M61
Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

This document provides minimal inhibitory concentration breakpoints and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M38 and M51.

A CLSI supplement for global application.
Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

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Abstract

Clinical and Laboratory Standards Institute document M61—Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi includes minimal inhibitory concentration and quality control tables developed following the guidance in CLSI documents M38\textsuperscript{1} and M51.\textsuperscript{2} The data in the tables are valid only when the methodologies in CLSI documents M38\textsuperscript{1} and M51\textsuperscript{2} are followed. Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.


The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If you or your organization is not a member and would like to become one, or to request a copy of the catalog, contact us at: Telephone: +1.610.688.0100; Fax: +1.610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.
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**Suggested Citation**


**Previous Editions:**
M51-S1: May 2010
M61-Ed1: November 2017
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Foreword

The breakpoints and interpretive categories provided in this document are generated using the reference method for antifungal susceptibility testing of filamentous fungi described in CLSI document M38. These methods may be used for:

- Routine antifungal testing of patient isolates to guide therapy and classify isolates as susceptible or resistant to antifungal agents for which clinical breakpoints have been established
- Evaluation of commercial devices that will be used in medical laboratories
- Testing of new agents or systems by drug or device manufacturers

Results generated by reference methods, such as those described in CLSI documents, may be used by regulatory authorities to evaluate commercial susceptibility testing device performance as part of the device approval process. Regulatory clearance indicates that the commercial susceptibility testing device provides results that are substantially equivalent to those generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer’s approved package insert.

NOTE: Fungal taxonomy has undergone major changes in recent years. The dual (asexual and sexual stages) nomenclature has been abolished, and fungal species are constantly being reclassified and renamed according to improved molecular characterization. Species names listed in CLSI document M38 were revised to reflect the most recent taxonomic changes (at the time of publication), based on classification by DNA bar coding. Information on updated fungal species classification is publicly available.

Overview of Changes

This document replaces the previous edition of the approved document, M61-Ed1, published in 2017. Several changes were made in this edition, including:

- **Table 1. Minimal Inhibitory Concentration Breakpoints for Select Antifungal Agents Against Aspergillus fumigatus:**
  - Added new table

  **NOTE:** The minimal inhibitory concentration (MIC) breakpoints and interpretive categories for voriconazole were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2019. These MIC breakpoints and interpretive categories are tentative and are open for comment for one year from the publication of M61.

- **Table 2. Recommended 24-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:**
  - Added new table with information from previous edition’s Table 1 (which was separated into three QC tables based on incubation time)

  **NOTE:** The MIC QC ranges for manogepix and rezafungin were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2020. These QC ranges are tentative and are open for comment for one year from the publication of M61.
Added QC ranges for:
- Manogepix
  - *Candida albicans* ATCC® 90028
  - *Candida parapsilosis* ATCC® 22019
  - *Aspergillus flavus* ATCC® 204304
- Rezafungin
  - *C. parapsilosis* ATCC® 22019
  - *Candida krusei* ATCC® 6258

Added 24-hour QC ranges originally published in CLSI document M60 for:
- Amphotericin B
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Anidulafungin
  - *C. krusei* ATCC® 6258
- Fluconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Fluocytosine
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Isavuconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Itraconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Ketoconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Micafungin
  - *C. krusei* ATCC® 6258
- Posaconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258
- Voriconazole
  - *C. parapsilosis* ATCC® 22019
  - *C. krusei* ATCC® 6258

Revised QC ranges for *C. parapsilosis* ATCC® 22019 for:
- Anidulafungin
- Micafungin
Revised “Paecilomyces variotii ATCC® MYA-3630” to “Hamigera insecticola (previously identified as Paecilomyces variotii) ATCC® MYA-3630”

Added footnote regarding caspofungin susceptibility testing

Deleted footnote regarding anidulafungin concentration ranges

- **Table 3. Recommended 48-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:**
  - Added new table with information from previous edition’s Table 1 (which was separated into three QC tables based on incubation time)

**NOTE:** The MIC QC ranges for orolofim and rezafungin were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2020. These QC ranges are tentative and are open for comment for one year from the publication of M61.

- Added QC ranges for:
  - Anidulafungin
    - *C. parapsilosis* ATCC® 22019
  - Manogepix
    - *C. albicans* ATCC® 90028
    - *C. parapsilosis* ATCC® 22019
    - *A. flavus* ATCC® 204304
    - *A. fumigatus* ATCC® MYA-3626
  - Micafungin
    - *C. parapsilosis* ATCC® 22019
  - Rezafungin
    - *C. parapsilosis* ATCC® 22019
    - *C. krusei* ATCC® 6258
  - Orolofim
    - *A. flavus* ATCC® MYA-3631
    - *A. fumigatus* ATCC® MYA-3626

- Revised QC ranges for *C. parapsilosis* ATCC® 22019 for:
  - Itraconazole
  - Posaconazole

- Revised:
  - *Fusarium* spp. (*Fusarium verticillioides* [moniliforme] ATCC® MYA-3629 and *Fusarium solani* ATCC® MYA-3636) reference ranges from MIC to minimal effective concentration and adjusted the within-range percentage
  - “Paecilomyces variotii ATCC® MYA-3630” to “Hamigera insecticola (previously identified as Paecilomyces variotii) ATCC® MYA-3630”
• Table 4. Recommended 48- to 96-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:
  – Added new table with information from previous edition’s Table 1 (which was separated into three QC tables based on incubation time)
  – **Deleted** footnote regarding anidulafungin concentration ranges

• Table 5. Recommended Zone Diameter Limits for Quality Control and Reference Strains Using Disk Diffusion Antifungal Susceptibility Testing Procedures:
  – Corrected reference range for *Paecilomyces variotii* ATCC® MYA-3630 for:
    o Caspofungin
  – Revised “*Paecilomyces variotii* ATCC® MYA-3630” to “*Hamigera insecticola* (previously identified as *Paecilomyces variotii*) ATCC® MYA-3630”
  – **Deleted** footnote regarding tentative QC zone diameter ranges

• Table 6. Solvents and Diluents for Preparation of Stock Solutions of Antifungal Agents:
  – Added:
    o Manogepix
    o Orolofim
    o Rezafungin
  – Added footnotes regarding dimethyl sulfoxide and manufacturer recommendations

**NOTE:** The content of this document is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

**Key Words**

Antifungal agent,azole, breakpoint, disk diffusion, echinocandin, filamentous fungi, interpretive category, microbroth dilution, minimal inhibitory concentration, quality control, susceptibility testing
<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Species</th>
<th>MIC Breakpoints and Interpretive Categories, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voriconazole</td>
<td><em>A. fumigatus</em></td>
<td>S ≤ 0.5, I 1, R ≥ 2</td>
</tr>
</tbody>
</table>

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

Footnote

a. Interpretive breakpoints were derived from a collection of sequence-confirmed isolates of *A. fumigatus sensu stricto* and are not applicable to other members of the *A. fumigatus* species complex.

NOTE: Information in boldface type is new or modified since the previous edition.
Table 2. Recommended 24-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures

<table>
<thead>
<tr>
<th>Organism</th>
<th>Purpose</th>
<th>Antifungal Agent</th>
<th>MIC/MEC Range, µg/mL</th>
<th>MIC/MEC Mode, µg/mL</th>
<th>MICs/MECs Within Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em> ATCC® 90028</td>
<td>QC</td>
<td>Manogepix</td>
<td>0.004–0.015</td>
<td>0.008</td>
<td>100</td>
</tr>
<tr>
<td><em>Candida krusei</em> ATCC® 6258¹,²</td>
<td>QC</td>
<td>Amphotericin B</td>
<td>0.5–2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anidulafungin</td>
<td>0.03–0.12</td>
<td>0.06</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caspofungin⁵</td>
<td>0.12–1</td>
<td>0.5</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluconazole</td>
<td>8–64</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine</td>
<td>4–16</td>
<td>8</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isavuconazole</td>
<td>0.06–0.5</td>
<td>0.25</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>0.12–1</td>
<td>0.5</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketoconazole</td>
<td>0.12–1</td>
<td>0.5</td>
<td>95.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micafungin</td>
<td>0.12–0.5</td>
<td>0.25</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posaconazole</td>
<td>0.06–0.5</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rezafungin</td>
<td>0.015–0.12</td>
<td>0.03</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.06–0.5</td>
<td>0.25</td>
<td>98.3</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em> ATCC® 22019¹,²</td>
<td>QC</td>
<td>Amphotericin B</td>
<td>0.25–2</td>
<td>0.5</td>
<td>97.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anidulafungin</td>
<td>0.25–2</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caspofungin</td>
<td>0.25–1</td>
<td>0.5</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluconazole</td>
<td>0.5–4</td>
<td>2</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flucytosine</td>
<td>0.06–0.25</td>
<td>0.12</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isavuconazole</td>
<td>0.015–0.06</td>
<td>0.06</td>
<td>90.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>0.06–0.5</td>
<td>0.25</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketoconazole</td>
<td>0.03–0.25</td>
<td>0.06/0.12</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manogepix</td>
<td>0.008–0.03</td>
<td>0.015</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micafungin</td>
<td>0.5–2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posaconazole</td>
<td>0.03–0.25</td>
<td>0.12</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rezafungin</td>
<td>0.25–2</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.016–0.12</td>
<td>0.06</td>
<td>100</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> ATCC® 204304</td>
<td>QC</td>
<td>Manogepix</td>
<td>0.004–0.03</td>
<td>0.008</td>
<td>99.2</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> ATCC® MYA-3626 ³</td>
<td>Reference (MEC)</td>
<td>Anidulafungin</td>
<td>≤0.015</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em> ATCC® MYA-3633 ³</td>
<td>Reference (MEC)</td>
<td>Anidulafungin</td>
<td>≤0.015</td>
<td>N/A</td>
<td>99.6</td>
</tr>
<tr>
<td><em>Hamigera insecticola</em> (previously identified as <em>Paecilomyces variotii</em>) ATCC® MYA-3630</td>
<td>Reference (MEC)</td>
<td>Anidulafungin</td>
<td>≤0.015</td>
<td>N/A</td>
<td>100</td>
</tr>
</tbody>
</table>

Abbreviations: ATCC®, American Type Culture Collection; MEC, minimal effective concentration; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

**Footnotes**

a. ATCC® is a registered trademark of the American Type Culture Collection.
Table 3. (Continued)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Purpose</th>
<th>Antifungal Agent</th>
<th>MIC/MEC Range, µg/mL</th>
<th>MIC/MEC Mode, µg/mL</th>
<th>MICs/MECs Within Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.5–4</td>
<td>2</td>
<td>98.7</td>
</tr>
<tr>
<td>ATCC® MYA-3626&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td>Itraconazole</td>
<td>0.25–2</td>
<td>1</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.25–1</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>QC</td>
<td>Manogepix</td>
<td>0.008–0.06</td>
<td>0.03</td>
<td>98.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orolofim</td>
<td>0.008–0.06</td>
<td>0.03</td>
<td>98.2</td>
<td></td>
</tr>
<tr>
<td>A. fumigatus ATCC® MYA-3627&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>0.5–4</td>
<td>2</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>≥ 16</td>
<td>&gt; 16</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.25–1</td>
<td>0.5</td>
<td>99.2</td>
</tr>
<tr>
<td>Aspergillus terreus ATCC® MYA-3633&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>2–8</td>
<td>4</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.25–1</td>
<td>0.5</td>
<td>99.2</td>
</tr>
<tr>
<td>Fusarium solani ATCC® MYA-3636&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Reference (MEC)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Anidulafungin</td>
<td>&gt; 8</td>
<td>N/A</td>
<td>87</td>
</tr>
<tr>
<td>Fusarium verticillioides (moniliforme) ATCC® MYA-3629&lt;sup&gt;5,6&lt;/sup&gt;</td>
<td>Reference</td>
<td>Amphotericin B</td>
<td>2–8</td>
<td>4</td>
<td>99.6</td>
</tr>
<tr>
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<td></td>
<td>Itraconazole</td>
<td>≥ 16</td>
<td>&gt; 16</td>
<td>97.9</td>
</tr>
<tr>
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<td>Posaconazole</td>
<td>0.5–2</td>
<td>1</td>
<td>98.1</td>
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<td></td>
<td>Voriconazole</td>
<td>1–4</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Fusarium verticillioides (moniliforme) ATCC® MYA-3629&lt;sup&gt;5,6&lt;/sup&gt;</td>
<td>Reference (MEC)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Anidulafungin</td>
<td>&gt; 8</td>
<td>N/A</td>
<td>87.5</td>
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<tr>
<td>Hamigera insecticola</td>
<td>QC</td>
<td>Amphotericin B</td>
<td>1–4</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>(previously identified as Paecilomyces variotii) ATCC® MYA-3630&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td>Isavuconazole</td>
<td>0.06–0.5</td>
<td>0.12</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Itraconazole</td>
<td>0.06–0.5</td>
<td>0.12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posaconazole</td>
<td>0.03–0.25</td>
<td>0.06</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole</td>
<td>0.015–0.12</td>
<td>0.06</td>
<td>100</td>
</tr>
</tbody>
</table>

Abbreviations: ATCC®, American Type Culture Collection; MEC, minimal effective concentration; MIC, minimal inhibitory concentration; N/A, not applicable; ND, not determined; QC, quality control.

Footnotes

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

b. The percentage of manogepix minimal inhibitory concentrations (MICs) within range for C. parapsilosis ATCC® 22019 and A. flavus ATCC® 204304 was less than the threshold value of 95% necessary for quality control isolates. These values may be assessed and adjusted in the future based on Tier 3 data.

c. The MIC ranges for A. flavus ATCC® 204304 are based on data from a collaborative study<sup>3,4</sup> that were not obtained according to the process described in CLSI document M23.<sup>7</sup> However, A. flavus is the only mold for which reproducible reference limits for ravuconazole have been established, so it is included in this table.

d. Although the anidulafungin concentration range in the study was 0.015 to 32 µg/mL, off-scale MICs > 32 µg/mL from that study are reported in this table as > 8 µg/mL for consistency with the recommended routine testing range for this compound.<sup>6</sup>

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 3

