Isavuconazole Breakpoints for *Aspergillus fumigatus*

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1 Foreword

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Using the CLSI voluntary consensus process, the Subcommittee on Antifungal Susceptibility Testing develops standards that promote accurate antifungal susceptibility testing and appropriate reporting. The subcommittee reviews data from various sources and studies (eg, *in vitro*, pharmacokinetic/pharmacodynamic [PK/PD], and clinical studies) to establish antifungal susceptibility test methods, breakpoints, epidemiological cutoff values, and QC ranges.

The details of the necessary and recommended data for selecting appropriate breakpoints and QC ranges, as well as how the data are presented for evaluation, are described in CLSI M23.[1] CLSI antifungal breakpoints are provided in CLSI M27M44S[2] and CLSI M38M51S.[3]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods, QC parameters, and the manner in which breakpoints are established may be refined to ensure more accurate results. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should always be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment. For more information, visit www.clsi.org.

This CLSI rationale document is based on the need for guidance on the interpretation of antifungal agents for invasive aspergillosis. Aspergillosis, which is most commonly caused by *Aspergillus fumigatus*, is the most frequent invasive hyalohyphomycosis. Invasive aspergillosis is a devastating disease that occurs predominantly in immunocompromised individuals, particularly those with profound neutropenia. Isavuconazole is the most recent triazole developed that demonstrated significant activity against *A. fumigatus* in patients with invasive aspergillosis. However, *A. fumigatus* isolates with mutations in the *CYP51* gene are known to produce elevated minimal inhibitory concentrations (MICs) to triazoles and contribute to therapeutic failures. Therefore, antifungal susceptibility guidance is needed to alert clinicians to the likelihood of resistance, so that alternate therapeutic strategies can be considered.

2 Introduction

Isavuconazole is a triazole antifungal agent with broad-spectrum *in vitro* activity against both yeasts and filamentous fungi. It functions through the inhibition of the cytochrome P450-dependent 14α-lanosterol demethylase, which interrupts ergosterol synthesis in fungi. Isavuconazole was compared with voriconazole in a randomized controlled trial of patients with invasive aspergillosis. A total of 527 patients, who were categorized as having an invasive mold infection, including 231 with proven or probable invasive aspergillosis, were randomized in this study. Both treatment groups were equivalent (ie, non-inferior) as determined from primary mortality and secondary clinical response endpoints.[4] Based on these results, isavuconazole is considered a first-line therapy for the treatment of invasive aspergillosis and is approved by the US Food and Drug Administration and by authorities in the European Union and Japan for treatment of invasive aspergillosis.[5,6]

There is now increasing concern for the development of azole resistance in *A. fumigatus*, which is caused primarily by mutations within the *CYP51A* gene that encodes the enzyme Cyp51, also known as 14α-lanosterol demethylase. Resistance to isavuconazole and other azoles in *Aspergillus* can develop with clinical or environmental exposure to these antifungals.[7-10] Patients with infections caused by azole-resistant strains reportedly have worse clinical outcomes and higher mortality rates when treated with voriconazole.[11,12]

To assist clinicians treating patients with invasive aspergillosis, in January 2023, CLSI approved clinical breakpoints for isavuconazole against *A. fumigatus sensu stricto* (see Table 1).

Table 1. Current CLSI Isavuconazole Breakpoints[a]

Organism Group	Interpretive Categories and MIC Breakpoints, µg/mL		
	S	I	R
A. fumigatus	≤ 1	2	≥4

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.
[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 *Aspergillus* species complex.

No historical CLSI isavuconazole breakpoints are being replaced by the current isavuconazole breakpoints. The breakpoints presented in Table 1 are the original breakpoints for this drug-microorganism combination.

An isavuconazole MIC of 2 µg/mL is in the I interpretive category. The I category is different than the susceptible-dose dependent interpretive category used for fluconazole against *Candida* species. Instead, the I category provides a necessary buffer zone for antifungal susceptibility testing to avoid major and very major errors caused by inherent variability in the *in vitro* diagnostic test method.[2] Available data do not allow isolates with test results in the I range to be clearly categorized as either S or R. Voriconazole MIC results against *A. fumigatus* can serve as a surrogate for isavuconazole interpretations, if available, as follows:

- If susceptible to voriconazole, isavuconazole can be reported as S.
- If resistant to voriconazole, isavuconazole can also be reported as R.
- If intermediate to both isavuconazole and voriconazole, both antifungals can be reported as I.

If voriconazole MICs are not available, the laboratory should consider repeating isavuconazole susceptibility testing or sending to a reference laboratory for MIC confirmation. Sequencing of the *CYP51A* gene is also an option, although it is currently not widely available and is performed only for research purposes.

MIC values of 1 µg/mL and 2 µg/mL are close to the wild-type distribution and within one dilution of the I and R categories for both isavuconazole and voriconazole. Because of the inherent variability of antifungal MIC testing and with ±2 dilutions set as essential agreement, MIC values should be interpreted carefully within that MIC range.

Although isavuconazole and voriconazole against *A. fumigatus* MIC results are often the same or within two dilutions, there are cases where categorical agreement is not achieved. A study of 1882 *A. fumigatus* isolates with MIC results for both isavuconazole and voriconazole against each strain demonstrated categorical agreement of 94.58% (1780 out of 1882 isolates).[13] Minor errors (ie, one drug classified as intermediate and the other as susceptible or resistant) were reported in 5.15% of isolates (97 of 1882 isolates), and major errors (ie, susceptible for isavuconazole but resistant for voriconazole) were reported in 0.27% of the total