An international evaluation of MIC distributions and ECV definition for *Fusarium* species identified by molecular methods for the CLSI broth microdilution method

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Abstract

CLSI epidemiological cutoff values (ECVs) of antifungal agents are available for various *Candida* spp., *Aspergillus* spp. and the Mucorales. However, those categorical endpoints have not been established for *Fusarium* spp., mostly due to the difficulties associated with collecting sufficient CLSI MICs for clinical isolates identified according to the currently recommended molecular DNA-PCR based identification methodologies. CLSI MIC distributions were established for 53 *Fusarium dimerum* species complex (SC), 10 *F. fujikuroi*, 82 *F. proliferatum*, 20 *F. incarnatum-equiseti* SC, 226 *F. oxysporum* SC, 608 *F. solani* SC, and 151 *F. verticillioides* isolates originating in 17 laboratories (Argentina, Australia, Brazil, Canada, Europe, Mexico and the United States). According to the CLSI guidelines for ECV definition, ECVs encompassing ≥97.5% of pooled statistically modeled MIC populations were as follows: amphotericin B ECVs of 4 µg/ml (*F. verticillioides*) and 8 µg/ml (*F. oxysporum* SC and *F. solani* SC); posaconazole ECVs of 2 µg/ml (*F. verticillioides*), 8 µg/ml (*F. oxysporum* SC), and 32 µg/ml (*F. solani* SC); voriconazole ECVs of 4 µg/ml (*F. verticillioides*), 16 µg/ml (*F. oxysporum* SC) and 32 µg/ml (*F. solani* SC); and itraconazole ECVs of 32 µg/ml (*F. oxysporum* SC and *F. solani* SC). Insufficient data precluded ECV definition for the other species. Although these ECVs could aid in detecting non-WT isolates with reduced susceptibility to the agents evaluated, the relationship between molecular mechanisms of resistance (gene mutations) and MICs needs to be investigated for *Fusarium* spp.

Introduction

While the genus *Fusarium* and its teleomorphic (sexual) forms encompass a variety of species, only some have been associated with human disease. Identification of *Fusarium* isolates to the accepted phylogenetic species complexes/species level is essential (1-4) but challenging, as important taxonomic changes were made and is still in a state of flux for some genera. Following the results of DNA sequencing studies, prevalent well known fungal genera were divided into several new genera. By 2013, the consensus (International Code of Nomenclature for Algae) was to continue using certain well known generic names and that each fungal species should have only a single name, including those in the genus *Fusarium*. In addition, its well known anamorphs, as they have been used in the present paper, ought to be used instead of the known teleomorphs (e.g., *Haemonectria* and *Gibberella*) (1-3). However, the perception is that new generic changes would be suggested such as the establishment of the

genus *Bisifusarium* to include the more commonly known members of the *F. dimerum* SC and the name *Neocosmospora solani* to replace *Fusarium solani* (5). The most frequent causes of fungal infections are among the members of the three complexes, *F. solani* species complex (SC), *F. oxysporum* SC, *F.* (*Giberella*) *fujikuroi* SC, which includes among others *F. verticillioides* and *F. proliferatum*, and to a lesser extent both *F. dimerum* SC and *F. incarnatum-equiseti* SC; their distribution could be region dependent (4,6-9). Common clinical presentations among non-immunocompromised patients are onychomycosis, keratitis, allergic disease (sinusitis and bronchopulmonary disease), and in the immunocompromised host (e.g., prolonged neutropenia and T-cell immunodeficiency) disseminated disease as well as other severe invasive infections (1,4,6-12). Amphotericin B lipid formulations, voriconazole and posaconazole, and to a lesser extent itraconazole, have been recommended or used for the treatment and prophylaxis of *Fusarium* infections, in addition to surgical debridement and reversal of immunosuppression (13-18). The survival rate is low with some reports suggesting 30% or less for fusariosis, especially among patients with persistent neutropenia (15-21). Most successful therapeutic treatment of invasive disease is usually associated with neutrophil recovery, a major factor in making the setting of clinical breakpoints so challenging. The new formulations of itraconazole and posaconazole have improved bioavailability and reduced variability in exposure among subjects (22,23). However, the efficacy of these formulations in the treatment of fusariosis has not been established.

A reproducible procedure for testing the antifungal susceptibilities of *Fusarium* spp. is described by the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests in the M38-A2 document (24). However, neither species-specific clinical breakpoints (BPs) and/or epidemiological cutoff values (ECVs) have been established for this fungal group. The main reason, as for other less prevalent fungal species, is the lack of both clinical trials and knowledge regarding molecular resistance mechanisms for *Fusarium* spp. As a consequence, information on the relationships between resistance mechanisms, low and high MICs and clinical response to therapy is not available. However, it is still possible to define ECVs. These are calculated based on MIC distributions ($\geq$100 MICs/species/agent) from multiple independent laboratories ($\geq$3) (25,26, CLSI documents on ECVs under development). ECVs can identify non-wild type (non-WT [often harboring molecular mechanisms of resistance]) isolates or those isolates less susceptible to the antifungal agent being evaluated. Although amphotericin B and triazole MIC data have been reported for a variety of *Fusarium* spp., most available data are either for isolates identified only to the genus level, or by non-molecular methods, or the number of isolates evaluated was small (2,4,27-30). Therefore, there

was the need to pool data from multiple laboratories in order to define ECVs for *Fusarium* spp.

The purpose of the present study was (i) to define WT-susceptibility endpoint MIC distributions of the three most prevalent species/complexes (*F. oxysporum* SC, *F. solani* SC and *F. verticillioides*) using aggregated CLSI M38-A2 broth microdilution MIC data originating from 16 of the 17 participant laboratories and (ii) to propose ECVs for amphotericin B, voriconazole, posaconazole and itraconazole for these agents/species/complexes combinations for which the number of isolates was ≥113 and originated from ≥7 independent laboratories. Amphotericin B, voriconazole, posaconazole and itraconazole pooled MIC distributions comprising 10 to 82 isolates for less prevalent species/complexes (e.g., *F. dimerum* SC, *F. fujikuroi*, *F. incarnatum-equiseti* SC, *F. proliferatum*) also were collated. We aggregated a total of 10 to 608 MICs (species and antifungal agent dependent) as obtained in the 17 participant laboratories (Argentina, Australia, Brazil, Canada, Europe, Mexico, and the United States).

Materials and Methods

Isolates. Each isolate was recovered from unique clinical specimens from patients with mostly the following infections: eye, skin (some times both cutaneous, nail and other organ), sinus, pulmonary, and invasive disease (blood, lymph nodes). Antifungal susceptibility testing was performed according to the CLSI broth microdilution method (M38-A2) at the following medical centers: VCU Medical Center, Richmond, VA; Hospital São Paulo, Escola Paulista de Medicina-UNIFESP, São Paulo, Brazil; Instituto Nacional de Enfermedades Infecciosas "Dr. C. G. Malbrán" Buenos Aires, Argentina; Institut national de santé publique du Québec, Laboratoire de santé publique du Québec, Sainte-Anne-de-Bellevue, Québec, Canada; Provincial Laboratory, Alberta Health Services, Edmonton, Canada; University Hospitals Case Medical Center and Case Western Reserve University, Cleveland, OH; Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México; Facultat de Medicina, IISPV, URV**,** Reus, Spain; National Mycology Reference Centre, SA Pathology, Adelaide, Australia; Canisius Wilhelmina Hospital, Nijmegen, Netherlands; The Instituto Adolfo Lutz Reference Center São Paulo, Brazil; Hospital General Universitario Gregorio Marañón, Madrid, Spain; JMI Laboratories, North Liberty, IA; Mycology Department, The Instituto Adolfo Lutz Reference Center São Paulo, Brazil; Grupo Fleury, São Paulo, Brazil; Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milano, Italy; University of Texas Health Science Center, San Antonio, TX.

Identification of the isolates was performed in each laboratory using conventional methods (both macroscopic and microscopic characteristics on potato dextrose agar) (1,31) and confirmed by DNA-PCR based molecular assays (e.g., mostly sequencing and amplification of the β-tubulin (BenA), the translation elongation factor 1α [TEF], or the largest subunit of RNA polymerase [RPB1] and/or the second largest subunit of RNA polymerase [RPB2], as well as the analysis of the internal transcribed spacer 1 [ITS1] and ITS2 regions (1,4,9,32,33). CLSI MICs for each of the four agents were aggregated for 53 *F. dimerum* SC (including 1 *F. delphinoides* isolate), 10 *F. fujikuroi*, 82 *F. proliferatum*, 20 *F. incarnatum-equiseti* SC, 226 *F. oxysporum* SC, 608 *F. solani* SC (including 11 *F. falciforme* isolates), and 151 *F. verticillioides* isolates originating from between 3 to 16 of the 17 independent laboratories (data shown in Table 1). Additionally, insufficient MIC data (<10 isolates from 2 to 3 laboratories) were provided for other members of the *F. fujikuroi* SC identified as *F. sacchari, F. subglutinans*, and *F. thapsinum* (data not shown). Since molecular resistance mechanisms have not been elucidated for *Fusarium* spp. and any antifungal agent, none of the isolates were evaluated for gene mutations.

MIC data for at least one of the following three quality control (QC) isolates, *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6258 and *Paecilomyces variotii* ATCC MYA-3630 and/or the reference *Aspergillus flavus* ATCC 204304 isolate were reported by the participant laboratories (24).

Antifungal susceptibility testing. MICs of amphotericin B and the three triazoles for each available isolate in the total set (Tables 1 and 2) were obtained in each center by CLSI broth microdilution method (standard RPMI-1640 broth [0.2% dextrose] and final inoculum concentrations that ranged from 0.4 X 10^4 to 5 X 10^4 CFU/ml); MICs were the lowest drug concentrations that produced complete growth inhibition (100%) at 48 h (24). MICs for the QC *Candida* strains were obtained after 48 h using the 50% (triazoles) and 100% (amphotericin B) growth inhibition criteria (24). These MICs were within the recommended MIC limits with the following exceptions: discrepant MICs for both the *C. krusei* and *C. parapsilosis* QC isolates and the triazoles were observed, but the agreement (97.7 to 99.7%) was similar or higher than those listed in the M38-A2 document (24); the modes were within one dilution.

Definitions. As previously defined in the “Introduction”, the WT is the population of strains in a species/drug combination with no detectable acquired resistance mechanisms. The ECV (or CO_{WT}) is the highest MIC that would categorize an isolate as WT (without known mechanisms of resistance) or alternatively, the critical drug concentration value that may identify

169 those strains with decreased susceptibility to the agent being evaluated (non-WT isolates), or
170 potentially resistant (25,26,34).

171 **Data analysis.** The analysis of the data was performed as previously reported in various
172 studies and following the CLSI guidelines set forth for this purpose (25,26,34, CLSI documents
173 on ECVs under development). Briefly, after listing the MIC distributions of each species/species
174 complex/agent from each laboratory in an Excel spreadsheet, they were reviewed for
175 skewed/abnormal distributions (e.g., mostly mode at the lowest concentration tested or
176 bimodal), which were not included in the statistical analysis. As per CLSI recommendations
177 (CLSI documents on ECVs under development) and following the examination of global WT
178 modal MIC variability, distributions for each antifungal agent and species/species complex were
179 pooled with the qualifying data. ECVs were calculated for each pooled distribution by the
180 previously reported iterative statistical technique that captured at least 95%, 97.5% and 99% of
181 the modeled WT population (not the observed MICs) (34). In addition, we evaluated the inherent
182 variability (approximately within one doubling dilution) of susceptibility testing and the presence
183 of outlier laboratories in each pooled distribution.

184

185 **Results and Discussion**

186 Susceptibility testing should aid in predicting patient response to therapy, which is the
187 specific role of the BP (35,36). The CLSI has only established species-specific BPs for testing
188 the susceptibility of some *Candida* spp. to echinocandins, fluconazole and voriconazole (36).
189 The reason for this dearth of BPs is that their establishment requires particular steps: (i) WT
190 MIC distributions and ECVs for each species and agent being evaluated, (ii) the agent
191 pharmacokinetic and pharmacodynamic (PK/PD) parameters, (iii) knowledge of the relationship
192 between mechanisms of resistance and MICs, and more importantly, (iv) the correlation of MICs
193 and clinical response to treatment with the specific agent from clinical trials (34-36). Data for
194 these steps are not available for *Fusarium* spp. However, we have gathered MIC distributions
195 for *F. oxysporum* SC, *F. solani* SC, and *F. verticillioides* (the three species more commonly
196 associated with human disease) and three triazoles and amphotericin B. Although ECVs were
197 not proposed for the other species evaluated due to insufficient data, their pooled MIC
198 distributions have been listed in Table 1; the CLSI criteria require a minimum of 100
199 MICs/species from at least three laboratories and EVCs calculated by the iterative statistical
200 method (CLSI documents on ECVs under development). It is expected that the proposed ECVs
201 would separate the two populations (WT and non-WT) that are present in the MIC distribution of

a species and agent combination. Although they would not differentiate between susceptible (treatable) and resistant (non-treatable) isolates as BPs do, our proposed ECVs can help to identify those isolates that are more likely to harbor acquired molecular mutations conferring microbial resistance (non-WT isolates). This is an important addition in the absence of BPs for *Fusarium* spp.

Table 1 depicts the pooled MIC distributions for the four agents and *Fusarium* complexes/species evaluated. In general the MIC distributions were typical for each antifungal agent and species, where 2 to 5 two-fold dilution concentrations surround the modal MIC. The exceptions were itraconazole and some voriconazole distributions being skewed to the right. In addition, the distributions from each laboratory were comparable as their modal MICs for each species/species complex/agent combination were mostly within 1 two-fold dilution of one another with three exceptions. The amphotericin B mode for *F. oxysporum* SC was one dilution higher in one of the contributing laboratories (4 µg/ml versus 1 to 2 µg/ml in the other laboratories) while posaconazole and voriconazole modes were one dilution lower for *F. oxysporum* SC and *F. verticillioides* (1 µg/ml versus 2 to 4 µg/ml in the other laboratories) (data not shown in Tables 1 and 2). Most amphotericin B modes were 2 µg/ml, the exceptions were the lower modes for *F. dimerum* SC and *F. fujikuroi* and the higher mode for *F. incarnatum-equiseti* SC (Table 1). Among the triazoles, the highest values were observed when testing itraconazole (modes 8 to ≥16 µg/ml). Posaconazole and voriconazole modes ranged from 0.5 to 8 µg/ml and 2 to 8 µg/ml, respectively, with the lowest modes for *F. verticillioides* and the highest for both *F. solani* SC and *F. dimerum* SC. The MIC data for 2 to 11 (agent dependent) isolates of *F. falciforme* were similar to those of their *F. solani* SC with one exception, the eight posaconazole MICs for this species were >16 µg/ml; the same applied for the 4 to 8 isolates of the other three siblings of the *F. fujikuroi* SC (*F. sacchari, F. subglutinans*, and *F. thapsinum*) where all itraconazole MICs were >16 µg/ml instead (data not shown in Table 1). Although some of the distributions for the less prevalent species are small, these results underline the need for identification to the species or complex level in addition to antifungal susceptibility testing.

While the *in vitro* activities of the four antifungal agents evaluated are similar to those previously reported for *Fusarium* isolates (both CLSI and EUCAST MICs) (2,4,27-30), overall our MIC ranges are wider (Tables 1 and 2). In addition to our aggregated itraconazole data, this was more evident with amphotericin B MICs for both *F. proliferatum* and *F. oxysporum* SC as well as with voriconazole MICs for *F. solani* SC. However, the number of isolates for each pooled distribution was higher than those tested in prior studies (2,4,27-30) (15 to 22 isolates for

more prevalent species) and perhaps a better representation of the range of susceptibility to these agents. Nevertheless, the most frequent MIC (when provided) was similar to those in the present study. To our knowledge pooled MIC data are not available for the less prevalent species. Based on these data and the widespread geographical region from which our pooled MIC data originated, we assume that our data are valid.

As mentioned above, the CLSI has set forth criteria for the calculation of species-specific ECVs based on unmodified CLSI methodologies for MIC determination (≥100 isolates originating in at least three independent laboratories/species/agent) as well as the ECV percentage (the ≥97.5% values) to be calculated by the iterative statistical technique (CLSI documents on ECVs under development). Since ≥97.5% values risk classifying some isolates with acquired resistance mechanisms as WT, we have also provided the ≥95% and ≥99% ECVs. Values were either the same, or the ≥97.5/≥99% ECVs separated by one dilution. Table 2 depicts the ECVs for the aggregated MIC distributions that met the CLSI criteria: amphotericin B, itraconazole, posaconazole and voriconazole versus *F. oxysporum* SC, *F. solani* SC and *F. verticillioides*. Insufficient data precluded the calculation of ECVs for the combination of itraconazole and *F. verticillioides* and any other species. The ECVs of amphotericin B were 4 µg/ml (*F. verticillioides*) and 8 µg/ml (*F. oxysporum* SC and *F. solani* SC); these values are actually above what anecdotally is considered the notional "breakpoint" for resistance among some *Aspergillus* spp. (2 µg/ml). Similarly high ECVs were observed among *Aspergillus* spp., *Mucor circinelloides* and *Rhizopus arrhizus* (25,38). As expected, the highest ECVs were those of the three triazoles and *F. solani* SC (32 µg/ml). Lower posaconazole and voriconazole ECVs were calculated for *F. verticillioides* (2 and 4 µg/ml, respectively) and *F. oxysporum* SC (8 and 16 µg/ml, respectively). These triazole ECVs are mostly higher than their expected maximal, variable and dose dependent trough levels of each of the agents (22,23,39) and highlight the intrinsic resistant nature of *Fusarium* spp. The same applies to amphotericin B values.

Although case series of *Fusarium* infections have been reported throughout the years (4,6,8,15,19-21), an indication of the potential correlation between MICs for *Fusarium* spp. and response to treatment was only found in a recent report (21), where CLSI MICs for seven *Fusarium* isolates identified by molecular methods, antifungal therapy (voriconazole or both voriconazole and amphotericin B), and clinical response was documented for patients with invasive fusariosis. Favorable clinical responses were reported for two of the seven patients infected with *F. verticillioides* (voriconazole MICs of 2 and 4 µg/ml, respectively); according to our voriconazole ECV for this species, both infecting strains would be considered WT isolates (Table 2). Of the four patients infected with *F. solani*, the correlation was only evident in one

patient (favorable clinical response versus a voriconazole MIC of 4 µg/ml, or another WT strain). The remaining three patients failed therapy; two of them were treated with both voriconazole and amphotericin B (amphotericin B MICs, 4 µg/ml and voriconazole MICs, >8 µg/ml or >16 µg/ml). These amphotericin B MICs could be considered WT and both voriconazole MICs more likely non-WT, although the final MIC endpoint was not given (voriconazole ECV for *F. solani* SC, 32 µg/ml). However, it is important to keep in mind that categorization of an isolate as WT does not indicate that the isolate is susceptible (treatable), given that ECVs do not predict clinical response to therapy. Similarly, other factors preclude correlations of in vitro and clinical response to therapy in other studies, where cultures, species and especially MICs are not reported and the response was influenced by the site of infection, the underlying disease and/or the reversal of immunosuppression. In addition, the molecular mechanisms of resistance have not been evaluated in any *Fusarium* isolate causing human disease as it has been for *Candida* and *Aspergillus*. As found with the Mucorales, the molecular biology of *Fusarium* spp. resistance needs to be investigated.

In conclusion, species-specific amphotericin B ECVs comprising ≥97.5% of the modeled populations of 4 µg/ml (*F. verticillioides*) and 8 µg/ml (*F. oxysporum* SC and *F. solani* SC); posaconazole ECVs of 2 µg/ml (*F. verticillioides*), 8 µg/ml (*F. oxysporum* SC), and 32 µg/ml (*F. solani* SC); voriconazole ECVs of 4 µg/ml (*F. verticillioides*), 16 µg/ml (*F. oxysporum* SC) and 32 µg/ml (*F. solani* SC); and itraconazole ECVs of 32 µg/ml (*F. oxysporum* SC and *F. solani* SC) have been proposed based on CLSI data from multiple laboratories. ECVs were mostly one dilution lower using ≥95% of the modeled populations, which could be more clinically relevant. Similar to the ECVs for *Candida* spp. and *Aspergillus* spp., the proposed ECVs for the more prevalent *Fusarium* spp. may aid in the detection of strains with acquired mechanisms of resistance (non-WT) to the agents evaluated. However, ECVs are not BPs and cannot predict clinical response to therapy and categorization as WT would not mean that the isolate is treatable or susceptible. Also, as for the Mucorales, knowledge regarding molecular mechanisms of resistance and their relationship with MICs is needed.

Acknowledgments

We would like to thank M. Castanheira (JMI Laboratories, North Liberty, IA), V.R. de Azevedo Bastos (Universidade Federal de São Paulo, São Paulo, Brazil) and A. Fothergill (University of Texas Health Science Center, San Antonio, TX) for their collaboration.

Disclosures: None.

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Table 1. Pooled MIC distributions of amphotericin B and three triazoles for species of *Fusarium* from between 3 and 16 laboratories as determined by the CLSI broth microdilution method[a]

Agent	Species or species complex (SC)[b]	No. of labs	No. of isolates tested	No. of isolates with MIC (μg/ml) of:[c]							
				≤0.25	0.5	1	2	4	8	16	>16
Amphotericin B	*F. dimerum* SC[d]	8	50	3	7	**16**	13	5	5	1	
	F. fujikuroi[e]	3	10		1	**6**	3				
	F. proliferatum[e]	10	82	1	5	16	**31**	22	5	1	1
	F. verticillioides[e]	9	151		1	27	**84**	28	6	5	
	F. incarnatum-equiseti SC[f]	6	20		3	3	5	**6**	3		
	F. oxysporum SC[g]	14	226	1	10	37	**107**	61	8	2	
	F. solani SC[h]	15	608	8	46	120	**265**	125	29	15	
Itraconazole	*F. dimerum* SC[d]	7	45			3	1		15	**25**	1
	F. fujikuroi[e]	3	10							1	**9**
	F. proliferatum[e]	10	60			1		4	14	**21**	20
	F. verticillioides[e]	7	96			2	4	5	27	**41**	17
	F. incarnatum-equiseti SC[f]	6	20			1	1	2	**8**	6	2
	F. oxysporum SC[g]	9	148			2	2	4	29	**87**	24
	F. solani SC[h]	11	338		2	1	7	5	90	**220**	13
Posaconazole	*F. dimerum* SC[d]	7	48		1	2	3	5	**25**	11	1
	F. fujikuroi[e]	3	10		2	3	**4**	1			
	F. proliferatum[e]	9	49			7	**16**	6	8	5	7
	F. verticillioides[e]	7	113	15	**43**	33	9	3			10
	F. incarnatum-equiseti SC[f]	6	19		3	2	5	**6**	2	1	
	F. oxysporum SC[g]	10	146		1	20	**53**	37	13	22	
	F. solani SC[h]	8	357			8	15	42	**163**	113	16
Voriconazole	*F. dimerum* SC[d]	7	53			3	9	15	**24**	2	
	F. fujikuroi[e]	3	10				2	**5**	1	2	
	F. proliferatum[e]	10	74			3	10	**29**	24	6	2
	F. verticillioides[e]	8	143		1	25	**70**	35	2	2	8
	F. incarnatum-equiseti SC[f]	6	20		1	2	5	**8**	3		1
	F. oxysporum SC[g]	13	200		5	10	36	**94**	47	5	3
	F. solani SC[h]	16	555		3	9	51	123	**243**	119	7

[a]MICs determined by the CLSI M38-A2 method (24).
[b]Species complexes or species as identified by molecular methods (1,4,9,32,33).
[c]The highest number in each row (showing the most frequently obtained MIC or the mode) in boldface.
[d]Refers to the *Fusarium dimerum* species complex.
[e]Members of the *Fusarium* (*Gibberella) fujikuroi* species complex.

[f]Refers to the *Fusarium incarnatum-equiseti* species complex, syn. *F. semisectum*.
[g]Refers to the *Fusarium oxysporum* species complex.
[h]Refers to the *Fusarium solani* species complex.

Table 2. Epidemiologic cutoff values (ECVs) of amphotericin B, itraconazole, posaconazole, and voriconazole for two clinically relevant *Fusarium* species complexes and *F. verticillioides* as determined by the CLSI broth microdilution method[a]

Species or Species complex	Antifungal agent[b]	MIC (µg/ml)	Calculated statistical ECV (µg/ml)[c]			
		Range	Mode[d]	≥ 95%	≥97.5%	≥99%
F. verticillioides	AMB	0.5-16	2	4	4	4
	ITR	1->16	16	ND	ND	ND
	POS	≤0.25->16	0.5	2	2	2
	VOR	0.5->16	2	4	4	8
F. oxysporum SC	AMB	≤0.25-16	2	4	8	8
	ITR	1- >16	16	32	32	32
	POS	0.5-16	2	8	8	8
	VOR	0.5->16	4	8	16	16
F. solani SC	AMB	≤0.25-16	2	4	8	8
	ITR	0.5- >16	16	16	32	32
	POS	1->16	8	32	32	32
	VOR	0.5->16	8	16	32	32

[a]ECVs were defined for pooled distributions for >100 isolates and from > 3 laboratories using CLSI document M38-A2 methodology (24,34).
[b]AMB, amphotericin B; ITR, itraconazole; POS, posaconazole; VOR, voriconazole.
[c]Calculated ECVs comprising >95%, >97.5% and >99% of the statistically modeled population. ND, not determined due to insufficient data.
[d]MIC most frequently obtained for each distribution.

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