Voriconazole Breakpoints for *Aspergillus fumigatus*

CLSI rationale document FR01
May 2024

Nathan P. Wiederhold, PharmD
University of Texas Health Science Center at San Antonio
USA

David Andes, MD
University of Wisconsin – Madison Medical School
USA

Andrew M. Borman, BSc, PhD
UK Health Security Agency
UK

Philippe J. Dufresne, PhD, RMCCM
Institut national de santé publique du Québec
Canada

Shawn R. Lockhart, PhD, D(ABMM), F(AAM)
Centers for Disease Control and Prevention
USA

Gary W. Procop, MD, MS, MEd
American Board of Pathology
USA

On behalf of the Working Group on *A. fumigatus* Breakpoints and the CLSI Subcommittee on Antifungal Susceptibility Tests

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 Tests 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. Voriconazole is one of the first triazoles 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 voriconazole 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

Voriconazole 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.[4,5] Voriconazole was compared with amphotericin B in a randomized controlled trial of patients with invasive aspergillosis.[6,7] A total of 277 patients, who were categorized as having definite or probable invasive aspergillosis of the lungs, were included in this study. More patients treated with voriconazole achieved a satisfactory global response compared with those treated with amphotericin B (53% vs 32%, respectively, $P < 0.0001$). Similarly, more patients treated with voriconazole (71%) were alive on day 84 of the trial compared with those treated with amphotericin B (58%). Based on these results, voriconazole is considered a first-line therapy for the treatment of invasive aspergillosis[8] and is approved by the US Food and Drug Administration and by authorities in the European Union and Japan for treatment of invasive aspergillosis.

There is increasing concern for the development of azole resistance in *A. fumigatus*,[9] which is caused primarily by mutations within the *CYP51A* gene that encodes the enzyme Cyp51, also known as 14α-lanosterol demethylase. Resistance to voriconazole and other azoles in *Aspergillus* can develop with clinical or environmental exposure to these antifungal agents.[10-13] Patients with infections caused by azole-resistant strains reportedly have higher mortality rates.[9,14,15] To assist clinicians treating patients with invasive aspergillosis, in June 2020, CLSI published clinical breakpoints for voriconazole against *A. fumigatus sensu stricto* (see Table 1).

Table 1. Current CLSI Voriconazole Breakpoints[a,b]

Organism Group	Interpretive Categories and MIC Breakpoints, µg/mL		
	S	I	R
A. fumigatus	≤ 0.5	1	≥ 2

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.
[a] Last reviewed June 2019; first published in CLSI M61-Ed2.[16]
[b] 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.

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

Regarding breakpoint harmonization, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) also established breakpoints[17] for *A. fumigatus* and voriconazole in 2012 and updated them in 2020. When converted to CLSI format, these breakpoints are S ≤ 1 µg/mL and R ≥ 2 µg/mL, with no intermediate category.

An MIC of 2 µg/mL is associated with an area of technical uncertainty, and EUCAST recommends reporting these values with the following comment: "In some clinical situations (non-invasive infections forms) voriconazole can be used provided sufficient exposure is ensured."

3 Standard Dosages and Pharmacokinetic Data

Standard intravenous and oral dosages that are used clinically, as well as pharmacokinetic (PK) data for voriconazole, are listed in Table 2.

Table 2. Voriconazole Dosages and PK Data

	IV	Oral
Dosage regimen[8,18,19]	6 mg/kg every 12 h on day 1, then 4 mg/kg every 12 h daily (n = 33) on day 7 in adults	400 mg twice daily on day 1, then 200 mg twice daily (n = 23) at steady state in adults; oral therapy can be used at 200–300 mg (or 3–4 mg/kg) every 12 h
C_{max} ± SD (% CV), µg/mL	Median 4.6 (range: 2.48-9.92)	3.57 ± 1.73 (% CV 48.5)
C_{min} ± SD (% CV), µg/mL	Median 2.08 (range: 0.38-7.35)	1.40 ± 1.29 (% CV 88.0)
CL, mL/min	Not provided	192.5 ± 141.5 (% CV 73.5)
V_d[20]	4.66 L/kg	
$T_{1/2}$, h[21]	6–9	11.31 ± 9.87 (% CV 87.3)
AUC_{0-12h}, µg*h/mL	37.6 (range: 13.7–104)	26.20 ± 18.58 (% CV 70.9)
Protein binding	58% bound	

Abbreviations: AUC_{0-12h}, area under the concentration-time curve over a 12-hour dosing interval; CL, overall clearance; C_{max}, maximum observed plasma concentration; C_{min}, minimum observed plasma concentration; % CV, coefficient of variation expressed as a percentage; h, hour(s); IV, intravenous; PK, pharmacokinetic; SD, standard deviation; $T_{1/2}$, terminal elimination half-life; V_d, volume of distribution.

NOTE: Voriconazole exhibits nonlinear PK with wide interpatient variability; marked intrapatient variability has also been reported.[22] Similar variability has been demonstrated in pediatric populations.[23-27] Several factors influence the PK of voriconazole, including saturable metabolism, polymorphisms in cytochrome P450 2C19, and drug-drug interactions. Therapeutic drug monitoring to maintain concentrations in the therapeutic window (eg, trough levels of 1-6 µg/mL) is commonly recommended.[28-30]

The adult dosages used for breakpoint determination are shown in Table 3.