INTENDED USE
The Sensititre® susceptibility system is an in vitro diagnostic product for susceptibility testing of non-fastidious yeast including Candida species, Cryptococcus species, Aspergillus species and miscellaneous other rapid growing yeast species. It is a micro broth method that provides qualitative quantitative Minimum Inhibitory Concentration (MIC) results in a dried plate format. TREK Diagnostic Systems manufactured broth has only been validated with Sensititre® Products.

PRINCIPLES OF USE
The Sensititre® yeast susceptibility test is a colorimetric microdilution test. Each plate is dosed with antifungal agents at appropriate dilutions, and a colorimetric indicator. Results are read manually by observing the lowest antifungal concentration showing inhibition of growth (as evidenced by no color change).

PRECAUTIONS
Results should be used as an aid to selecting the drug of choice for treatment Only personnel trained in susceptibility testing techniques should use the system Since living microorganisms used with this product can be infectious to the user, proper handling and disposal methods should be used.

STORAGE AND SHELF LIFE
The plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat. Each plate is packaged in foil with a silica gel desiccant. Do not use the plate or broth if past its expiration date, or the desiccant color is not blue or orange or the foil pouch is damaged. Inoculate the plate within 5 hours of removal from the pouch.

PROCEDURE
Materials Included:
YeastOne® susceptibility plate
Adhesive seal

Materials Not Included [TREK Inc Product code]:
Sensititre® demineralised water [T3339]
Sensititre® yeast susceptibility inoculum broth [Y3462]
Sensititre® doseheads (for use with Autolnuculator®/AIM®) [E3010]
Sensititre® Autolnuculator®[V3010]/ Sensititre® AIM® [V3020]
Sensititre® Vizion® [V2021]
Sensititre® Nephelometer ® [V3011]
SPECIMEN COLLECTION AND PREPARATION
Specimens should be collected, transported, stored and then plated on to primary isolation medium to give isolated colonies using standard procedures. (1)

SELECTION OF SUSCEPTIBILITY TEST BROTH
Sensititre® approved broths are performance tested for use in Sensititre® susceptibility products.

INOCULATION PROCEDURE (Candida and Cryptococcus 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 the Yeast isolate, and emulsify into sterile water. Vortex mix the suspension for 15 seconds, ensuring that the suspension is uniform. If clumping occurs, allow them 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 suspension into 11 ml of YeastOne® inoculum broth, to give an inoculum of 1.5 - 8 X 10^3 CFU/ml.
Steps 1 and 2 should be completed in 15 minutes.
3. Transfer 100µl by either:
a. Sensititre® AutoInoculator®/ Sensititre AIM®. Replace the tube cap with a Sensititre® single-use dosehead and inoculate the plate according to the AutoInoculator® /AIM® instructions.
Remove the test tube dosehead combination from the AutoInoculator® /AIM® 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 using an appropriate pipette.
Inoculate broth into a plate within 15 minutes.
4. A check of the colony count should be done 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.
INOCULATION PROCEDURE (*Aspergillus* spp.) (5-7, 22)

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

1. Subculture from Sabouraud dextrose agar onto Tasashio or potato dextrose agar. Incubate for 7 days at 35°C to obtain adequate sporulation.
2. Collect conidia with a cotton swab and suspend in sterile saline with Tween.
3. Allow heavy particles to settle for 3 to 5 minutes.
4. Collect supernatant and mix with a vortex mixer.
5. Adjust turbidity of supernatant to 80 to 82% transmittance at 530nm measured with a spectrophotometer equivalent to an inoculum of 0.6 – 5 x 10⁶ cfu/ml. Alternatively, adjust to a 0.5 McFarland Standard (22).
6. Add 100 µl to 11ml of YeastOne® inoculum broth to give a final inoculum of 0.5-5 x 10⁴ cfu/ml.
7. Transfer 100µl by either:
   a. **Sensititre® AutoInoculator®/Sensititre® AIM®**. Replace the tube cap with a Sensititre® single-use dosehead and inoculate the plate according to the AutoInoculator® /AIM® instructions.

Remove the test tube dosehead combination from the AutoInoculator® /AIM® 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 using an appropriate pipette.
   Inoculate broth into a plate within 15 minutes.

8. A check of the colony count should be done by removing 10 µl from the positive control well and plating onto Sabauroud dextrose agar (SDA). A correct inoculum will produce 50-500 colonies.
9. Cover all wells with the adhesive seal. Avoid creases as these can lead to skips.

**INCUBATION**

Minimally incubate the plates for 24-25 hours at 35°C in a non-CO₂ incubator.
*Cryptococcus* species should be incubated for 72 hours.
*Aspergillus* species should be incubated for 48 to 72 hours.

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

Up to 3 plates can be stacked if not incubated in the ARIS®.

**READING TEST RESULTS**

Plates may be read visually under normal laboratory lighting using a manual mirror viewer or by using 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 red (positive). Some yeasts 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 posaconazole, voriconazole, fluconazole, itraconazole and ketoconazole. (see details for reading below).

1. Examine the positive growth well after 24 hours incubation (*Candida* species). If the growth well is red, the endpoints for the antifungals can be interpreted. If the well is still blue or only faintly purple, re-incubate for an additional 24 hours and re-examine.
DO NOT READ TURBIDITY IN THE SENSITITRE YEASTONE® PLATES.

Read Only Color Change.

2. The MIC is the lowest concentration of an antifungal agent that substantially inhibits growth of the organism as detected by a color change. The amount of color change in the wells containing the agent should be compared to the color of the positive growth-control wells.

3. No growth occurs when there is no change in the blue indicator in any dilution of an antifungal. The organism is susceptible to the lowest concentration of antifungal.

4. 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.

5. The organism is resistant to the highest concentration of antifungal when growth is seen in all wells. The MIC endpoint should be recorded as “greater than” (> the highest concentration.

6. For *Aspergillus* species read the MIC as the lowest concentration with a blue color.

INTERPRETATION OF RESULTS

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

<table>
<thead>
<tr>
<th>Well Concentration µg/ml</th>
<th>R = RED: Positive growth indication</th>
<th>B = BLUE: Negative growth indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  2  4  8  16  32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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.
The first well showing a distinct color change as compared to the positive growth well is the MIC.

Flucytosine and Azole Antifungals. *Candida albicans*, *C. glabrata* and *C. tropicalis* with flucytosine and azoles, such as fluconazole, itraconazole, ketoconazole, voriconazole and posaconazole 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 colour 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 colour 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, (1) a comment should accompany the test result reported.

Trailing endpoint: This occurs when a slight color change persists and is often identical in several concentrations. The MIC should be read as the first well showing a less intense color change compared to the positive growth control well.

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

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

From time to time we are encountering paradoxical growth in the higher concentrations of Itraconazole on the Sensititre yeast panels which results in pinking of these wells.

The paradoxical effect also known as the Eagle phenomenon refers to an observation where an increase in the antimicrobial concentration beyond a certain point paradoxically
results in an increase to the number of bacteria that survive. An explanation could be that as the concentration is too high, the agent might be self-antagonising the receptor with which it binds (penicillin binding proteins, for example, in the case of a penicillin).

Resolution
The growth in the high concentration should be ignored unless you have growth in all of the other concentrations of Itraconazole. In the example below the 0.5µg/ml well highlighted with the black square is where the MIC result should be recorded.

If you have any other questions or concerns please contact the technical support department on +441342318777 or email techsupport@trekds.co.uk. Alternatively please contact +1-800-871-8909 or email techsupport@trekds.com in the USA.

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 red 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.

QUALITY CONTROL
Frequency of quality control testing should be according to local guidelines (1). Inoculum should be cultured onto a suitable medium to check for purity. Test results are invalid if a mixed culture is detected. All Sensititre® plates include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells.

For user quality control of the MIC system, the following culture(s) from the American Type Culture Collection (ATCC®) are recommended:

<table>
<thead>
<tr>
<th>Candida krusei*</th>
<th>ATCC® 6258</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida parapsilosis</td>
<td>ATCC® 22019</td>
</tr>
</tbody>
</table>

*ATCC now lists these organisms as Issatchenkia orientalis. Results should not be reported if QC results are not in range.
Expected QC values are provided in Table 3.

Contact Sensititre® Distributor or TREK Diagnostic Systems in the event that quality control discrepancies cannot be resolved.

**TABLE 3. Recommended 24 and 48-hour MIC ranges (µg/ml) or Quality Control strains.**

Ranges that are different from CLSI quality control ranges are underlined (1).

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Candida krusei ATCC 6258</th>
<th>Candida parapsilosis ATCC 22019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hour</td>
<td>48 hour</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.5 - 2</td>
<td>1 - 4</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>0.03 - 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0.12 - 0.25</td>
<td>0.25 - 1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>8 - 64</td>
<td>16 - 128</td>
</tr>
<tr>
<td>5-Flucytosine</td>
<td>4 - 16</td>
<td>8 - 32</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.12 - 0.5</td>
<td>0.25 - 1</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.12 - 0.25</td>
<td>0.25 - 1</td>
</tr>
<tr>
<td>Micafungin</td>
<td>0.06 - 0.25</td>
<td>0.12 - 0.5</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.06 - 0.5</td>
<td>0.12 - 1</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.06 - 0.5</td>
<td>0.12 - 1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Susceptible</th>
<th>Susceptible dose dependent</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Non-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin</td>
<td>&lt; 2</td>
<td></td>
<td></td>
<td>&gt; 2</td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>&lt; 2</td>
<td></td>
<td></td>
<td>&gt; 2</td>
<td></td>
</tr>
<tr>
<td>Fluconazole*</td>
<td>&lt; 8</td>
<td>16 - 32</td>
<td>&gt; 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Flucytosine</td>
<td>&lt; 4</td>
<td>8 - 16</td>
<td>&gt; 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&lt; 0.125</td>
<td>0.25 - 0.5</td>
<td>&gt; 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td>&lt; 2</td>
<td></td>
<td></td>
<td>&gt; 2</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>&lt; 1</td>
<td>2</td>
<td>&gt; 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Isolates of Candida krusei are assumed to be intrinsically resistant to fluconazole and their MICs should not be interpreted using this scale.

**NOTE 1:** Shown are the breakpoints (µg/mL) for Candida spp. Against the indicated agents. If minimal inhibitory concentrations (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.

Please refer to CLSI (1) for more information concerning interpretation of results.

**LIMITATIONS**

1. Sensititre YeastOne® plates are for use with non-fastidious yeast including Candida species, Cryptococcus species, Aspergillus species and miscellaneous rapid growing...
yeast species. They are not intended for fastidious or slow growing yeast such as *Histoplasma* or *Blastomyces*, and filamentous fungi.

2. Comparison between the Sensititre YeastOne® at 24 hours and the CLSI reference method at 48 hour 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.

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. Colour 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 performance of voriconazole with *Cryptococcus* species and rapid growing yeast species has not been determined. Voriconazole MIC’s should therefore only be reported for *Candida* species.

9. Use only with Sensititre® approved yeast susceptibility inoculum broth. The use of other broths could result in error.

10. As with any in-vitro susceptibility testing method, the results of testing should be correlated with the patient’s clinical response to prescribed therapy.

11. Performance has only been established for Amphoteracin B, Itraconazole Posaconazole and Voriconazole with *Aspergillus* species.
12. Reference 5 showed high level (>99% agreement) with CLSI method for amphotericin B and Aspergillus species. Lower agreement was seen with itraconazole, A. fumigatus, A. flavus and A. terreus had >90% agreement whilst A. nidulans had 85% and A. ustus 33% on panels incubated for 48 hours with a 10³ cfu/ml inoculum. Agreement over 90% was observed with an inoculum of 10⁴ cfu/ml and 72 hours incubation with itraconazole and amphotericin B (6).

13. Correlation of the MIC for Caspofungin to the treatment outcome following caspofungin use has not been fully established. (9)

14. Performance of the YeastOne® with Anidulafungin, Caspofungin, and Micafungin with Cryptococcus, Aspergillus species and rapid growing yeast species other than Candida species has not been established. Anidulafungin, Caspofungin and Micafungin MIC's should therefore only be reported for Candida species.

15. Performance of the YeastOne® with Posaconazole with Cryptococcus has not been established.

16. Only instruments supported by Sensititre i.e. a simple mirror viewer, Sensitouch, Vizion, Sensititre Autoreader, Optiread and ARIS instruments must be used to report results with CE IVD and FDA cleared Sensititre products, any other system used will not be supported.

PERFORMANCE
Panels read either manually or are designed to give comparable performance to CLSI reference microbroth procedure. Comparable performance is defined as > 90% agreement to within a doubling dilution of the reference MIC (1).

For further information contact TREK Diagnostic systems or your local distributor

BIBLIOGRAPHY


**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.mcsdiagnostics.com or by contacting MCS Diagnostics.

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