Thermo SCIENTIFIC
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# Instructions for Use

## *Thermo Scientific* ***Sensititre YeastOne Susceptibility Plates***

| 042 -YEAST - TAIWAN CID10616 |
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| Revision Date:01st April 2020 |

# THERMO SCIENTIFIC SENSITITRE YEASTONE SUSCEPTIBILITY PLATES

For *in vitro* Diagnostic Use

**For more information including plate layout and QC ranges, please refer to www.trekds.com/techinfo. Plate code and batch number required.**

## *Intended Use*

The Thermo Scientific™ Sensititre™ YeastOne™ susceptibility plate is an *in vitro* diagnostic microbroth dilution method in a dried 96-well microplate format that provides quantitative minimum inhibitory concentration (MIC) results for non-fastidious yeast including *Candida* species

## Principles of Use

Sensititre YeastOne is a colorimetric microbroth dilution test. Each microplate is dosed with antifungal agents at appropriate dilutions and a colorimetric indicator.

Results are manually read by observing the lowest antifungal concentration that exhibits no growth (as evidenced by no color change).

## Precautions

Results should be used as an aid in selecting the drug of choice for treatment.
Only trained personnel should operate the system.
Proper organism handling and disposal methods should be used.

## Storage and Shelf Life

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 (1) the desiccant color is not orange, (2) the expiration date has passed, or (3) the foil pouch is damaged.

## Materials

### Included:

Sensititre YeastOne plate

Adhesive seal

### Not Included [Product Code]:

Thermo Scientific™Sensititre™ Demineralised Water [T3339]

Sensititre YeastOne Broth [Y3462]

Thermo Scientific™Sensititre™ Doseheads (for use with Thermo Scientific™Sensititre™ AIM™ Automated Inoculation Delivery System) [E3010]

Sensititre AIM System [V3020]

Thermo Scientific™Sensititre™ Vizion™ Digital MIC Viewing System [V2021]

Thermo Scientific™Sensititre™ Nephelometer [V3011]
Manual Viewer [V4007]
0.5 McFarland Turbidity Standard [E1041]
Bacteriological Loop
20µl Pipette
Sterile Inoculum Reservoir
100ul Pipette and Disposable Tips

## Quality Control Strains

Fungal Growth Agar Plates e.g Sabauroud Dextrose Agar (SDA)
Incubator 34 - 36°C, non-CO2
Vortex Mixer

## Specimen Collection and Preparation

Specimens should be collected, transported, stored and plated onto primary isolation medium using standard procedures1.

## Selection of Susceptibility Test Broth

Sensititre System approved broths are performance tested for use with the Sensititre System.

# Inoculation Procedure (Candida Testing)

Allow all broths to come to room temperature before use.

Plates should be inoculated within 5 hours of removal from the pouch. A final organism density of approximately 1.5 – 8 x $10^3$ CFU/ml is recommended.

Steps 1 and 2 should be completed within 15 minutes.

1. Pick several well isolated colonies of >1mm diameter from a pure 24-hour culture (Sabauroud Dextrose Agar) of the yeast isolate and emulsify into demineralised water. Mix well ensuring the suspension is uniform, vortex if required. If clumping occurs, allow the suspension to settle before adjusting the density. Adjust to a 0.5 McFarland standard visually or with a Sensititre Nephelometer·
2. Transfer 20 µl of the suspension into 11 ml of Sensititre YeastOne Broth to give a final inoculum of 1.5 – 8 x $10^3$ CFU/ml.
3. Transfer 100µl of the final suspension to the Sensititre YeastOne Plate within 15 minutes of completing step 2 by either:
    a. **Sensititre AIM System -** Replace the tube cap with a Sensititre Single-use Dosehead and inoculate the plate according to the Sensititre AIM user manual. Remove the test tube dosehead combination from the Sensititre AIM System 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

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 prevent well skips

## Incubation

Incubate plates at 35˚C in a non-$CO_2$ incubator.
Incubation temperatures over 35°C may affect performance.

- *Candida* species should be incubated for 24 to 25 hours (refer to step 1 under READING TEST RESULTS section)

## 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 Sensititre Vizion System 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 yeast species may not change the indicator completely to red but display more of a purpling. Some organisms may show a slight purpling in posaconazole, voriconazole, fluconazole, itraconazole and ketoconazole.

**1.** Examine the positive growth well after incubation
   a. *Candida* species should be incubated for 24 to 25 hours (refer to step 1 under READING TEST RESULTS section)
**2.** If the growth well is red, the endpoints for the antifungals can be determined. For *Candida* species, if the well is blue or faint purple, re-incubate for an additional 24 hours and re-examine.

**3.** The MIC is the lowest concentration of an antifungal agent that substantially inhibits growth of the organism as detected by a color change.

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

## Interpretation of Results

*TABLE 1. Illustration and the interpretation of test results that may occur*

| Well Concentration µg/ml | | | | | | | **R = RED: Positive growth indication**<br>**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 techniques, these occurrences are not common.

## 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*

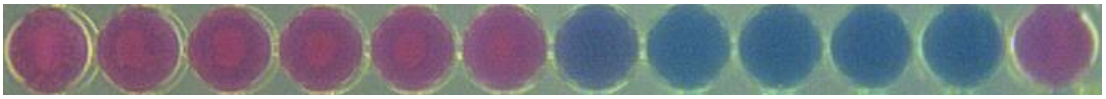
MIC end points should be determined after 24 hours of incubation at 35°C. The MIC should be read as the first well showing a less intense color change as compared to the positive control well.

## 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 YeastOne Panels which results in pinking of these wells.

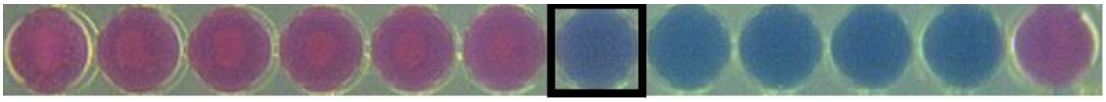
The paradoxical effect also known as the Eagle phenomenon refers to an observation where an increase in the antimicrobic concentration beyond a certain point paradoxically results in an increase to the number of bacteria that survive. An explanation could be that as the concentration is too high, the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



0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16

## 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.



0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16

If you have any other questions or concerns please contact the technical support department on Phone: +44 (0) 1256 694287 | Fax: +44 (0) 1256 463388 or see page 12 for list of emails.

## 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 conducted 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

The following cultures from the American Type Culture Collection (ATCC™) are recommended for user quality control:

| | |
|---|---|
| *Issatchenkia orientalis (Candida krusei)* | ATCC 6258 |
| *Candida parapsilosis* | ATCC 22019 |

Results should **not** be reported if QC results are not in range.

The inoculation, reading and interpretation of Sensititre YeastOne susceptibility plates when testing for user quality control should be performed as described on the preceding section.

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

| Antifungal Agent | *Issatchenkia orientalis* ATCC6258 | | *Candida parapsilosis* 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 | 0.5-4 | <u>2-8</u> |
| Itraconazole | 0.12-1 | 0.25-1 | 0.06-0.5 | 0.06-0.5 |
| Micafungin | 0.06-0.5* | 0.12 – 0.5 | 0.5-2 | 0.5-4 |
| Posaconazole | 0.06-0.5 | 0.12-1 | 0.03-0.25 | 0.06-0.25 |
| Voriconazole | 0.06-0.5 | 0.12-1 | 0.015-0.12 | 0.03-0.25 |

*** Sensititre range**

Expected QC values are provided in CLSI M27

Isolates of *Issatchenkia orientalis (Candida krusei)* are assumed to be intrinsically resistant to fluconazole and their MICs should not be interpreted using this scale

**NOTE 1:** If minimal inhibitory concentrations (MICs) for *Candida* spp. 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 ug/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. They are not intended for fastidious or slow growing yeast such as *Histoplasma* or *Blastomyces*, and filamentous fungi.
2. Comparison between the Sensititre YeastOne System 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, refer to CLSI Antifungal Susceptibilities for yeast Standard M27.
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. Use only with Sensititre System approved yeast susceptibility inoculum broth. The use of other broths could result in error.
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. Correlation of the MIC for Caspofungin to the treatment outcome following caspofungin use has not been fully established.
11. Only instruments supported by the Sensititre system i.e. a simple mirror viewer, Sensititre Vizion, , 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 manually are designed to give comparable performance to CLSI reference microbroth procedure. Comparable performance is defined as > 90% essential and categorical agreement to within a doubling dilution of the reference MIC (1).

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