Reference Method for Broth Dilution
Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement

This document provides updated tables for the CLSI antimicrobial susceptibility testing standard M27-A3.
An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process.
Clinical and Laboratory Standards Institute
Advancing Quality in Health Care Testing

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Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement

Abstract


The tabular information in this document presents the most current information for drug selection, interpretation, and quality control.


The data in the interpretive tables in this supplement are valid only if the methodology is followed in CLSI document M27-A3—Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition.
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Table 1. Interpretive Guidelines for *In Vitro* Susceptibility Testing of *Candida* spp.

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Susceptible (S)</th>
<th>Susceptible-dose dependent (S-DD) a</th>
<th>Intermediate (I) b</th>
<th>Resistant (R)</th>
<th>Nonsusceptible (NS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin c</td>
<td>≤2</td>
<td>-</td>
<td>-</td>
<td>&gt;2</td>
<td>-</td>
</tr>
<tr>
<td>Caspofungin c</td>
<td>≤2</td>
<td>-</td>
<td>-</td>
<td>&gt;2</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole d</td>
<td>≤8</td>
<td>16-32</td>
<td>≥64</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole e</td>
<td>≤4</td>
<td>8-16</td>
<td>≥32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole f</td>
<td>≤0.125</td>
<td>0.25-0.5</td>
<td>≥1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micafungin c</td>
<td>≤2</td>
<td>-</td>
<td>-</td>
<td>&gt;2</td>
<td>-</td>
</tr>
<tr>
<td>Voriconazole c</td>
<td>≤1</td>
<td>2</td>
<td>≥4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**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.

**NOTE 2:** The MIC breakpoints in boldface type were adopted at a meeting of the subcommittee held on 9 June 2007 in Boston, MA. These breakpoints are considered tentative for one year and are open for comments. There is no Resistant category assigned for the echinocandin agents; isolates with higher MICs may be described as nonsusceptible.

**Footnotes**

a. Susceptibility is dependent on achieving the maximal possible blood level. For fluconazole, doses of 400 mg/day or more may be required in adults with normal renal function and body habitus. For itraconazole, measures to assure adequate drug absorption and plasma itraconazole concentrations of >0.5 µg/mL may be required for optimal response.

b. The susceptibility of these isolates is not certain, and the available data do not permit them to be clearly categorized as either “susceptible” or “resistant.”

c. For these drugs, the data are based substantially on experience with non-neutropenic patients with candidemia, and their clinical relevance in other settings is uncertain.

d. For fluconazole, these guidelines are based on extensive experience with mucosal and invasive infections due to *Candida* spp. It is also pertinent that the 8-µg/mL upper boundary for the susceptible range of fluconazole is not known with certainty—the data would permit selection of either 4 or 8 µg/mL for this cutoff. When an isolate is identified as *Candida glabrata* and the MIC is ≤ 32, patients should receive a maximum dosage regimen of fluconazole. Expert consultation on selection of a maximum dosage regimen may be useful. Finally, isolates of *Candida krusei* are assumed to be intrinsically resistant to fluconazole, and their MICs should not be interpreted using this scale.

e. Flucytosine MIC breakpoints are based largely on historical data and partially on the drug’s pharmacokinetics.

f. For itraconazole, the data are based entirely on experience with mucosal infections, and data supporting breakpoints for invasive infections due to *Candida* spp. are not available.
Table 1. (Continued)

References:


Table 2. Solvents and Diluents for Preparation of Stock Solutions of Antifungal Agents

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Solvent (Full Strength and Intermediate Solutions)</th>
<th>Diluent (Final Concentrations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>DMSO*</td>
<td>Medium</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Water</td>
<td>Medium</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>Water</td>
<td>Medium</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Water</td>
<td>Medium</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
<tr>
<td>Micafungin</td>
<td>Water</td>
<td>Medium</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
<tr>
<td>Ravuconazole</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>DMSO</td>
<td>Medium</td>
</tr>
</tbody>
</table>

* DMSO = dimethyl sulfoxide is potentially toxic.
Table 3. Scheme for Preparing Dilutions of Water-Soluble Antifungal Agents to Be Used in Broth Dilution Susceptibility Tests

<table>
<thead>
<tr>
<th>Step</th>
<th>Concentration (μg/mL)</th>
<th>Source</th>
<th>Volume (mL)</th>
<th>+ Medium (mL)</th>
<th>= Intermediate Concentration (μg/mL)</th>
<th>= Final Concentration at 1:10 (μg/mL)</th>
<th>Log₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5120</td>
<td>Stock</td>
<td>1 mL</td>
<td>7</td>
<td>640 μg/mL</td>
<td>64</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>640</td>
<td>Step 1</td>
<td>1.0</td>
<td>1.0</td>
<td>320</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>640</td>
<td>Step 1</td>
<td>1.0</td>
<td>3.0</td>
<td>160</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>Step 3</td>
<td>1.0</td>
<td>1.0</td>
<td>80</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>Step 3</td>
<td>0.5</td>
<td>1.5</td>
<td>40</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>160</td>
<td>Step 3</td>
<td>0.5</td>
<td>3.5</td>
<td>20</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>Step 6</td>
<td>1.0</td>
<td>1.0</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>Step 6</td>
<td>0.5</td>
<td>1.5</td>
<td>5</td>
<td>0.5</td>
<td>-1</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>Step 6</td>
<td>0.5</td>
<td>3.5</td>
<td>2.5</td>
<td>0.25</td>
<td>-2</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>Step 9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.25</td>
<td>0.125</td>
<td>-3</td>
</tr>
<tr>
<td>11</td>
<td>2.5</td>
<td>Step 9</td>
<td>0.5</td>
<td>1.5</td>
<td>0.625</td>
<td>0.0625</td>
<td>-4</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>Step 9</td>
<td>0.5</td>
<td>3.5</td>
<td>0.3125</td>
<td>0.03125</td>
<td>-5</td>
</tr>
</tbody>
</table>

Table 4. Scheme for Preparing Dilution Series of Water-Insoluble Antifungal Agents to Be Used in Broth Dilution Susceptibility Tests

<table>
<thead>
<tr>
<th>Step</th>
<th>Source</th>
<th>Volume (mL)</th>
<th>+ Solvent (eg, DMSO)*</th>
<th>= Intermediate Concentration (μg/mL)</th>
<th>= Final Concentration at 1:100 (μg/mL)</th>
<th>Log₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>1600</td>
<td>0.5</td>
<td>1600 μg/mL</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1600</td>
<td>Stock</td>
<td>0.5</td>
<td>800</td>
<td>8.0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1600</td>
<td>Stock</td>
<td>0.5</td>
<td>400</td>
<td>4.0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1600</td>
<td>Stock</td>
<td>0.5</td>
<td>200</td>
<td>2.0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>Step 4</td>
<td>0.5</td>
<td>100</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>Step 4</td>
<td>0.5</td>
<td>50</td>
<td>0.5</td>
<td>-1</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>Step 4</td>
<td>0.5</td>
<td>25</td>
<td>0.25</td>
<td>-2</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>Step 7</td>
<td>0.5</td>
<td>12.5</td>
<td>0.125</td>
<td>-3</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>Step 7</td>
<td>0.5</td>
<td>6.25</td>
<td>0.0625</td>
<td>-4</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>Step 7</td>
<td>0.5</td>
<td>3.13</td>
<td>0.0313</td>
<td>-5</td>
</tr>
</tbody>
</table>

*Dimethyl sulfoxide

<table>
<thead>
<tr>
<th>Organism</th>
<th>Purpose</th>
<th>Antifungal Agent</th>
<th>MIC Range (µg/mL)</th>
<th>% of MICs Within Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>QC</td>
<td>Amphotericin B</td>
<td>0.25-1.0</td>
<td>99.1</td>
</tr>
<tr>
<td>ATCC® 22019</td>
<td></td>
<td>Fluconazole</td>
<td>2.0-8.0</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>0.06-0.25</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketoconazole</td>
<td>0.06-0.25</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>0.12-0.5</td>
<td>98.6</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>QC</td>
<td>Amphotericin B</td>
<td>0.25-2.0</td>
<td>99.5</td>
</tr>
<tr>
<td>ATCC® 6258</td>
<td></td>
<td>Fluconazole</td>
<td>16-64</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>0.12-0.5</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketoconazole</td>
<td>0.12-0.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>4.0-16</td>
<td>96.8</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.5-2.0</td>
<td>91.9</td>
</tr>
<tr>
<td>ATCC® 90028</td>
<td></td>
<td>Fluconazole</td>
<td>0.25-1.0</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>0.5-2.0</td>
<td>95.0</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.25-1.0</td>
<td>99.5</td>
</tr>
<tr>
<td>ATCC® 24433</td>
<td></td>
<td>Fluconazole</td>
<td>0.25-1.0</td>
<td>95.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>1.0-4.0</td>
<td>91.9</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.5-2.0</td>
<td>96.4</td>
</tr>
<tr>
<td>ATCC® 90018</td>
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<td>Fluconazole</td>
<td>0.25-1.0</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>≤0.12-0.25</td>
<td>99.5</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.5-2.0</td>
<td>93.7</td>
</tr>
<tr>
<td>ATCC® 750</td>
<td></td>
<td>Fluconazole</td>
<td>1.0-4.0</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>≤0.12-0.25</td>
<td>99.5</td>
</tr>
</tbody>
</table>

NOTE: ATCC® is a registered trademark of the American Type Culture Collection.

* As Issatchenka orientalis is now known to be the sexual form (the teleomorph) of *C. krusei*, it would be technically correct to use *I. orientalis* as the name for this fungus. However, this change would confuse most users and the far more widely used name *C. krusei* is retained.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal Agent</th>
<th>24-Hour Range</th>
<th>Mode</th>
<th>% Within Range</th>
<th>48-Hour Range</th>
<th>Mode</th>
<th>% Within Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida parapsilosis ATCC® 22019</td>
<td>Amphotericin B</td>
<td>0.25-2.0</td>
<td>0.5</td>
<td>97.1</td>
<td>0.5-4.0</td>
<td>2.0</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.25-2.0</td>
<td>1.0</td>
<td>95.0</td>
<td>0.5-2.0</td>
<td>1.0</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.25-1.0</td>
<td>0.5</td>
<td>96.7</td>
<td>0.5-4.0</td>
<td>1.0</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>0.06-0.25</td>
<td>0.12</td>
<td>99.2</td>
<td>0.12-0.5</td>
<td>0.25</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>0.5-4.0</td>
<td>2.0</td>
<td>98.2</td>
<td>1.0-4.0</td>
<td>2.0</td>
<td>98.1</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.12-0.5</td>
<td>0.25</td>
<td>95.8</td>
<td>0.12-0.5</td>
<td>0.25</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>0.03-0.25</td>
<td>0.06/0.12</td>
<td>97.5</td>
<td>0.06-0.5</td>
<td>0.12</td>
<td>98.3</td>
</tr>
<tr>
<td><strong>Micafungin</strong></td>
<td><strong>Posaconazole</strong></td>
<td><strong>0.5-2</strong></td>
<td><strong>1</strong></td>
<td><strong>100.0</strong></td>
<td><strong>0.5-4</strong></td>
<td><strong>1</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Ravuconazole</strong></td>
<td>0.06-0.25</td>
<td>0.12</td>
<td>96.7</td>
<td>0.06-0.25</td>
<td>0.12</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td><strong>Voriconazole</strong></td>
<td>0.016-0.12</td>
<td>0.06</td>
<td>95.8</td>
<td>0.03-0.25</td>
<td>0.06</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.016-0.12</td>
<td>0.06</td>
<td>100.0</td>
<td>0.03-0.25</td>
<td>0.06</td>
<td>100.0</td>
</tr>
<tr>
<td>Candida krusei ATCC® 6258</td>
<td>Amphotericin B</td>
<td>0.5-2.0</td>
<td>1.0</td>
<td>100.0</td>
<td>1.0-4.0</td>
<td>2.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.03-0.12</td>
<td>0.06</td>
<td>97.9</td>
<td>0.03-0.12</td>
<td>0.06</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.12-1.0</td>
<td>0.5</td>
<td>98.8</td>
<td>0.25-1.0</td>
<td>0.5</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>Flucytosine (5-FC)</td>
<td>4.0-16</td>
<td>8.0</td>
<td>97.5</td>
<td>8.0-32</td>
<td>16</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>8.0-64</td>
<td>16</td>
<td>100.0</td>
<td>16-128</td>
<td>32</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.12-1.0</td>
<td>0.5</td>
<td>95.8</td>
<td>0.25-1.0</td>
<td>0.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>0.12-1.0</td>
<td>0.5</td>
<td>95.4</td>
<td>0.25-1.0</td>
<td>0.5</td>
<td>99.6</td>
</tr>
<tr>
<td><strong>Micafungin</strong></td>
<td><strong>Posaconazole</strong></td>
<td><strong>0.12-0.5</strong></td>
<td><strong>0.25</strong></td>
<td><strong>99.6</strong></td>
<td><strong>0.12-0.5</strong></td>
<td><strong>0.25</strong></td>
<td><strong>99.0</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Ravuconazole</strong></td>
<td>0.06-0.5</td>
<td>0.25</td>
<td>100.0</td>
<td>0.12-1.0</td>
<td>0.5</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td><strong>Voriconazole</strong></td>
<td>0.06-0.5</td>
<td>0.25</td>
<td>93.3</td>
<td>0.25-1.0</td>
<td>0.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06-0.5</td>
<td>0.25</td>
<td>98.3</td>
<td>0.12-1.0</td>
<td>0.5</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**NOTE 1:** The MIC QC ranges in boldface type were adopted at a meeting of the subcommittee held on 20 January 2007 in Tampa, FL. These breakpoints are considered tentative for one year and are open for comments.

**NOTE 2:** ATCC® is a registered trademark of the American Type Culture Collection.

**NOTE 3:** The MIC for anidulafungin, caspofungin, and micafungin is the lowest concentration at which a score of 2 (prominent decrease in turbidity; see CLSI document M27-A3, Section 7.6.3) is observed after 24 hours incubation.
Table 7. Composition of RPMI 1640 Medium (with glutamine and phenol red but without bicarbonate)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>g/L Water</th>
<th>Constituent</th>
<th>g/L Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-arginine (free base)</td>
<td>0.200</td>
<td>Biotin</td>
<td>0.0002</td>
</tr>
<tr>
<td>L-asparagine (anhydrous)</td>
<td>0.050</td>
<td>D-pantothenic</td>
<td>0.00025</td>
</tr>
<tr>
<td>L-aspartic acid</td>
<td>0.020</td>
<td>Choline chloride</td>
<td>0.003</td>
</tr>
<tr>
<td>L-cystine • 2HCl</td>
<td>0.0652</td>
<td>Folic acid</td>
<td>0.001</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>0.020</td>
<td>Myoinositol</td>
<td>0.035</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>0.300</td>
<td>Niacinamide</td>
<td>0.001</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.010</td>
<td>PABA (para-aminobenzoic acid)</td>
<td>0.001</td>
</tr>
<tr>
<td>L-histidine (free base)</td>
<td>0.015</td>
<td>Pyridoxine HCl</td>
<td>0.001</td>
</tr>
<tr>
<td>L-hydroxyproline</td>
<td>0.020</td>
<td>Riboflavin</td>
<td>0.0002</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>0.050</td>
<td>Thiamine HCl</td>
<td>0.001</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.050</td>
<td>Vitamin B₁₂</td>
<td>0.000005</td>
</tr>
<tr>
<td>L-lysine • HCl</td>
<td>0.040</td>
<td>Calcium nitrate • H₂O</td>
<td>0.100</td>
</tr>
<tr>
<td>L-methionine</td>
<td>0.015</td>
<td>Potassium chloride</td>
<td>0.400</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>0.015</td>
<td>Magnesium sulfate (anhydrous)</td>
<td>0.04884</td>
</tr>
<tr>
<td>L-proline</td>
<td>0.020</td>
<td>Sodium chloride</td>
<td>6.000</td>
</tr>
<tr>
<td>L-serine</td>
<td>0.030</td>
<td>Sodium phosphate, dibasic (anhydrous)</td>
<td>0.800</td>
</tr>
<tr>
<td>L-threonine</td>
<td>0.020</td>
<td>D-glucose</td>
<td>2.000</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>0.005</td>
<td>Glutathione, reduced</td>
<td>0.001</td>
</tr>
<tr>
<td>L-tyrosine • 2Na</td>
<td>0.02883</td>
<td>Phenol red, Na</td>
<td>0.0053</td>
</tr>
<tr>
<td>L-valine</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8. Modifications for Special Circumstances

<table>
<thead>
<tr>
<th>Drug</th>
<th>Organism</th>
<th>Modification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td><em>Candida</em> spp.</td>
<td>Use of Antibiotic Medium 3 and a 24-hour end point has enhanced detection of resistance in some reports, but this medium is not standardized, substantial lot-to-lot variability is possible, and experience has varied.</td>
<td>See Section 7.7.1 of CLSI document M27-A3. 1, 2</td>
</tr>
<tr>
<td>All drugs</td>
<td><em>C. neoformans</em></td>
<td>Use of Yeast Nitrogen Base may enhance the growth of <em>C. neoformans</em> and improve the clinical relevance of antifungal MICs.</td>
<td>3, 4</td>
</tr>
<tr>
<td>All drugs</td>
<td>All organisms</td>
<td>Supplementation of the test medium so it contains glucose at a final concentration of 20 g/L may simplify end-point determination.</td>
<td>5</td>
</tr>
</tbody>
</table>

**NOTE:** These modifications are not a part of the formal CLSI document M27-A3 methodology and the utility of each of these modifications remains to be established. This table is provided solely as a reference for laboratories that are interested in studying adaptations of CLSI document M27-A3 that may enhance its utility under specific circumstances.

**References:**

Summary of Delegate Comments and Committee Responses

M27-S3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement

1. M27-S3 clearly communicates results of studies of 24- and 48-hour reference MIC ranges for microdilution testing of both established and newly introduced antifungal agents. This document offers laboratory professionals essential, current information for broth dilution antifungal susceptibility testing of yeasts in a concise, tabular format. I only have one minor suggestion for CLSI’s consideration: to include the applicability of individual tables to microdilution or macrodilution (or both) testing.

- The tables that are specific to micro- or macrodilution are titled as such—the others apply to both. No change has been made to the supplement.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—A Quality Management System Model for Health Care. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

- Documents & Records
- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Process Control
- Information Management
- Occurrence Management
- Assessments—External and Internal
- Process Improvement
- Customer Service
- Facilities & Safety

M27-S3 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—Application of a Quality Management System Model for Laboratory Services defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

M27-S3 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials Publications section on the following page.

<table>
<thead>
<tr>
<th>Preexamination</th>
<th>Examination</th>
<th>Postexamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination ordering</td>
<td>Sample collection</td>
<td>Sample transport</td>
</tr>
<tr>
<td>M24</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Adapted from CLSI/NCCLS document HS1—A Quality Management System Model for Health Care.
Related CLSI Reference Materials*


M11-A7  Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007). This standard provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

M23-A2  Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001). This document addresses the required and recommended data needed for the selection of appropriate interpretive standards and quality control guidelines for new antimicrobial agents.

M24-A  Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard (2003). This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, Nocardia spp., and other aerobic actinomycetes.

M29-A3  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

M38-A  Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard (2002). This document addresses the selection of antifungal agents; preparation of antifungal stock solutions and dilutions for testing; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive fungal infections.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.
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Anna Longwell, PC

Aptum Oncology

Argus Ltd.

A/S Rosco

Associated Regional & University Pathologists

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AstraZeneca Pharmaceuticals

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Axis-Shield PoC AS

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Bayer Healthcare, LLC, Diagnostics Div. – Elkhart, IN

BD

BD Biosciences – San Jose, CA

BD Diagnostic Systems

BD Vacutainer Systems

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Bio-Detection Systems

BioMedica Laboratories SDN BHD

bioMérieux, Inc. (M03)

Bio-Mérieux, Inc. (NC)

Bio-Rad Laboratories, Inc. – France

Bio-Rad Laboratories, Inc. – Irvine, CA

Bio-Rad Laboratories, Inc. – Plano, TX

Blaine Healthcare Associates, Inc.

Braun Biosystems, Inc.

Canon U.S. Life Sciences, Inc.

Cephrad Pharmaceuticals, Inc.

Center for Measurement Standards/TRI

Centers for Disease Control and Prevention

Central States Research Centre, Inc.

Cepheid

Chen & Chen, LLC (IQUUM)

The Clinical Microbiology Institute

Comprehensive Cytomeric Consulting

Control Lab

Copan Diagnostics Inc.

Cosmetic Ingredient Review

Cubist Pharmaceuticals

Cumbre Inc.

Dale Behring Marburg GmbH – A Siemens Company

Dahl-Chase Pathology Associates PA

David G. Rhoads Associates, Inc.

Diagnostic Products Corporation

Diagnostica Stago

Docre, Inc.

DX Tech

Eiken Chemical Company, Ltd.

Elanco Animal Health

Emisphere Technologies, Inc.

Eurofins Medinet

Fio

Focus Diagnostics

Future Diagnostics B.V.

Genomic Health, Inc.

Gen-Probe

Genzyme Diagnostics

GlaxoSmithKline

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Habeg Regulatory Consulting

HistoGeneX N.V.

Icom Laboratories, Inc.

Immucor Corporation

Indiana State Department of Health

Instrumentation Laboratory

Japan Ass. of Clinical Reagents

Joanneau Research

Forschungsgesellschaft mbh

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Kaiser Permanente

K.C.J. Enterprises

Krooser Consulting

Laboratory Specialists, Inc.

LifeScan, Inc. (a Johnson & Johnson Company)

Liposcience

Mae Standards Consultant, LLC

Medical Decision Consultants, Inc.

Merck & Company, Inc.

Microrny, LLC

MicroPhage

Monogram, Inc.

MultiPhase Solutions, Inc.

NanoCen

Nanoeng, Point-of-Care Diagnostics Div.

Nanosphere, Inc.

Nihon Koden Corporation

Nisui Pharmaceutical Co., Ltd.

NIR, & Associates, Inc.

NorDx – Scarborough Campus

Novartis (Aberdeen, UK)

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Nuclart Pharmaceuticals

Olympus America, Inc.

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QSE Consulting

Quakert Clinical Laboratories

Quest Diagnostics Incorporated

Radiatorometer, Inc.

RCC CIBA S. A.

Repilide

Rib-X Pharmaceuticals

Roche Diagnostics GmbH

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Roche Diagnostics Ltd.

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Roche Molecular Systems

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Sandif Pasteur

Sarstedt, Inc.

Scherer Corporation

Sequenom, Inc.

Siemens Healthcare Diagnostics

Siemens Medical Diagnostics (CA)

Siemens Medical Solutions Diagnostics (DE)

Siemens Medical Solutions Diagnostics (NY)

Speciality Ranch Luxembourg

Sphere Medical Holding Limited

State of Alabama

Sifining Medical Innovations

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Targara Therapeutics, Inc

Tethys Bioscience, Inc.

TheraDoc

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Third Wave Technologies, Inc.

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ThromboVision Inc.

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All Children’s Hospital (FL)

Allergen General Hospital (PA)

Alpena General Hospital (MI)

Alta Bates Summit Medical Center (CA)

American University of Beirut Medical Center (NJ)

Anne Arundel Medical Center (MD)

Antelope Valley Hospital District (CA)

Arkansas Children’s Hospital (AR)

Arkansas Dept of Health

Public Health Laboratory (AR)

Arkansas Methodist Medical Center (AR)

Asian Medical Center (Seoul)

Asante Health Systems (OR)

Asi Group of Hospitals Ltd.

Assocacion Española Primera de Socorros Mutuos (Spain)

Aspiras Waunau Hospital (WI)

Atlantic City Medical Center (NJ)

Atlantic Health Sciences Corp.

Auburn Regional Medical Center (WA)

Augusta Medical Center (VA)

Aultman Hospital (OH)

Avera McKennan (SD)

Az Sint-Jan

Azienda Ospedale Di Lecce (Italy)

Baffa Regional Hospital (Canada)

Baptist Hospital for Women (TN)

Baptist Hospital of Miami (FL)

Bassett Army Community Hospital (AK)

Baton Rouge General (LA)

Baxter Regional Hospital (CA)

BayCare Health System (FL)

Baylor Health Care System (TX)

Bayou Pathology, APMC (LA)

Baystate Medical Center (MA)

BB & A.G. Ve AS, A.

Beacon Laboratories (Turkey)

Beche Medical Center (DE)

Belfield HSS Trust

Beloit Memorial Hospital (WI)
Reference Method for Broth Dilution
Antifungal Susceptibility Testing of Yeasts;
Third Informational Supplement

This document provides updated tables for the CLSI antimicrobial susceptibility testing standard M27-A3.

An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process.