

Only Fluconazole and Voriconazole are included in the M44-A guideline from CLSI (formerly NCCLS)(1) and interpretation zones are only available for Fluconazole so far. When MIC breakpoints are available from CLSI (2) they are used in the table below.

**Table 13.5-1 Interpretation for Yeasts**

**Mueller-Hinton Glucose Methylene Blue agar. Inoculum McFarland 0.5 undiluted. Incubation at 35 °C ± 2 °C ambient air for 20-24 hours. MICs according to M27-S3 (2007).**

| NEO-SENSITABS                               | POTENCY | CODE  | Zone diameter<br>in mm |                |      | Break-points<br>MIC µg/ml |       |
|---|---------|-------|------------------------|----------------|------|---------------------------|-------|
|   |         |       | S                      | I              | R    | S                         | R     |
| Amphotericin B                              | 10 µg   | AMPHO | ≥ 15                   | 14-10          | < 10 | ≤ 1                       | ≥ 2   |
| Caspofungin *) (24)                         | 5 µg    | CASP5 |                        |                |      |                           |       |
| C. albicans, C. tropicalis                  |         |       | ≥ 17                   | 16-15          | ≤ 14 | ≤ 0.25                    | ≥ 1   |
| C. parapsilosis,* C. guilliermondii         |         |       | ≥ 13                   | 12-11          | ≤ 10 | ≤ 2                       | > 2   |
| C. glabrata, C. krusei                      |         |       | ≥ 16                   | -              | -    | ≤ 0.5                     | -     |
| Fluconazole **) (27)                        | 25 µg   | FLUCZ |                        |                |      |                           |       |
| C. albicans, C. tropicalis, C. parapsilosis |         |       | ≥ 17                   | 16-14          | ≤ 13 | ≤ 2                       | ≥ 8   |
| C. glabrata                                 |         |       | -                      | ≥ 15<br>(SDD)  | ≤ 14 | ≤ 32<br>(SDD)             | ≥ 64  |
| Itraconazole (11)                           | 10 µg   | ITRAC | ≥ 23                   | 22-14<br>(SDD) | ≤ 13 | ≤ 0.12                    | > 0.5 |
| Ketoconazole                                | 15 µg   | KETOC | ≥ 28                   | 27-21          | ≤ 20 | ≤ 0.12                    | ≥ 0.5 |
| Posaconazole (9,12)                         | 5 µg    | POSAC | ≥ 17                   | 16-14<br>(SDD) | ≤ 13 | ≤ 1                       | > 2   |
| Voriconazole (23)                           | 1 µg    | VOR.1 |                        |                |      |                           |       |
| C. albicans, C. tropicalis, C. parapsilosis |         |       | ≥ 17                   | 16-15          | ≤ 14 | ≤ 0.12                    | ≥ 1   |
| C. krusei                                   |         |       | ≥ 15                   | 14-13          | ≤ 12 | ≤ 0.5                     | ≥ 2   |
| C. glabrata                                 |         |       | ≥ 16                   | -              | ≤ 15 | ≤ 0.5                     | > 0.5 |

\*) Tentative. There is cross-resistance between Caspofungin and the other echinocandins: Anidulafungin and Micafungin (19).

\*\*) *C. krusei* should be reported as resistant to Fluconazole (no matter the zone).

For further information on Susceptibility Testing of Yeasts, see Neo-Sensitabs User's Guide.

**Table 13.5-2 Mold Disk Diffusion Testing (14, 17,21)**

**Mueller-Hinton Plain. Incubation for 16-24 hours (zygomycetes), 24 hours (Aspergillus), 48 hours (other spp.). Temperature: 35-37°C**

**Inoculum:**

Prepare a suspension of sporulating colonies in 0.85% saline, add 1 drop of Tween 20. Allow heavy particles to settle for 3-5 minutes and the upper suspension is treated for 15 seconds in a vortex mixer. The density of the suspension is read on a spectrophotometer at 530 nm wave length and the optical density adjusted at 0.09 to 0.13 for Aspergillus.

| NEO-SENSITABS                        | POTENCY | CODE  | Zone diameter<br>in mm |   |   | Break-points<br>MIC µg/ml |       |
|--------------------------------------|---------|-------|------------------------|---|---|---------------------------|-------|
|                                      |         |       | S                      | I | R | S                         | R     |
| Amphotericin B<br>(zygomycetes only) | 10 µg   | AMPHO | ≥ 15                   | - | - | ≤ 1                       | -     |
| Caspofungin                          | 5 µg    | CASP5 | ≥ 17                   | - | - | ≤ 1                       | -     |
| Itraconazole                         | 10 µg   | ITRAC | ≥ 17                   | - | - | ≤ 1                       | ≥ 2   |
| Posaconazole                         | 5 µg    | POSAC | ≥ 23                   | - | - | ≤ 0.25                    | ≥ 0.5 |
| Voriconazole                         | 1 µg    | VOR.1 | ≥ 17                   | - | - | ≤ 1                       | ≥ 2   |

**Note:** The base medium should not be supplemented with neither 2% glucose nor 0.5% methylene blue dye.

Itraconazole susceptible strains should be reported as susceptible to both, Posaconazole and Voriconazole.

**Interpretation table for Local treatment**

In local treatment of fungal infections, a high concentration of antifungal is placed at site of the infection. Consequently other MIC breakpoints and zone interpretations should be used in those cases.

| Local Treatment<br>MH Glucose Methylene Blue Agar or Shadomy<br>McFarland 0.5 inoculum |   |                                       |              |
|--|---|---------------------------------------|--------------|
| <b>Susceptible</b>   | ≥ 20 mm   | ≥ 15 mm                               | ≥ 10 mm      |
| <b>Intermediate</b>  | 12-19 mm  | 10-14 mm                              | -            |
| <b>Resistant</b>   | ≤ 11 mm   | no zone                               | no zone      |
|  | Ciclopirox<br>Clotrimazole<br>Econazole<br>Fluconazole<br>Isoconazole<br>Ketoconazole<br>Miconazole<br>Tioconazole<br>Terbinafine | Natamycin<br>Nystatin<br>Itraconazole | Griseofulvin |

Fluorocytosine cannot be tested on MH-agar (antagonists), but has to be tested on Shadomy agar or similar.

**Table 13.5-3 Quality Control Zone Diameters (mm) Ranges**

**Mueller-Hinton Glucose Methylene Blue agar. Inoculum McFarland 0.5 undiluted. Incubation at 35 °C ± 2 °C for 20-24 hours.**

| NEO-SENSITABS  | POTENCY | CODE  | Zone diameter in mm              |                                      |                               |
|----------------|---------|-------|----------------------------------|--------------------------------------|-------------------------------|
|                |         |       | <i>C. albicans</i><br>ATCC 90028 | <i>C. parapsilosis</i><br>ATCC 22019 | <i>C. krusei</i><br>ATCC 6258 |
| Amphotericin B | 10 µg   | AMPHO | 20-27                            | 22-29                                | 18-25                         |
| Fluconazole    | 25 µg   | FLUCZ | 28-39                            | 22-33                                | -                             |
| Itraconazole   | 10 µg   | ITRAC | 21-30                            | 19-26                                | 16-22                         |
| Ketoconazole   | 15 µg   | KETOC | 31-42                            | 35-45                                | 22-29                         |
| Voriconazole   | 1 µg    | VOR.1 | 31-42                            | 28-37                                | 23-31                         |
| Caspofungin    | 5 µg    | CASP5 | 15-22                            | 13-23                                | 16-22                         |
| Posaconazole   | 5 µg    | POSAC | 24-34                            | 25-36                                | 23-31                         |

Carrillo-Munoz et al (30) determined Posaconazole susceptibility of clinical yeast isolates with Neo-Sensitabs and a microdilution method. Complete agreement between Posaconazole Neo-Sensitabs and the microdilution was 92.3 % after 24 hours incubation. The authors conclude that the Agar Diffusion with Posaconazole Neo-sensitabs can improve Posaconazole susceptibility testing due to its excellent correlation and reduced percentage of disagreements in comparison with microdilution testing.

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