













Method-Dependent Epidemiological Cutoff Values for Detection of Triazole Resistance in *Candida* and *Aspergillus* Species for the Sensititre YeastOne Colorimetric Broth and Etest Agar Diffusion Methods

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ABSTRACT Although the Sensititre Yeast-One (SYO) and Etest methods are widely utilized, interpretive criteria are not available for triazole susceptibility testing of *Candida* or *Aspergillus* species. We collected fluconazole, itraconazole, posaconazole, and voriconazole SYO and Etest MICs from 39 laboratories representing all continents for (method/agent-dependent) 11,171 *Candida albicans*, 215 *C. dubliniensis*, 4,418 *C. glabrata* species complex, 157 *C. guilliermondii* (*Meyerozyma guilliermondii*), 676 *C. krusei* (*Pichia kudriavzevii*), 298 *C. lusitaniae* (*Clavispora lusitaniae*), 911 *C. parapsilosis sensu stricto*, 3,691 *C. parapsilosis* species complex, 36 *C. metapsilosis*, 110 *C. orthopsilosis*, 1,854 *C. tropicalis*, 244 *Saccharomyces cerevisiae*, 1,409 *Aspergillus fumigatus*, 389 *A. flavus*, 130 *A. nidulans*, 233 *A. niger*, and 302 *A. terreus* complex isolates. SYO/Etest MICs for 282 confirmed non-wild-type (non-WT) isolates were included: *ERG11* (*C. albicans*), *ERG11* and *MRR1* (*C. parapsilosis*), *cyp51A* (*A. fumigatus*), and *CDR2* and *CDR1* overexpression (*C. albicans* and *C. glabrata*, respectively). Interlaboratory modal agreement was superior by SYO for yeast species and by the Etest for *Aspergillus* spp. Distributions fulfilling CLSI criteria for epidemiological cutoff value (ECV) definition were pooled, and we proposed SYO ECVs for *S. cerevisiae* and 9 yeast and 3 *Aspergillus* species and Etest ECVs for 5 yeast and 4 *Aspergillus* species. The posaconazole SYO ECV of 0.06 $\mu\text{g/ml}$ for *C. albicans* and the Etest itraconazole ECV of 2 $\mu\text{g/ml}$ for *A. fumigatus* were the best predictors of non-WT isolates. These findings support the need for method-dependent ECVs, as, overall, the SYO appears to perform better for susceptibility testing of yeast species and the Etest appears to perform better for susceptibility testing of *Aspergillus* spp. Further evaluations should be conducted with more *Candida* mutants.

KEYWORDS *Aspergillus* spp., *Candida glabrata*, *Candida albicans*, Etest MICs for fungal mutants, Etest method ECVs, SYO MICs for fungal mutants, SYO method ECVs, antifungal resistance, triazole ECVs

The triazoles (fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) are the current treatments for severe candidiasis and aspergillosis (e.g., as first-line or prophylactic, adjunctive, empirical, transition from another agent, or salvage therapies) (1–3). These fungal infections may cause elevated levels of morbidity and mortality among immunocompromised patients (3–5). The impact of azole resistance and its prevalence has been widely recognized, and various mechanisms of mutational resistance have been elucidated in the four most common species of *Candida*, especially in *Candida albicans*, and in *Aspergillus fumigatus* (6–10). In most *Candida* isolates, azole resistance (or unusually high or increased MICs) are mostly associated with two main molecular mechanisms, among others: an increase (overexpression) of the azole target azole sterol demethylase or alterations (amino acid substitutions) in either the gene *ERG11*, as the enzyme is encoded during the fungal ergosterol biosynthesis pathway, or the *MRR1* transcriptional regulator (6, 8, 9). However, in the case of *C. glabrata*, azole resistance has frequently been related to the overexpression or alteration of the *PDR1* gene, which regulates efflux pumps (7). On the other hand, the main

azole resistance mechanism in *A. fumigatus* is due to alterations of the *cyp51A* gene (10).

Azole susceptibility testing (yielding MICs) is recommended for all bloodstream and other clinically relevant *Candida* isolates (1). Although routine MIC determination for *Aspergillus* species isolates is not usually recommended during initial aspergillosis therapy, MICs have an important role in identifying potentially resistant isolates, e.g., isolates from patients failing therapy (2). There are several antifungal susceptibility methods for the determination of MICs for isolates of both *Candida* and *Aspergillus*, including the broth microdilution M27 and M38 reference methods by the Clinical and Laboratory Standards Institute (CLSI) (11, 12) and the Antifungal Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (13) (http://www.eucast.org/ast_of_fungi/). In addition, the colorimetric broth microdilution Sensititre Yeast-One (SYO; Trek Diagnostic System, Cleveland, OH) method as well as the agar diffusion Etest (bioMérieux, Marcy l'Etoile, France) method, among other commercial assays, are widely utilized for antifungal susceptibility testing in the clinical laboratory; these methods are more practical and less time-consuming for routine use (14–16).

The objective of earlier studies evaluating the performance of the SYO and Etest methods involved the comparison of azole MICs obtained by these methods with those obtained by the reference assays for prevalent species of *Candida* and *Aspergillus* (17–19). Some of those early studies also evaluated the agreement on the ranking of isolates within existent categorical endpoints with little attention to the critical issue of interlaboratory reproducibility. Recently, triazole MIC data for *A. fumigatus* and *C. glabrata* mutant strains obtained by these commercial methods have been reported (20–24). However, a lack of suitable clinical data has precluded the establishment of breakpoints (BPs) for the categorical interpretation of triazole MICs for either *Candida* or *Aspergillus* spp. by these two methods. Therefore, both assays rely on available CLSI BPs for *Candida* spp. as interpretive categories as well as for quality control (QC) (14, 16). The proposal of SYO/Etest epidemiological cutoff values (ECVs) for susceptibility testing of either *Candida* or *Aspergillus* isolates with amphotericin B or the echinocandins has revealed substantial method-dependent differences between some of those values, despite the regulatory requirement to show equivalence to the reference method before marketing (25, 26). Those results emphasize the need to establish method-dependent triazole ECVs for these two widely used commercial methods for testing the susceptibility of *Candida* and *Aspergillus* isolates to the triazoles in the clinical laboratory.

For the last 2 years, we have gathered available triazole MICs obtained by both the SYO and Etest assays for isolates of prevalent and nonprevalent yeast species (*C. albicans*, *C. dubliniensis*, *C. glabrata* species complex, *C. guilliermondii* [*Meyerozyma guilliermondii*], *C. krusei* [*Pichia kudriavzevii*], *C. lusitanae* [*Clavispora lusitanae*], and the *C. parapsilosis* species complex [including *C. parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. tropicalis*]), *Saccharomyces cerevisiae*, and five *Aspergillus* species complexes (*A. fumigatus* [including *A. fumigatus sensu stricto*], *A. flavus*, *A. nidulans*, *A. niger*, and *A. terreus*). Additional SYO MIC distributions for the less prevalent or common yeast species *C. famata* (*Debaryomyces hansenii*), *C. kefyr* (*Kluyveromyces marxianus*), and *C. metapsilosis* were also reported when they originated from at least three laboratories and had comparable modes. From here on we use the most common clinical names. These triazole MICs were submitted from 39 independent worldwide laboratories (method/agent/species dependent) in order (i) to define the MIC distributions obtained by each commercial susceptibility testing method/agent and species; (ii) to examine the suitability of these distributions for the ECV setting, including the evaluation of interlaboratory modal agreement; and (iii) to define ECVs for each species/agent/method that fulfilled the CLSI criteria for ECV definition (modal compatibility among the laboratories, at least 100 MICs for each species/method/agent that originated in ≥ 3 independent laboratories) using the iterative statistical method at the 97.5% cutoff value (27–29) or the second numerical derivative method when the putative wild-type (WT) mode was at the lowest concentration in the distribution (30).

Although the majority of the isolates evaluated were not assessed for mechanisms of resistance, we also collected MIC data for 282 known or confirmed mutants (non-wild type [non-WT]) obtained by both methods, as follows: SYO and Etest MICs for *C. albicans* (*ERG11*), SYO MICs for *C. parapsilosis* (*ERG11*, *MRR1*) mutants and/or strains with overexpression of the *CDR2* gene, SYO MICs for *C. glabrata* strains with overexpression of the *CDR1* gene, and SYO and Etest MICs for *A. fumigatus sensu stricto* strains harboring *cyp51A* mutations. These data were submitted mostly from European laboratories as well as from Argentina, Thailand, and South Africa and one published Etest study (20). SYO data for 58 *C. glabrata* *PDR* gene mutants also were submitted, but those data were not included due to the large modal variability for the nonmutants compared with the global modes.

RESULTS AND DISCUSSION

Antimicrobial susceptibility testing for clinical isolates is most useful when either method- and species-dependent BPs or ECVs are available for the isolate and agent evaluated. The BP categorizes the isolate as either susceptible or resistant, and the ECV categorizes the isolate as either wild type (WT; no detectable phenotypic resistance) or non-WT (more likely harboring resistance mechanisms) (27). Since ECVs are based solely on *in vitro* data (either MIC or minimal effective concentration [MEC] results), classification of an isolate as a presumptive WT cannot directly predict a successful therapeutic outcome. Classification of an isolate as a non-WT indicates that it could harbor acquired mechanisms of resistance to the agent being evaluated and would less likely respond to contemporary therapy (27). However, the putative mechanism of resistance would not necessarily be known in order to categorize a strain as non-WT. CLSI BPs are based on *in vitro* and clinical data, genetic mechanisms of resistance, as well as pharmacokinetic/pharmacodynamics (PK/PD) parameters (27, 28). EUCAST ECVs and BPs are based on MIC distributions and PK/PD parameters (http://www.eucast.org/ast_of_fungi/). Therefore, when the BP is available for the isolate and agent being evaluated, that is the value that should be used. To our knowledge, method-dependent SYO or Etest ECVs or BPs for the four triazoles evaluated have not been proposed for the categorization of *Candida* or *Aspergillus* isolates. Our ECVs were defined following the criteria recently published by the CLSI (27). They were based on either SYO or Etest triazole MIC distributions that originated from 3 to 30 (SYO) or 3 to 11 (Etest) laboratories (species and agent dependent) (Tables 1 to 4) (27). As mentioned before, SYO MICs were submitted from multiple laboratories for the following mutants: 59 *C. albicans* *ERG11* (4 laboratories) and 39 *A. fumigatus sensu stricto* *cyp51A* (5 laboratories) mutants. Etest MICs were submitted from multiple laboratories for 81 *A. fumigatus* *cyp51A* (7 laboratories and one published study [20]) mutants (Tables 1, 2, and 5). SYO MICs were received from single laboratories for the following mutants: 13 *C. glabrata* and 2 *C. albicans* mutants with overexpression of the *CDR1* and *CDR2* genes, respectively, and 78 *C. parapsilosis* mutants (49 *ERG11* mutants and 29 *MRR1* mutants). Etest MICs were received from single laboratories for 10 *C. albicans* (*ERG11*) mutants (not listed in Table 1, 2, or 5). The MICs for these confirmed mutants provided a preliminary assessment of the utility of our proposed ECVs in recognizing the non-WT strains. Therefore, since BPs are not available for these commercial methods, the ECVs proposed in the present study could help the clinician and laboratory personnel in identifying isolates with possible acquired resistance mechanisms or could be useful for surveillance or epidemiological studies.

Although SYO MICs for the species evaluated originated from 30 of the 39 participant centers, exclusions were made according to the CLSI criteria for ECV definition (Table 1) (27). During data consolidation, individual SYO MIC distributions for *Candida* and *Aspergillus* were not included in the ECV analysis when they were bimodal, when the particular mode for a distribution was more than 1 to 2 dilutions from the global mode, when there were less than five isolates in the distribution or when the modes were aberrant or not defined. MIC distributions were also excluded when the MIC data for the QC isolates were outside the recommended range (14, 16). The total number of

TABLE 1 SYO pooled triazole MIC distributions for species of *Candida*, *Saccharomyces*, and *Aspergillus*^a

Agent and species ^b	No. of isolates	No. of labs used/ total no. ^c	No. of isolates with MIC (μg/ml) of ^d :											
			0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
Fluconazole														
<i>C. albicans</i>	11,171	28/30	12	1,016	4,252	4,152	978	238	122	82	78	33	49	159
Confirmed <i>ERG11</i> mutants	59	4/4		3	1	4	3		1	2	2		43	
<i>C. dubliniensis</i>	195	7/10	48	64	57	13	6	3	2			1	1	
<i>C. famata</i>	23	3/6		1	2	11	5	3	1					
<i>C. glabrata</i>	4,418	30/30	13	9	23	64	152	375	1,049	1,330	691	216	496	
<i>C. guilliermondii</i>	153	8/13	2	1	6	20	36	46	19	10	6	4	3	
<i>C. kefyr</i>	55	3/4	13	25	15	2								
<i>C. krusei</i>	537	15/16	1	1	1			3	8	43	193	220	67	
<i>C. lusitaniae</i>	298	12/12	16	41	75	99	43	12	5	1	4	1	1	
<i>C. parapsilosis</i>	3,691	28/30	89	502	1,210	958	421	221	151	82	27	19	11	
<i>C. parapsilosis sensu stricto</i>	911	5/5	18	118	282	216	121	74	53	19	6	4		
<i>C. metapsilosis</i>	36	4/4		1	2	17	10	5		1				
<i>C. orthopsilosis</i>	110	5/5	3	4	29	43	15	8	4	2		1	1	
<i>C. tropicalis</i>	1,854	24/28	20	82	270	701	482	129	53	19	24	14	60	
<i>S. cerevisiae</i>	244	3/3	4	3	9	40	70	76	26	10	4	2		
Itraconazole														
<i>C. albicans</i>	7,843	27/30	69	995	2,696	2,754	905	164	77	27	14	7	9	126
Confirmed <i>ERG11</i> mutants	59	4/4			2	2	2	5	11	3	7	4	23	
<i>C. dubliniensis</i>	125	6/8	13	21	47	27	7	2	5	1			2	
<i>C. famata</i>	18	3/5	1	1	2	3	7	3	1					
<i>C. glabrata</i>	3,594	29/30	12	19	42	112	428	1,335	910	195	71	26	444	
<i>C. guilliermondii</i>	149	9/13	3		8	31	55	37	10	2			3	
<i>C. kefyr</i>	45	3/3	5	10	17	12	1							
<i>C. krusei</i>	574	13/16	4	3	14	69	283	156	33	2	1		9	
<i>C. lusitaniae</i>	171	8/11	11	12	52	60	28	7	1					
<i>C. parapsilosis</i>	3,353	23/30	209	570	1,098	1,150	252	59	13	1	1			
<i>C. parapsilosis sensu stricto</i>	730	4/5	68	79	237	254	83	6	3					
<i>C. metapsilosis</i>	32	4/4	3	3	12	11	1	2						
<i>C. orthopsilosis</i>	88	3/4	2	13	35	26	12							
<i>C. tropicalis</i>	1,399	23/29	14	51	138	513	508	126	16	4	1	5	23	
<i>S. cerevisiae</i>	41	3/3	1	1	3	21	11	2	2					
<i>A. niger</i>	233	6/7	18	23	48	69	44	17	6			1	7	
Posaconazole														
<i>C. albicans</i>	6,729	27/30	596	2,768	2,318	587	175	96	56	32	10	3	60	28
Confirmed <i>ERG11</i> mutants	59		1	3	1	4	9	8	7	7	1	1	24	
<i>C. dubliniensis</i>	185	7/8	35	56	63	25	4		2					
<i>C. glabrata</i>	2,999	25/29	4	5	28	39	50	153	590	1,145	579	62	251	93
<i>C. guilliermondii</i>	111	9/12	3	1	9	15	27	35	18	3				
<i>C. kefyr</i>	40	3/3			7	13	13	5	2					
<i>C. krusei</i>	562	13/15	1	1	3	20	90	264	151	25	5		2	
<i>C. lusitaniae</i>	172	11/11	17	49	58	36	10		1	1				
<i>C. parapsilosis</i>	3,085	26/30	136	538	1,091	915	297	86	11	7	1	1		2
<i>C. parapsilosis sensu stricto</i>	670	5/5	40	127	206	193	69	31	2				2	
<i>C. metapsilosis</i>	17	3/4	3	7	4	3								
<i>C. orthopsilosis</i>	30	3/4	5	14	7	4								
<i>C. tropicalis</i>	1,366	23/29	16	50	107	250	408	336	147	22	6		17	7
<i>S. cerevisiae</i>	41	3/3			3	6	20	9	2	1				
Voriconazole														
<i>C. albicans</i>	8,747	29/30	5,947	1,691	481	222	111	82	47	22	10	15	76	43
Confirmed <i>ERG11</i> mutants	59	4/4	3	4	4	1	4	1	7	6	3	5	2	19
<i>C. dubliniensis</i>	215	7/9	182	21	5	3	1	2			1			
<i>C. famata</i>	25	3/5	5	10	4	2	2	2						
<i>C. glabrata</i>	3,255	24/30	23	29	65	189	486	911	824	340	136	156	82	14
<i>C. guilliermondii</i>	157	11/12	8	10	32	46	34	10	11	4	1		1	
<i>C. kefyr</i>	55	3/3	46	8	1									
<i>C. krusei</i>	676	14/16	2	1	1	16	108	291	199	42	11	3	1	1
<i>C. lusitaniae</i>	248	11/12	120	70	32	15	4	1	2	4				
<i>C. parapsilosis</i>	2,670	26/30	1,213	695	364	210	103	50	16	10	9			

(Continued on next page)

TABLE 1 (Continued)

Agent and species ^b	No. of isolates	No. of labs used/ total no. ^c	No. of isolates with MIC ($\mu\text{g/ml}$) of ^d :											
			0.008	0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥ 16
Voriconazole			0.008	0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥ 16
<i>C. parapsilosis sensu stricto</i>	718	5/5	261	185	122	80	47	12	5	4	2			
<i>C. metapsilosis</i>	30	3/4	2	10	11	4	2	1						
<i>C. orthopsilosis</i>	20	3/4	1	8	3	6	2							
<i>C. tropicalis</i>	1,637	19/28	45	92	227	466	443	200	70	25	20	9	23	17
<i>S. cerevisiae</i>	41	3/3	1	3	17	15	2	2	1					
<i>A. fumigatus</i>	903	8/8	2	7	35	64	157	396	179	33	8	7	7	8
Confirmed <i>cyp51A</i> mutants	39	5/5					3	4	8	3	4	5	2	10
<i>A. flavus</i>	389	6/7	5	1	14	32	89	139	59	29	16	1	0	4
<i>A. niger</i>	74	3/6				1	9	19	33	12				
<i>A. terreus</i>	302	5/6	6	5	16	19	48	122	69	15	2			

^aIncluding the complexes of *C. glabrata*, *C. parapsilosis*, and *Aspergillus* spp. (the *cyp51A* mutants are *A. fumigatus sensu stricto*).

^bNewly accepted taxonomic names or reclassifications are as follows: *C. famata* (*D. hansenii*), *C. guilliermondii* (*M. guilliermondii*), *C. kefyri* (*K. marxianus*), *C. krusei* (*P. kudriavzevii*), and *Candida lusitanae* (*Clavispora lusitanae*).

^cTotal number of laboratories included in the ECV definition pool/total number of laboratories that submitted data.

^dData are from between 3 and 30 laboratories and were determined by the colorimetric broth microdilution SYO method (14); the highest number in each row (showing the most frequent, or mode, MIC) is in bold.

isolates for which SYO MICs for the 12 *Candida* species and the four triazoles from 3 to 30 independent laboratories were pooled for ECV definition ranged from 11,171 to 17, with data points for *C. parapsilosis sensu stricto*, *C. parapsilosis* species complex, *C. metapsilosis*, *C. orthopsilosis*, *C. famata*, *C. kefyri*, and *S. cerevisiae* being included. The SYO MIC distributions for the 59 *C. albicans* and 39 *A. fumigatus sensu stricto* mutants from multiple laboratories are also listed in Table 1. In the case of SYO data for *Aspergillus* spp., interlaboratory modal consensus was an overall issue given that, of the submitted data for five species, ECVs were proposed only for voriconazole (3 of 4 species) and itraconazole (*A. niger*) (Tables 1 and 3). Of the 903 *A. fumigatus* listed in Table 1, 71% (640 data points) were identified as *A. fumigatus sensu stricto* and 29% (263 data points) were identified as *A. fumigatus* species complex (identification by morphological methods, matrix-assisted laser desorption ionization–time of flight [MALDI-TOF] mass spectrometry, or molecular methods [e.g., β -tubulin and calmodulin sequencing]) (31). *Candida* isolates also were identified to the species level by biochemical tests, MALDI-TOF mass spectrometry, and/or molecular methods in the laboratories submitting the data (31, 32); *C. parapsilosis* and *C. glabrata* were submitted mainly as species complex isolates (Table 1).

Table 1 also depicts the SYO modes for *Candida* and *Aspergillus* species. The lowest SYO fluconazole modes (0.25 $\mu\text{g/ml}$) were for *C. albicans*, *C. dubliniensis*, and *C. kefyri*, and the highest mode was for *C. krusei* (64 $\mu\text{g/ml}$). A similar modal diversity was noted among posaconazole MICs (modes, 0.01 $\mu\text{g/ml}$ for *C. albicans* and 1 $\mu\text{g/ml}$ for *C. glabrata*). However, itraconazole and voriconazole modes were mostly 0.06 $\mu\text{g/ml}$ to 0.12 $\mu\text{g/ml}$ and 0.008 to 0.03 $\mu\text{g/ml}$, respectively. The exceptions were itraconazole modes for *C. glabrata* (0.5 $\mu\text{g/ml}$) and *C. guilliermondii* and *C. krusei* (0.25 $\mu\text{g/ml}$) and voriconazole modes for *C. guilliermondii* and *C. tropicalis* (0.06 $\mu\text{g/ml}$) and *C. glabrata* and *C. krusei* (0.25 $\mu\text{g/ml}$). Most SYO modes for the *C. parapsilosis* complex were ± 1 doubling dilution, but all posaconazole modes for the four species in the complex were 0.03 $\mu\text{g/ml}$. SYO voriconazole modes for *Aspergillus* spp. and the itraconazole mode for *A. niger* ranged from 0.12 to 0.5 $\mu\text{g/ml}$. As expected, SYO modes for the *C. albicans* and *A. fumigatus* mutants were much higher than those for the nonmutant isolates, and we observed an overlap between both groups of MICs among the lower drug concentrations (Table 1). Therefore, the SYO data for *Candida* spp. showed excellent modal agreement, while most SYO data points for *Aspergillus* spp. were unsuitable for the ECV definition pool, as previously reported among SYO posaconazole data for *A. fumigatus* (23).

Eleven of the 39 laboratories contributed Etest MICs for the four more prevalent

TABLE 2 Etest triazole pooled MIC distributions for species of *Candida* and *Aspergillus*^a

Agent and species ^b	No. of isolates	No. of labs used/ total no. ^c	No. of isolates with MIC (μg/ml) of: ^d												
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
Fluconazole			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
<i>C. glabrata</i>	356	7/10			1	4	13	34	50	79	88	36	13	28	10
<i>C. parapsilosis</i>	639	9/9	3	19	68	131	153	138	66	21	9	7	20		4
<i>C. tropicalis</i>	368	9/10	3	5	11	61	96	120	47	11	4	1	4	4	1
Itraconazole			≤0.004	0.008	0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
<i>C. albicans</i>	975	8/9	7	55	145	237	295	150	27	19	16	6	7	5	6
<i>C. krusei</i>	101	3/3				1		2	9	36	35	7	7	1	3
<i>C. tropicalis</i>	165	5/8	2	12	23	39	30	22	15	11	7	2	1	1	
<i>A. fumigatus</i>	1,112	10/10	4	1	3	30	56	157	483	268	73	12	5	20	
Confirmed <i>cyp51A</i> mutants	81	8/8				1			1		1	7	4	67	
<i>A. flavus</i>	250	7/8		1	4	19	37	103	69	16	1				
<i>A. nidulans</i>	130	4/4		1	1	13	39	34	23	11	7			1	
<i>A. niger</i>	176	4/5			1	1	2	5	25	71	45	17	5	4	
Posaconazole			≤0.004	0.008	0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
<i>C. albicans</i>	305	4/6	6	29	94	102	44	17	9	3	1				
<i>C. krusei</i>	48	3/3					1	5	17	16	7				2
<i>C. parapsilosis</i>	162	4/5	8	26	51	37	23	9	3	2			1		2
<i>C. tropicalis</i>	101	4/5		9	21	32	21	4	8	3	1		1	1	
<i>A. flavus</i>	204	7/7		1	4	14	70	96	17	2					
<i>A. niger</i>	168	4/5			5	16	58	73	15	1					
<i>A. terreus</i>	194	5/5			8	47	105	27	4	2			1		
Voriconazole			≤0.004	0.008	0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
<i>C. albicans</i>	2,159	8/11	485	803	491	158	104	42	22	20	11	5	7	1	10
<i>C. glabrata</i>	551	8/9		7	11	20	37	105	143	96	63	30	15	14	10
<i>C. krusei</i>	130	6/6			1	2	3	20	30	45	24	5		2	
<i>C. parapsilosis</i>	506	7/9	4	20	46	97	167	100	39	15	4	4	8	1	1
<i>C. tropicalis</i>	260	6/10	4	4	12	28	82	88	22	14	5			1	
<i>A. fumigatus</i>	1,409	11/11	1	6	2	30	132	633	473	100	19	7	3	2	1
Confirmed <i>cyp51A</i> mutants	75	8/8					5	6	7	8	13	15	3	1	17
<i>A. flavus</i>	257	7/7			1		18	84	103	39	10	1			1
<i>A. niger</i>	173	4/5		2	3	22	37	81	25	1	1				1

^aIncluding the complexes of *C. parapsilosis*, *C. glabrata*, and *Aspergillus* species (the *cyp51A* mutants are *A. fumigatus sensu stricto*).

^bNewly accepted taxonomic name or reclassification: *C. krusei* (*P. kudriavzevii*).

^cTotal number of laboratories included in the ECV definition pool/total number of study laboratories and one published study (20) that submitted data.

^dData are from between 3 and 11 laboratories and were determined by the agar diffusion Etest method (15); the highest number in each row (showing the most frequent, or mode, MIC) is in bold.

Candida spp., *C. krusei*, and *Aspergillus* spp. Eight laboratories, as well as a published study (20), contributed Etest itraconazole and itraconazole data for the 75 and 81 *A. fumigatus sensu stricto* mutants, respectively (Tables 2 and 5). A total of 64% (712 of the 1,112 itraconazole MICs) of the *A. fumigatus* isolates and most *Candida* isolates were identified at the species level (31, 32), but *C. glabrata* and *C. parapsilosis* were mainly identified as the species complex. Therefore, we were unable to provide the potential antifungal susceptibility differences among the species in the *C. parapsilosis* species complex by the Etest like we did by the SYO method (Table 1). The modal variability among the Etest MIC distributions entering the ECV definition data pool also precluded our ECV definition for *C. albicans* and fluconazole, *C. glabrata* and both itraconazole and posaconazole, *C. parapsilosis* and itraconazole, and *C. krusei* and fluconazole. However, most Etest data points for the *Aspergillus*/agent combinations were suitable for the ECV definition pool, although we observed modal discrepancies for itraconazole and voriconazole versus *A. terreus*. Consequently, we collected more suitable Etest data for *Aspergillus* spp., while the overall SYO data for *Candida* spp. were superior. The lowest Etest modes were for *C. parapsilosis* versus fluconazole and posaconazole (0.5 and

0.01 $\mu\text{g/ml}$, respectively), *C. tropicalis* versus itraconazole (0.03 $\mu\text{g/ml}$), and *C. albicans* versus voriconazole (0.008 $\mu\text{g/ml}$). All Etest modal values for *Aspergillus* spp. ranged from 0.12 to 0.25 $\mu\text{g/ml}$, except for the itraconazole modes for *A. fumigatus* (0.5 $\mu\text{g/ml}$) and for *A. niger* (1 $\mu\text{g/ml}$). Etest modes for the *A. fumigatus* mutants were also much higher than those for the nonmutant isolates.

Tables 3 and 4 depict the proposed ECOFFinder program SYO and Etest triazole ECVs, respectively, for 97.5% of the modeled MIC population for the species and triazole combinations that fulfilled the CLSI criteria for ECV calculation (27). There was no need to weigh the data since none of the individual distributions contributed $\geq 50\%$ of the total. In addition to SYO ECVs for the prevalent *Candida* spp., fluconazole ECVs were proposed for *C. orthopsilosis* (4 $\mu\text{g/ml}$) and *S. cerevisiae* (16 $\mu\text{g/ml}$) (Table 3). Although fluconazole ECVs for *C. parapsilosis sensu stricto* and species complex were the same (2 $\mu\text{g/ml}$), the other ECVs for *C. parapsilosis sensu stricto* were 1 dilution higher. To our knowledge, ECVs for *C. parapsilosis sensu stricto* or any other member of this complex and for *S. cerevisiae* are not yet available for the reference methods (26, 28; http://www.eucast.org/ast_of_fungi/). Due to aberrant modes by the Etest, we only defined voriconazole Etest ECVs of 0.03 to 2 $\mu\text{g/ml}$ for five *Candida* spp. and ECVs of 0.12 to 64 $\mu\text{g/ml}$ for the other three agents and three to four species (Table 4). However, we proposed ECVs for three to four relevant *Aspergillus* spp. (2, 4, 5). Inconsistent itraconazole and voriconazole modes for *A. terreus* from four laboratories as well as insufficient posaconazole and voriconazole MICs for *A. nidulans* (data were submitted from only two laboratories) precluded definition of an ECV for these two species/agents (27) (data not shown in Table 2). In Table 6, we compared our SYO and Etest ECVs with the approved CLSI ECVs listed in the new edition of the M59 document (26). In general, SYO ECVs were 1 to 2 dilutions higher than those for the CLSI or Etest methods. In some instances, such as for fluconazole and voriconazole versus *C. glabrata*, among others, SYO and CLSI ECVs of 64 and 8 $\mu\text{g/ml}$ and 2 and 0.25 $\mu\text{g/ml}$, respectively, have been defined (26). All these observations underscore the need for method-dependent ECVs in order to properly categorize the MIC for the infecting isolate being evaluated as either WT or non-WT. It also demonstrates that while commercial systems can successfully establish equivalence according to FDA criteria, the pooling of data from multiple laboratories can more easily detect differences between these assays and the reference method, at least in what is measured as the wild type.

As mentioned above, the main role of the ECV is to identify the strains that could harbor intrinsic or acquired resistance mechanisms (non-WT or mutant isolates) (27, 28). CLSI MICs for *Candida* and *Aspergillus* mutants are readily available in the literature (6–10, 33–36), but they are scarce by the commercial methods (20–24). A total of 162 SYO and Etest MICs for *C. albicans*, *C. glabrata*, and *C. parapsilosis* mutants were received. The number of SYO MICs above the ECVs of the four triazoles for the 59 *ERG11* *C. albicans* mutants was agent dependent. The posaconazole ECV of 0.06 $\mu\text{g/ml}$ recognized the highest percentage of mutants (55/59; 93%), followed by the itraconazole ECV of 0.12 $\mu\text{g/ml}$ (53/59; 90%), the voriconazole ECV of 0.01 $\mu\text{g/ml}$ (52/59; 88%), and the fluconazole SYO ECV of 1 $\mu\text{g/ml}$ (48/59; 81%). These *C. albicans* mutants had the following *ERG11* substitutions: F145L, Y132H, S442F, S405F, G464S, A114S, G464S, F145T, T220L, and P98A (alone or in combination). Although high CLSI triazole MICs have been documented for most of those substitutions (6, 8, 33–35), T220L and P98A (alone or in different combinations with E266D, G448R, V437I, V488I, K143R, and Y132H/X) have not been previously reported. Considering their high MICs of >8 $\mu\text{g/ml}$ (Table 1), it seems that these strains could also harbor combined resistance mechanisms (e.g., the most common efflux pump overexpression plus *erg11* overexpression and/or an *erg11* mutation). These molecular combinations are due to aneuploidy (duplication of chromosome 5 or multiplication of its long arm). However, we did not receive efflux pump overexpression data for the 59 *C. albicans* mutants. On the other hand, in Table 5 we list the *C. albicans* and *A. fumigatus* mutants that, according to our method-dependent ECVs, could be categorized as either WT (for which MICs are less than or equal to each ECV) and/or non-WT (for which MICs are greater than the ECV).

TABLE 3 Method-dependent SYO ECOFFinder ECVs of four triazoles for species of *Candida*, *Saccharomyces*, and *Aspergillus*^a

Agent and species ^b	No. of isolates	No. of labs used/total no.	MIC ($\mu\text{g/ml}$)		ECV ($\mu\text{g/ml}$) ^c
			Range	Mode	
Fluconazole					
<i>C. albicans</i>	11,171	28/30	0.06 to ≥ 128	0.25	1
<i>C. dubliniensis</i>	195	7/10	0.12 to 64	0.25	1
<i>C. glabrata</i>	4,418	30/30	0.12 to ≥ 128	16	64
<i>C. guilliermondii</i>	153	8/13	0.12 to ≥ 128	4	16
<i>C. krusei</i>	537	15/16	0.12 to ≥ 128	64	128
<i>C. lusitaniae</i>	298	12/12	0.12 to ≥ 128	1	4
<i>C. parapsilosis</i>	3,691	28/30	0.12 to ≥ 128	0.5	2
<i>C. parapsilosis sensu stricto</i>	911	5/5	0.12 to 64	0.5	2
<i>C. orthopsilosis</i>	110	5/5	0.12 to ≥ 128	1	4
<i>C. tropicalis</i>	1,854	24/28	0.12 to ≥ 128	1	4
<i>S. cerevisiae</i>	244	3/3	0.12 to 64	4	16
Itraconazole					
<i>C. albicans</i>	7,843	27/30	0.008 to ≥ 16	0.06	0.12
<i>C. dubliniensis</i>	125	6/8	0.01 to ≥ 16	0.06	0.25
<i>C. glabrata</i>	3,594	29/30	0.01 to ≥ 16	0.5	2
<i>C. guilliermondii</i>	149	9/13	0.01 to ≥ 16	0.25	1
<i>C. krusei</i>	574	13/16	0.01 to ≥ 16	0.25	1
<i>C. lusitaniae</i>	171	8/11	0.01 to 1	0.12	0.5
<i>C. parapsilosis</i>	3,353	23/30	0.01 to 4	0.12	0.25
<i>C. parapsilosis sensu stricto</i>	730	4/5	0.01 to 1	0.12	0.5
<i>C. tropicalis</i>	1,399	23/29	0.01 to ≥ 16	0.12	0.5
<i>A. niger</i>	233	6/7	0.01 to ≥ 16	0.12	1
Posaconazole					
<i>C. albicans</i>	6,729	27/30	0.008 to ≥ 16	0.01	0.06
<i>C. dubliniensis</i>	185	7/8	0.008 to 1	0.03	0.12
<i>C. glabrata</i>	2,999	25/29	0.008 to ≥ 16	1	4
<i>C. guilliermondii</i>	111	9/12	0.008 to 1	0.25	1
<i>C. krusei</i>	562	13/15	0.008 to 8	0.25	1
<i>C. lusitaniae</i>	172	11/11	0.008 to 1	0.03	0.12
<i>C. parapsilosis</i>	3,085	26/30	0.008 to ≥ 16	0.03	0.12
<i>C. parapsilosis sensu stricto</i>	670	5/5	0.008 to 8	0.03	0.25
<i>C. tropicalis</i>	1,366	23/29	0.008 to ≥ 16	0.12	1
Voriconazole					
<i>C. albicans</i>	8,747	29/30	0.008 to ≥ 16	0.008	0.01 ^d
<i>C. dubliniensis</i>	215	7/9	0.008 to 2	0.008	0.01 ^d
<i>C. glabrata</i>	3,255	24/30	0.008 to ≥ 16	0.25	2
<i>C. guilliermondii</i>	157	11/12	0.008 to ≥ 16	0.06	0.5
<i>C. krusei</i>	676	14/16	0.008 to ≥ 16	0.25	1
<i>C. lusitaniae</i>	248	11/12	0.008 to 1	0.008	0.03 ^d
<i>C. parapsilosis</i>	2670	26/30	0.008 to 2	0.008	0.01 ^d
<i>C. parapsilosis sensu stricto</i>	718	5/5	0.008 to 2	0.008	0.03 ^d
<i>C. tropicalis</i>	1,637	19/28	0.008 to ≥ 16	0.06	0.5
<i>A. fumigatus</i>	903	8/8	0.008 to ≥ 16	0.25	1
<i>A. flavus</i>	389	6/7	0.008 to ≥ 16	0.25	1
<i>A. terreus</i>	302	5/6	0.008 to 2	0.25	1

^aIncluding the complexes of *C. parapsilosis*, *C. glabrata*, and *Aspergillus* species. Modal variability or insufficient data precluded the proposal of ECVs for some species of both *Candida* and *Aspergillus*.

^bNewly accepted taxonomic names or reclassifications are as follows: *C. guilliermondii* (*M. guilliermondii*), *C. krusei* (*P. kudriavzevii*), and *Candida lusitaniae* (*Clavispora lusitaniae*).

^cECOFFinder ECVs for 97.5% of the statistically modeled population based on MICs obtained by the colorimetric broth microdilution SYO method (14, 29), except where indicated by a superscript c, referring to footnote c. The proposed method-dependent SYO ECV for *A. fumigatus* and posaconazole is 0.06 $\mu\text{g/ml}$, as reported elsewhere (23). *C. krusei* is intrinsically resistant to fluconazole regardless of the MIC.

^dECV as estimated using the second derivative method (30).

Isolates with those substitutions have been reported as both susceptible and resistant using CLSI methodologies and BPs (8, 33–35). Regarding data from the single laboratories, the SYO MICs of the four agents for the 2 *C. albicans* strains and 11 of the 13 *C. glabrata* strains from single laboratories with overexpression of the *CDR2* and *CDR1*

TABLE 4 Method-dependent Etest ECOFFinder ECVs of four triazoles for species of *Candida* and *Aspergillus*^a

Agent and species ^b	No. of isolates	No. of labs used/total no. ^c	MIC ($\mu\text{g/ml}$)		ECV ($\mu\text{g/ml}$) ^d
			Range	Mode	
Fluconazole					
<i>C. glabrata</i>	356	7/10	0.12 to ≥ 128	8	64
<i>C. parapsilosis</i>	639	9/9	0.03 to ≥ 128	0.5	4
<i>C. tropicalis</i>	368	9/10	0.03 to ≥ 128	1	4
Itraconazole					
<i>C. albicans</i>	975	8/9	≤ 0.004 to ≥ 16	0.06	0.25
<i>C. krusei</i>	101	3/3	0.03 to ≥ 16	0.5	2
<i>C. tropicalis</i>	165	5/8	≤ 0.004 to 8	0.03	0.5
<i>A. fumigatus</i>	1,112	10/10	0.008 to ≥ 16	0.5	2
<i>A. flavus</i>	250	7/8	0.01 to 2	0.25	1
<i>A. nidulans</i>	130	4/4	0.01 to 8	0.12	1
<i>A. niger</i>	176	4/5	0.03 to ≥ 16	1	4
Posaconazole^e					
<i>C. albicans</i>	305	4/6	≤ 0.004 to 1	0.03	0.12
<i>C. parapsilosis</i>	162	4/5	≤ 0.004 to ≥ 16	0.01	0.12
<i>C. tropicalis</i>	101	4/5	0.008 to 8	0.03	0.12
<i>A. flavus</i>	204	7/7	0.01 to 1	0.25	0.5
<i>A. niger</i>	168	4/5	0.03 to 1	0.25	0.5
<i>A. terreus</i>	194	5/5	0.03 to 4	0.12	0.25
Voriconazole					
<i>C. albicans</i>	2,159	8/11	≤ 0.004 to ≥ 16	0.008	0.03
<i>C. glabrata</i>	551	8/9	0.008 to ≥ 16	0.25	2
<i>C. krusei</i>	130	6/6	0.01 to 8	0.5	2
<i>C. parapsilosis</i>	506	7/9	≤ 0.004 to ≥ 16	0.06	0.25
<i>C. tropicalis</i>	260	6/10	≤ 0.004 to 8	0.12	0.5
<i>A. fumigatus</i>	1,409	11/11	≤ 0.004 to ≥ 16	0.12	0.5
<i>A. flavus</i>	257	7/7	0.01 to ≥ 16	0.25	0.5
<i>A. niger</i>	173	4/5	0.01 to ≥ 16	0.25	1

^aIncluding the complexes of *C. parapsilosis*, *C. glabrata*, and *Aspergillus* spp. Variability or insufficient data precluded the proposal of ECVs for some species of both *Candida* and *Aspergillus*.

^bNewly accepted taxonomic name or reclassification: *C. krusei* (*P. kudriavzevii*).

^cTotal number of laboratories included in the ECV definition pool/total number of laboratories that submitted data (including data from one published study [20]).

^dECOFFinder ECVs for 97.5% of the statistically modeled population based on MICs obtained by the commercial agar diffusion Etest method (15, 29). The proposed method-dependent Etest ECV for *A. fumigatus* and posaconazole was 0.25 $\mu\text{g/ml}$, as reported elsewhere (23).

gene efflux pumps, respectively, were above the four ECVs (data not shown in Table 5). However, only the fluconazole and voriconazole ECVs (2 and 0.03 $\mu\text{g/ml}$, respectively) recognized >96% of the 78 *C. parapsilosis* mutants. Therefore, the potential ability of our SYO ECVs to recognize $\geq 90\%$ of the isolates with mechanisms of resistance among the most prevalent *Candida* spp. (*C. albicans*, *C. glabrata*) provided a preliminary indication of their clinical value. More data points for other *Candida* mutants would better assess the utility of the SYO method for yeast testing in the clinical laboratory.

In the present study, a total of 75 and 81 Etest MICs (for voriconazole and itraconazole, respectively) and 39 SYO MICs (for voriconazole) for *A. fumigatus sensu stricto cyp51A* mutants were evaluated. Our proposed Etest itraconazole ECV of 2 $\mu\text{g/ml}$ for *A. fumigatus* had performance in recognizing the *cyp51A* mutants (78/81; 96%) superior to that of the voriconazole Etest ECV of 0.5 $\mu\text{g/ml}$ (50/75; 67%) and the SYO ECV of 1 $\mu\text{g/ml}$ (26/39; 67%) (Table 5). Etest itraconazole MICs were above the ECV for isolates with the following mutations: 48 isolates with TR34/L98 (59%), 12 with G54 (15%), 9 with M220 (11%), 5 with G448S (6%), and 7 with miscellaneous mutations (9%), including 2 with TR46/Y121F mutations (data not listed in Table 5). However, *cyp51A* G54 changes have been linked in the literature with cross-resistance to both itraconazole and posaconazole, and the M220 change has been linked with either high or low triazole MICs (36). An overlap between posaconazole MICs for nonmutants and those

TABLE 5 Triazole SYO and Etest MICs for selected confirmed *C. albicans* ERG11 and *A. fumigatus sensu stricto* cyp51 mutants^a

Species, agent	Method and mutation	No. of mutants with MIC (µg/ml) of ^b :							Total no. of mutants with MIC less than or equal to ECV/total no. tested ^b
		0.12	0.25	0.5	1	2	4	≥8	
Fluconazole, <i>C. albicans</i>	SYO	0.12	0.25	0.5	1	2	4	≥8	
	E266D			1	2			7	3
	E266D/V4881				1			7	1
	V112I/G450R			1				3	1
	K128T			2				1	2
	D116E/K128T/V159I	3	1						4
									11/59
Itraconazole, <i>C. albicans</i>	SYO	≤0.06	0.12	0.25	0.5	1	2	≥8	
	E266D		1					7	2
	E266D/V4881		1					7	1
	V112I/G450R	1			4			1	1
	D116E/K128T/ V159I	3						1	3
Posaconazole, <i>C. albicans</i>	SYO	≤0.06	0.12	0.25	0.5	1	2	≥8	
	V112I/G450R	1			2	1			1
	D116E/K128T/ V159I	3						1	3
Voriconazole, <i>C. albicans</i>	SYO	≤0.01	0.03	0.06	0.12	0.25	0.5	≥1	
	E266D	1	1		2			6	1
	E266D/V4881	1						7	1
	K128T	1	1					1	1
	D116E/K128T	4						4	4
									7/59
Itraconazole, <i>A. fumigatus</i>	Etest	≤0.06	0.12	0.25	0.5	1	2	≥8	
	G448S				1			4	1
	M220K						1		1
	I301T	1						1	1
									3/81
Voriconazole, <i>A. fumigatus</i>	Etest	<0.06	0.12	0.2	0.5	1	2	≥4	
	TR34		1		2	14	11	10	3
	G54E/R/W	2	2	5	3				12
	M220I/K/R/T/V		3	1	4		2	1	8
	G138C			1					1
	I301T	1							1
									25/75
Voriconazole, <i>A. fumigatus</i>	SYO	<0.06	0.12	0.25	0.5	1	2	≥4	
	TR34					1			1
	G54E/R/W		2	1	2				5
	M220I/K/T/V			2	3				5
	G138C			1					1
	I301T	1							1
									13/39

^aListed are SYO and Etest MICs for *C. albicans* and *A. fumigatus* mutants that were either below or above (shaded and nonshaded, respectively) each correspondent ECV among the total data points for the 59 *C. albicans* mutants and 75, 81, or 39 *A. fumigatus* mutants. Data were submitted from multiple participant laboratories (4 to 8) and a single published study (20).

^bThe proposed SYO ECVs were as follows: for *C. albicans* versus fluconazole, 1 µg/ml; for *C. albicans* versus itraconazole, 0.12 µg/ml; for *C. albicans* versus posaconazole, 0.06 µg/ml; for *C. albicans* versus voriconazole, 0.01 µg/ml; and for *A. fumigatus* versus voriconazole, 1 µg/ml. Etest ECVs were as follows: for *A. fumigatus* versus itraconazole, 2 µg/ml; and for *A. fumigatus* versus voriconazole, 0.5 µg/ml.

for a much larger number of mutants of *A. fumigatus* by three antifungal susceptibility methods (CLSI, EUCAST and Etest) has also been reported (23). These preliminary results for *Aspergillus* spp. indicated that the Etest appears to be a superior method for detecting mutations in *A. fumigatus* as well as for testing other *Aspergillus* spp. Once again, these results underscore the need for method-dependent ECVs. As far as the SYO data for *Aspergillus* spp. are concerned, further collaborative studies should evaluate the endpoint determination; both color change and growth inhibition have been reported in the literature.

In conclusion, we proposed method-dependent SYO and Etest ECVs for various

species/triazole combinations for which suitable data were available from multiple laboratories (3 to 30). Substantial data with excellent interlaboratory modal agreement were evaluated by the SYO method for *Candida* and other yeast species, including MIC distributions for the *C. parapsilosis* complex (*C. parapsilosis sensu stricto*, *C. metapsilosis*, and *C. orthopsilosis*) and *S. cerevisiae*. Because of that, we proposed SYO ECVs for 8 to 10 yeast species and the four triazoles evaluated, including *C. orthopsilosis* and *S. cerevisiae* versus fluconazole. We also provided MIC ranges and, more importantly, modes for other less prevalent yeast species. On the other hand, interlaboratory modal agreement by the Etest was better for *Aspergillus* than for yeast species. As a result, we proposed Etest ECVs of itraconazole, posaconazole, and voriconazole for three to four *Aspergillus* spp. and voriconazole ECVs for the four most prevalent *Candida* spp. and *C. krusei*. Finally, the SYO posaconazole ECV of 0.06 $\mu\text{g/ml}$ for *C. albicans* and the Etest itraconazole ECV of 2 $\mu\text{g/ml}$ for *A. fumigatus* were the best predictors in recognizing the non-WT or mutants (the highest percentage of MICs for mutants that were above the ECV). Although ECVs of fluconazole and voriconazole for *C. parapsilosis* recognized >96% of the non-WT isolates, the results were unsatisfactory with posaconazole and itraconazole ECVs. Data for mutants of other *Candida* spp. would better assess the method-dependent proposed ECVs. The SYO method appears to yield more suitable MIC data for testing most *Candida* spp. and the Etest for *Aspergillus* spp.

MATERIALS AND METHODS

Isolates. The *Candida* and other yeast isolates evaluated were mostly recovered from blood and other normally sterile sites from patients with candidemia or other deep infections (>90%) as well as superficial, oral, and vaginal infections and thrush. The *Aspergillus* isolates were also recovered from deep infections and sterile and other sites (mostly [>90%] bronchoalveolar lavage fluid and sputum) at the following medical centers: VCU Medical Center, Richmond, VA, USA; Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; Unité de Parasitologie, Mycologie, Département de Bactériologie Virologie Hygiène Mycologie Parasitologie, Créteil, France; Grupo de Infección Grave, Instituto de Investigación Sanitaria La Fe, Valencia, Spain; Unidad de Gestión Clínica de Enfermedades Infecciosas y Microbiología, Hospital de Valme, Seville, Spain; Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan; Public Health Ontario, Toronto, ON, Canada; Klinisk Mikrobiologi, Karolinska, Universitetlaboratoriet, Karolinska, Universitetssjukhuset, Stockholm, Sweden; Université Paris-Descartes, Faculté de Médecine, APHP, Hôpital Européen Georges Pompidou, Unité de Parasitologie-Mycologie, Service de Microbiologie, Paris, France; Laboratorio de Micología y Diagnóstico Molecular, Cátedra de Parasitología y Micología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Santa Fe, Argentina; Universidad Autónoma de Nuevo León, Mexico; National Institute for Communicable Diseases (Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses), a Division of the National Health Laboratory Service and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Hospital General Universitario Gregorio Marañón, Madrid, Spain; SA Pathology, National Mycology Reference Centre, Adelaide, South Australia, Australia; Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria; Departamento de Microbiología, Facultad de Medicina y Enfermería, Universidad de Córdoba, Córdoba, Spain; Servicio de Microbiología, Hospital Universitario Cruces, Barakaldo, Spain; Servicio de Microbiología, Hospital Universitario Central de Asturias, Asturias, Spain; Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Enfermería, Universidad del País Vasco/Euskal Herriko Unibertsitatea, UPV/EHU, Bilbao, Spain; Departamento de Biomedicina, Biotecnología y Salud Pública, Universidad de Cádiz, Cadiz, Spain; Hospital de Alcañiz, Alcañiz (Teruel), Spain; Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; Institute of Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy; University of Pittsburgh, Pittsburgh, PA, USA; Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland; Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, University of Technology, Gdansk, Poland; Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy; Mayo Clinic, Rochester, MN, USA; Microbiology Laboratory, Ospedale San Gerardo, Monza, Italy; Microbiology and Virology Unit IRCCS Policlinico San Matteo, Pavia, Italy; Microbiology Section, Humanitas Research Hospital, Milan, Italy; Microbiology Laboratory, A.O. Spedali Civili, Brescia, Italy; Microbiology Laboratory, Fondazione IRCCS Cà Granda O. Maggiore Policlinico, Milan, Italy; Microbiology Laboratory, Niguarda Hospital, Milan, Italy; Clinical Microbiology Laboratory, Attikon Hospital, Medical School, National and Kapodistrian, University of Athens, Athens, Greece; Laboratório Especial de Micologia, Disciplina de Infectologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil; Microbiology Institute, ASST Papa Giovanni XXIII, Bergamo, Italy; Unidad de Micología, Servicio de Microbiología, Hospital Universitario La Fe, Valencia, Spain; Microbiology-ASST Lariana, Como, Italy; and Medicina di Laboratorio, IRCCS Policlinico San Donato, Milan, Italy.

TABLE 6 Method-dependent ECVs of four triazoles for species of *Candida*, *Saccharomyces*, and *Aspergillus* by three susceptibility testing methods^a

Species ^b	Method-dependent ECV (μg/ml) for the following agent:											
	Fluconazole			Itraconazole			Posaconazole			Voriconazole		
	SYO	Etest	CLSI	SYO	Etest	CLSI	SYO	Etest	CLSI	SYO	Etest	CLSI
<i>C. albicans</i>	1	AM	0.5	0.12	0.25	NA	0.06	0.12	0.06	0.01	0.03	0.03
<i>C. dubliniensis</i>	1	ID	0.5	0.25	ID	NA	0.12	ID	0.25	0.01	ID	0.03
<i>C. glabrata</i>	64	64	8	2	8	4	4	ID	1	2	2	0.25
<i>C. guilliermondii</i>	16	ID	8	1	ID	NA	1	ID	0.5	0.5	ID	0.12
<i>C. krusei</i>	128	ID	32	1	2	1	1	ID	0.5	1	2	0.5
<i>C. lusitaniae</i>	4	ID	1	0.5	ID	0.5	0.12	ID	0.06	0.03	ID	0.06
<i>C. parapsilosis</i> species complex	2	4	1	0.25	AM	NA	0.12	0.12	0.25	0.01	0.25	0.03
<i>C. parapsilosis sensu stricto</i>	2	NA	NA	0.5	NA	NA	0.25	NA	NA	0.03	NA	NA
<i>C. tropicalis</i>	4	4	1	0.5	0.5	0.5	1	0.12	0.12	0.5	0.5	0.12
<i>S. cerevisiae</i>	16	ID	NA	ID	ID	NA	ID	ID	NA	ID	ID	NA
<i>A. fumigatus</i>	NA	NA	NA	AM	2	1	0.06 ^c	0.25 ^c	0.25 ^c	1	0.5	1
<i>A. flavus</i>	NA	NA	NA	AM	1	1	NA	0.5	0.5	1	0.5	2
<i>A. niger</i>	NA	NA	NA	1	4	4	NA	0.5	2	ID	1	2
<i>A. terreus</i>	NA	NA	NA	AM	AM	2	NA	0.25	1	1	AM	2

^aSYO and Etest ECVs proposed in the present study and CLSI M27 and M38 methods (11, 12). AM, aberrant modes, modal variability; ID, insufficient number of laboratories/isolates entering the ECV definition pool; NA, not available or not applicable.

^bNewly accepted taxonomic names or reclassifications are as follows: *C. guilliermondii* (*M. guilliermondii*), *C. krusei* (*P. kudriavzevii*), and *Candida lusitaniae* (*Clavispora lusitaniae*).

^cPosaconazole ECVs for *A. fumigatus* are reported elsewhere (23, 26); the SYO ECV for *C. orthopsilosis* was 4 μg/ml.

The submitted triazole MICs of the four triazoles for yeast species from 3 to 30 laboratories obtained by the SYO and/or Etest methods and evaluated for ECV definition were for (method/agent dependent) (Tables 1 and 2) 11,171 *C. albicans*, 215 *C. dubliniensis*, 4,418 *C. glabrata* species complex (including 349 *C. glabrata sensu stricto*), 157 *C. guilliermondii*, 676 *C. krusei*, 298 *C. lusitaniae*, 3,691 *C. parapsilosis* species complex, 922 *C. parapsilosis sensu stricto*, and 1,854 *C. tropicalis* isolates (Tables 1 and 2). SYO MICs for other, less common *Candida* and yeast species from at least three laboratories were collected for 25 *C. famata*, 55 *C. kefyr*, 36 *C. metapsilosis*, and 110 *C. orthopsilosis* isolates, and SYO data were also collected for 244 isolates of *S. cerevisiae*. In addition, we pooled SYO and mostly Etest data for the five most prevalent *Aspergillus* complexes originating from 3 to 11 independent laboratories, as follows (method agent dependent): 1,409 *A. fumigatus*, 389 *A. flavus*, 103 *A. nidulans*, 233 *A. niger*, and 302 *A. terreus* isolates.

We also received a total of 282 MICs for mutants: 59 SYO and 10 Etest MICs for *C. albicans* mutants (*Erg11* gene mutations), 2 *C. albicans* and 13 *C. glabrata* mutants (overexpression of CDR2 or CDR1 efflux pumps, respectively), and 78 *C. parapsilosis* mutants (*Erg11* and *MRR1*). SYO and Etest MICs were gathered for 39 and 81 *A. fumigatus sensu stricto* mutant isolates, respectively, with *cyp51A* gene mechanisms of resistance (TR34/L98H, G54, M220, and others) from five to seven participant laboratories and one previous Etest study (20) (Tables 1, 2, and 5). The isolates were identified at each medical center by conventional and molecular methodologies that included macro- and microscopic morphology, thermotolerance (incubation at 50°C), MALDI-TOF mass spectrometry, and β-tubulin and calmodulin sequencing (31, 32). Since molecular identification was not performed for all the isolates evaluated in the present study, we listed the nonmutant isolates in the respective tables as the complexes of *C. glabrata* or *C. parapsilosis* or *Aspergillus* spp. Strains of *A. fumigatus*, *C. albicans*, and *C. glabrata* that were submitted as having mutations were screened in the participant laboratories using published protocols (31, 36–38).

At least one of following quality control (QC) isolates and/or reference isolates was evaluated by the two methods in each of the participant laboratories: QC isolates *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *Paecilomyces variotii* ATCC MYA-3630 and reference isolates *A. fumigatus* ATCC MYA-3626 and *A. flavus* ATCC MYA-204304 (14, 16). MIC data were not included in the study unless the participant laboratories reported that their MICs for the individual QC isolates used in each center were within the expected MIC ranges.

Antifungal susceptibility testing. Triazole SYO and Etest MICs were obtained by the two commercial antifungal susceptibility methods by following the manufacturers' guidelines (14–16). The SYO MIC was the first blue or purple well after 24 h (*Candida*) or, mostly, 48 h (*Aspergillus*) of incubation. The Etest MIC was the lowest drug concentration at which the border of the growth-free elliptical inhibition intercepted the scale on the antifungal strip after 24 to 48 h, as needed; trailing growth was allowed solely for the definition of Etest MICs for *Candida* isolates.

Definitions. The definition of the ECV as a categorical endpoint has been widely described previously as well as above (27, 28). Briefly, the ECV is the highest MIC/MEC distribution for the WT population and is established by using reliable MIC/MEC distributions from at least three laboratories. A non-WT organism usually shows reduced susceptibility to the agent being evaluated compared to that of the WT (no phenotypic resistance) population. In addition to MIC distributions, the ECV calculation takes into account each laboratory distribution mode, the inherent variability of the test (usually within 1 doubling

dilution), and the fact that the ECV should encompass 95% to 97% of isolates. We used those same criteria and requirements for establishing our proposed Etest and SYO method-dependent ECVs. Most published ECVs are based on reference MIC distributions, and ECVs based on other methods could be different, as was shown in our study (Table 6).

Data collation and analyses. Triazole MICs were submitted from 39 independent laboratories worldwide (method/agent/species dependent) in order (i) to define the MIC distributions by each commercial susceptibility testing method/agent and species; (ii) to examine the suitability of these distributions for pooling prior to ECV setting, including the evaluation of interlaboratory modal agreement; and (iii) to estimate ECVs for each species/agent/method that fulfilled the CLSI criteria for ECV definition after pooling (at least 100 MICs for each species/method/agent that originated in ≥ 3 independent laboratories) (27, 28). ECVs were estimated by the iterative statistical method at the 97.5% cutoff value (29) or the second numerical derivative method when the putative wild-type mode was at the lowest concentration in the distribution (30) (Tables 2 and 4). SYO MIC distributions for less common yeast species (*C. famata* and *C. kefyr*) and *C. metapsilosis* were also reported when they originated from at least three laboratories and had comparable modes.

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