**PROCEDURE:** **FUNGAL MICS**

1. **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.

Sensititre® YeastOne Susceptibility plates are designed for **Research Use Only** in determining quantitative antifungal susceptibilities (MIC) of non-fastidious yeast, including *Candida* species and miscellaneous other rapid growing yeast species.

**TABLE 1. Antifungal agents and dilutions available on YEASTONE**

|  |  |  |
| --- | --- | --- |
| **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 | 0.008-16 |
| Posaconazole | PZ | 0.008-8 |
| Voriconazole | VOR | 0.008-8 |

1. **AVAILABILITY**

MIC’s are performed on all yeast isolated from blood cultures. Testing is limited to one yeast MIC per admission per patient. Other yeast MICs are performed, when clinically indicated, upon request.

1. **SPECIMEN COLLECTION**

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

1. **EQUIPMENT AND MATERIALS**
   1. Materials provided:
      1. YeastOne YO9 susceptibility plate
      2. Adhesive seals
   2. Materials available from TREK:
      1. Sensititre YeastOne inoculum broth
      2. Demineralized water, 5ml
      3. Sensititre Autolnoculator
      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
   3. Materials not provided:
      1. Quality control organisms (see QC section)
      2. Fungal growth medium agar plates (e.g. Sabauroud dextrose agar)
      3. Vortex mixer
      4. 35°Incubator (Non-C02)
      5. 20 µl pipette
2. **STORAGE**
   1. The Sensititre YeastOne Susceptibility plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat.
   2. 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.
   3. 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.
   4. The plates should be used prior to the expiration date printed on the label.
3. **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. QC is performed each day of testing. The following strains from the American Type Culture Collection (ATCC) are used:
      1. *Candida krusei\** ATCC 6258
      2. *Candida parapsilosis* ATCC 22019

\*ATCC now lists this organism as *Issatchenkia orientalis*

* 1. Yeast isolates should be maintained as described by The Clinical and Laboratory Standards Institute (CLSI), (Ref.B). The inoculation, reading and Interpretation of Sensititre YeastOne susceptibility plates when tested for user quality control should be performed as described in sections VII and VIII.
  2. Refer to Table 3 in the Thermo Scientific Yeast One Susceptibility procedure for recommended 24 and 48-hour MIC limits for two quality control strains as per Broth Microdilution CSLI M27 (Ref. B). Ranges that are different or additional to published quality control ranges submitted are underlined.

1. **PROCEDURE**

A final organism density of approximately 1.5-8x103 CFU/ml is recommended.

* 1. 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 x 103 CFU/ml.
     2. Transfer 100µl by either:
        1. Sensititre AIM Autoinoculator
           1. Replace the tube cap with a Sensititre single-use dosehead
           2. Press the inoculation volume until 100µl appears.
           3. Press the large green square to begin dispensing
        2. 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.**

1. **INTERPRETATION OF RESULTS**

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

* 1. 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 Trek Sensititre procedure for additional information. 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 yeasts may show a slight purpling in fluconazole, itraconazole and ketoconazole. Check purity test plates, results are invalid if a mixed culture is present. (See details for reading below).
     2. 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.
     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 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. Refer to Table 2 in the Thermo Scientific Yeast One Susceptibility procedure for illustration and interpretation of test results.

1. **REPORTING RESULTS**
   1. Refer to current CLSI M60, Table 1, for MIC interpretation reporting.
   2. Fluconazole will be reported with all other results suppressed. If fluconazole is resistant, add the comment, “Consult Lab for additional antifungals”
   3. Interpretation will be provided for Candida species listed in CSLI M60, Table 1, only. All other organisms will be reported with MIC values and an interpretation of “NI”.
   4. Interpretative breakpoints are not available for itraconazole , 5-flucytosine, posaconazole and amphotericin B. If one of these drugs is reported enter the MIC with an interpretation of NI
   5. 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.
   6. For fluconazole results falling into the S-DD (sensitive-dose dependent) category, an isolate comment should be added to the report stating: Susceptibility is dependent on achieving the maximal possible blood level. For fluconazole, doses if 400 mg/day or more may be required in adults with normal renal function and body habitus.
   7. 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.
   8. For Candida sp resistant to caspofungin and/or voriconazole do not report since the ability of the Sensititre system to detect resistance is unknown.
   9. Results should not be reported if QC results are not in range.
   10. Breakpoints may also be used for 48 hour readings if the 24h growth control shows insufficient growth.
2. **NOTES**
   1. 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 kruseii susceptible to fluconazole
   2. If Cryptcoccus susceptibilities are requested, they will be sent to the University of Texas.
3. **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 Antifungal Susceptibilities Standard M27 and M60 (Ref. B) 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. The ability of the Sensititre system to detect resistance to voriconazole and caspofungin is unknown because resistant strains of Candida sp.were not available at the time of comparative testing. Any nonsusceptible results should be confirmed by an alternative method.

1. **REFERENCES**
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   2. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Clinical and Laboratory Standards Institute, 940 Valley Road, Suite 1400, Wayne PA 19087.
   3. Findell, C. M., and Sherris, J.C., 1976. Susceptibility of *Enterobacter* to Cefamandole: Evidence for a High Mutation Rate to Resistance. Antifungal Agents and Chemotherapy, 9:970 - 974.
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   8. Espinel-Ingroff, A., M. Pfaller, S. A. Messer, C. C. Knapp, S. Killian, H. A. Norris, and M. A. Ghannoun. 1999. Multicentre comparison of the Sensititre YeastOne Colorimetric Antifungal Plate with the National Committee for Clinical Laboratory Standards M27-A Reference Method for Testing Clinical Isolates of Common and Emerging *Candida spp.,* and Other Yeasts and Yeast-Like Organisms. J. Clin. Microbiol. 37: 591-595.
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2. **REVISIONS**
   1. 1/1/2020 Added when clinically indicated under section II AVAILABILITY
   2. 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.
   3. 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
   4. 1/1/2020 Added or revised D,F,I and J under section IX REPORTING RESULTS
   5. 1/1/2020 Added refer to CSLI M60 for additional susceptibility guidance under section XI LIMITATIONS