0.12-8
Anidulafungin	AND	0.015-8
Caspofungin	CAS	0.03-4
Fluconazole	FZ	0.5-64
Isavuconazole	ISA	0.008-4
Itraconazole	IZ	0.015-4
Micafungin	MF	0.008-4
Posaconazole	PZ	0.008-4
Rezafungin	RZF	0.008-4
Voriconazole	VOR	0.008-2

II. AVAILABILITY

- A. MICs are performed on all *Candida* species isolated from blood cultures. Yeast susceptibilities can be referred to previous results for up to one month. A physician may request a susceptibility inside the one-month referral period.
Other *Candida* MICs are performed, when clinically indicated, upon request.

III. SPECIMEN COLLECTION

Test requires a 24-hour pure culture of yeast grown on Sabouraud dextrose agar.

IV. EQUIPMENT AND MATERIALS

- A. Materials provided:
1. YeastOne YO11 susceptibility plate
 2. Adhesive seals
- B. Materials available from TREK:
1. Sensititre YeastOne inoculum broth
 2. Demineralized water, 5ml
 3. Sensititre Autoinoculator
 4. Sensititre doseheads (for automated inoculation only)
 5. 100 µl pipette and tips for manual inoculation
 6. Manual viewer
 7. Plate configuration grid for manual viewer.

8. 0.5 McFarland turbidity standard
 9. Tips for manual inoculation
 10. Bacteriological loop
 11. Sterile inoculum reservoir
- C. Materials not provided:
1. Quality control organisms (see QC section)
 2. Fungal growth medium agar plates (e.g., Sabouraud dextrose agar)
 3. Vortex mixer
 4. 35°Incubator (Non-CO2)
 5. 20 µl pipette

V. STORAGE

- A. The Sensititre YeastOne Susceptibility plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat.
- B. 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.
- C. 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.
- D. The plates should be used prior to the expiration date printed on the label.

VI. QUALITY CONTROL

- A. 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.
- B. The potency of the antifungal agent dilutions should be checked by testing organisms with known endpoints. QC is performed weekly. The following strains from the American Type Culture Collection (ATCC) are utilized:
 1. *Candida krusei** ATCC 6258
 2. *Candida parapsilosis* ATCC 22019

*ATCC now lists this organism as *Issatchenkia orientalis*
- C. Yeast isolates should be maintained as described by The Clinical and Laboratory Standards Institute (CLSI). The inoculation, reading and Interpretation of Sensititre YeastOne susceptibility plates when evaluated for user quality control should be performed as described in sections VII and VIII.
- D. Refer to Table 3 in the Thermo Scientific Yeast One Susceptibility Instructions for Use for recommended 24 and 48-hour MIC limits for two quality control strains as per Broth Microdilution CSLI M27. Ranges that are different or additional to published quality control ranges submitted are underlined.

VII. PROCEDURE

- A final organism density of approximately $1.5-8 \times 10^3$ CFU/ml is recommended.
- A. Pick several well isolated colonies of >1mm diameter from a pure 24-hour culture of the yeast isolate and emulsify into 5 mls of sterile water. Mix well ensuring the suspension is uniform, vortex if required. If clumping occurs, allow them to settle before adjusting the density. Adjust to a 0.5 McFarland standard using the DEN-1 Densitometer.
 1. Transfer 20µl of the suspension into 11 ml of YeastOne inoculum broth, to give an inoculum of $1.5 - 8 \times 10^3$ CFU/ml.
 2. Transfer 100µl by either:
 - a. Sensititre AIM Autoinoculator
 - (1) Replace the tube cap with a Sensititre single-use dosing head.
 - (2) Press the inoculation volume until 100µl appears.

- (3) Press the large green square to begin dispensing.
 - b. Manual inoculation
 - (1) Pour the inoculum into a sterile inoculum reservoir.
 - (2) Using an appropriate pipette (8 channel multi-pipettor), carefully inoculate 100µl 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 the well.
 3. A check of the colony count should be done by removing 10µl from the positive control well and plating onto a Sabouraud dextrose agar. A correct inoculum will produce 10-80 colonies.
 4. Cover all wells with the adhesive seal. Avoid creases as they can lead to skips.
 5. Place plates in a stack of no more than three plates for incubation.
 6. Minimally incubate the plates for 24-25 hours at 35°C in a non-CO₂ incubator.
- *Incubation at temperatures over 35°C may affect the performance of these plates.**

VIII. INTERPRETATION OF RESULTS

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

A. Reading Test Results

1. 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 Vizion System. Refer to the Thermo Scientific Yeast One Susceptibility Instructions for Use 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 yeast 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. Check purity test plates, results are invalid if a mixed culture is present.
2. 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, reincubate for an additional 24 hours and examine for growth.
3. "No growth" in the antifungal solutions is recorded when there is no change in the blue indicator in the well.
4. 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 discernable color change. Trailing endpoints with Amphotericin B are not usually encountered.
5. For all other 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), i.e., the first blue or faint purple well.
6. If all dilutions for an antifungal agent demonstrate good color change the endpoint is recorded as (>) 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.
7. 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 4µg/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 MIC test performed.
8. For illustration and interpretation of test results, refer to Table 2 and reading notes in the Thermo Scientific Yeast One Susceptibility Instructions for Use.

IX. REPORTING RESULTS

- A. Refer to current CLSI M27M44S, Table 1, for MIC interpretation reporting.
- B. Fluconazole will be reported with all other results suppressed. For *Candida glabrata* only

- fluconazole and micafungin will be released. If fluconazole is resistant, add the comment, "Consult Lab for additional antifungals" Additional drug requests should be discussed on rounds. Refer to CSLI M27 Appendix A – Body Site reporting for *Candida* spp.
- C. Requests for isavuconazole should be sent to a reference laboratory.
 - D. Interpretation will be provided for *Candida* species listed in CSLI M27M44S Table 1, only. All other organisms will be reported with MIC values and an interpretation of "NI".
 - E. Interpretative breakpoints are not available for itraconazole, posaconazole and amphotericin B. If one of these drugs is requested enter the MIC with an interpretation of NI. Interpretive breakpoints are not available for voriconazole for *C. glabrata*, an interpretation of NI should be reported if this drug is requested.
 - F. Results will include a statement indicating that the susceptibilities were performed in a commercially produced microtiter tray which is not FDA approved for clinical use but was validated for use in our laboratory.
 - G. For fluconazole results falling into the S-DD (sensitive-dose dependent) category, an isolate comment should be added to the report stating: SDD: Susceptibility is dependent on the dose used, please contact ID pharmacy for dosing recommendations.
 - H. For *Candida krusei*, no MIC data will be provided for fluconazole. A resistant interpretation will be reported with the statement "All *Candida krusei* are intrinsically resistant to fluconazole.
 - I. *Candida* sp intermediate or resistant to caspofungin, voriconazole and micafungin should be sent to a reference lab for confirmation if release is requested. These isolates should be brought up on rounds.
 - J. Results should not be reported if QC results are not in range.
 - K. Breakpoints may also be used for 48-hour readings if the 24h growth control shows insufficient growth.

TREK SENSITITRE PANEL
YO11
<i>Candida</i> sp.
Amphotericin B
Anidulafungin
Caspofungin ^{de}
Fluconazole ^{acf}
Isavuconazole ^g
Itraconazole
Micafungin ^{bd}
Posaconazole
Rezafungin ^h
Voriconazole ^d

a-Routinely reported for all *Candida* sp. see note f

b- Routinely reported for *C. glabrata*

c- Report as R for *C.krusei* with no MIC data

d- Caspofungin, voriconazole and micafungin results that are I or R, if reported, need to be confirmed by a reference lab

e- A caspofungin result of 0.12 for *C. glabrata*, if reported, must be confirmed by a reference lab

f- *C.tropicalis* should be sent to reference lab for susceptibility testing

g. Requests for isavuconazole should be sent to a reference laboratory

h- Only sensitive interpretive category available

X. NOTES

- A. The following yeast bug/drug combinations should be brought up on rounds:

1. *Candida albicans* resistant to all azoles
 2. *Candida* sp. susceptible to azoles but resistant to echinocandins
 3. *Candida albicans* resistant to echinocandins
 4. *Candida* sp. resistant to micafungin
 5. *Candida krusei*, susceptible to fluconazole
 6. *Candida glabrata* when caspofungin MIC is 0.12 µg/ml
- B. Susceptibility requests on *Cryptococcus* sp. and miscellaneous rapid growing yeast species will be sent to the University of Texas.
 - C. *Cryptococcus* will be reported with a statement indicating that *Cryptococcus* sp. is resistant to all the echinocandins.
 - D. *Candida tropicalis* isolates should be sent to a reference lab for susceptibility testing due to YeastOne limitations.

XI. LIMITATIONS

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

- A. Testing of fungi and antifungal agents is inherently less precise than testing bacteria.
- B. For additional guidance, review of CLSI Antifungal Susceptibilities Standard M27 is encouraged.
- C. 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).
- D. Correlation of the MIC for caspofungin to the treatment outcome following caspofungin use has not been fully established.
- E. 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.
- F. Due to the low performance of voriconazole and fluconazole and rezafungin with *C. tropicalis*, isolates of *C. tropicalis* should be tested with an alternate method.
- G. Due to categorical agreement below 90% with the Sensititre YeastOne Susceptibility System when compared to the CLSI antifungal broth microdilution method caused by the occurrence of categorical errors and to avoid potential false susceptible results, perform an alternative method of testing prior to reporting results for the following antibiotic/organism combination(s): Caspofungin: *Candida glabrata* when the MIC is ≤ 0.12 µg/ml.
- H. The ability of the Sensititre YeastOne Susceptibility System to detect resistance with the following combination(s) is unknown because resistant strains were either not available or an insufficient number were encountered at the time of comparative testing: Caspofungin (0.015-16 µg/ml) and *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis*. If a resistant isolate is encountered, it should be submitted to a reference laboratory for further testing.

XII. REFERENCES

- A. CLSI *Performance Standards for Antifungal Susceptibility Testing of Yeasts* 3rd ed. CLSI supplement M27M44S. Clinical and Laboratory Standards Institute; 2022
- B. Thermo Scientific TM Sensititre TM YeastOne Susceptibility Plates 019-YORUO 25 April 2024
- C. Thermo Scientific TM Sensititre TM YeastOne Susceptibility Plates 018-PIYSTIVD-US December 2023.

XIII. REVISIONS

- A. 1/1/2020 Added when clinically indicated under section II AVAILABILITY
- B. 1/1/2020 Removed Table 2 under section VI QUALITY CONTROL and added Refer to Table

- 3 in the Thermo Scientific Yeast One Susceptibility procedure for recommended 24 and 48-hour MIC limits.
- C. 1/1/2020 Removed Table 3 and added refer to Table 2 in the Thermo Scientific Yeast One Susceptibility procedure for illustration and interpretation of test results under section VIII INTERPRETATION OF RESULTS
 - D. 1/1/2020 Added or revised D, F, I and J under section IX REPORTING RESULTS
 - E. 1/1/2020 Added refer to CLSI M60 for additional susceptibility guidance under section XI LIMITATIONS
 - F. 1/3/2025 Revised Table 1 to include drugs on newly validated YO11 panel
 - G. 1/3/2025 Removed susceptibility performed once per patient admission from section II AVAILABILITY
 - H. 1/3/2025 Revised section IX REPORTING RESULTS by replacing refer to CLSI M60 to refer to CLSIM27. Added table to aid in reporting of antifungals.
 - I. Updated section XI Limitations to reflect current Thermo Scientific TM Sensititre TM YeastOne Susceptibility Plates 018-PIYSTIVD-US