Method-dependent epidemiological cutoff values (ECVs) for detection of triazole resistance in *Candida* and *Aspergillus* species for the SYO colorimetric broth and Etest agar diffusion methods

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Running title: SYO and Etest Triazole ECVs for *Aspergillus* and *Candida*

Abstract

Although the Sensitrite 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 (SC), 157 *C.* (*Meyerozyma*) *guilliermondii*, 676 *C. krusei* (*Pichia kudriavzevii*), 298 *C.* (*Clavispora*) *lusitaniae*, 911 and 3,691 *C. parapsilosis sensu stricto* (*SS*) and *C. parapsilosis SC*, respectively, 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* complexes. SYO/Etest MICs for 282 confirmed non-WT isolates were included: *ERG11* (*C. albicans*), *ERG11* and *MRR1* (*C. parapsilosis*), *cyp51A* (*A. fumigatus*), and *CDR2*, *CDR*1 overexpression (*C. albicans* and *C. glabrata*, respectively). Interlaboratory modal agreement was superior by SYO for yeast spp., and by the Etest for *Aspergillus* spp. Distributions fulfilling CLSI criteria for ECV definition were pooled and we proposed SYO ECVs for *S. cerevisiae*, 9 yeast and 3 *Aspergillus* species, and Etest ECVs for 5 yeast and 4 *Aspergillus* species. The posaconazole SYO ECV of 0.06 µg/ml for *C. albicans* and the Etest itraconazole ECV of 2 µg/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 spp. and the Etest for *Aspergillus* spp. Further evaluations should be conducted with more *Candida* mutants.

Introduction

The triazoles (fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) are the current treatments for severe candidiasis and aspergillosis (e.g., first-line or prophylactic, adjunctive, empirical, transition from another agent, 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 been frequently related to the overexpression or alteration of the *PDR1* gene that 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 minimal inhibitory concentrations [MICs]) is recommended for all bloodstream and other clinically relevant *Candida* isolates (1). Although routine MIC determination for *Aspergillus* spp. 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]) as well as the agar diffusion Etest (bioMérieux, Marcy l'Etoile, France), 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 have been reported by these commercial methods (20-24). However, 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 CLSI available BPs for *Candida* spp. as interpretive categories as well as for quality control (QC) (14,16). The proposal of SYO/Etest ECVs (epidemiological cutoff values) 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 two years, we have gathered available triazole MICs by both SYO or Etest assays for isolates of prevalent and non-prevalent yeast species (*C. albicans*, *C. dubliniensis*, *C. glabrata* species complex, [SC], *C.* [*Meyerozyma*] *guilliermondii*, *C. krusei* [*Pichia kudriavzevii*], *C*. [*Clavispora*] *lusitaniae*, *C. parapsilosis* SC, including *C. parapsilosis sensu stricto* [*SS*], *C. orthopsilosis* and *C. tropicalis*), *Saccharomyces cerevisiae* and five *Aspergillus* species complexes (*A. fumigatus* [including *A. fumigatus SS*], *A. flavus*, *A. nidulans*, *A. niger*, and *A. terreus*). Additional SYO MIC distributions for less prevalent or common yeast species *C. famata* (*Debaryomyces hansenii*), *C. kefyr* (*Kluyveromyces marxianus*), and *C. metapsilosis* also were reported when they originated from at least three laboratories and had comparable modes. From here on we will be using the most "common" clinical names. These triazole MICs were submitted from 39 independent worldwide laboratories (method/agent/species dependent) in order: (i) to define MIC distributions by each commercial susceptibility testing method/agent and species; (ii) to examine the suitability of these distributions for 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 $\geq$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 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]) 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, *C. glabrata* MICs with overexpression of the *CDR1* gene, and SYO and Etest MICs for *A. fumigatus* SS harboring *cyp51A* mutations. These data were submitted mostly from European laboratories as well as from Argentina, Thailand, South Africa and one published Etest study (20). SYO data for 58 *PDR* gene *C. glabrata* mutants also were submitted, but those data were not included due to large modal variability of the non-mutants as 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 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 MEC results), classification of an isolate as a presumptively WT cannot directly predict a successful therapeutic outcome. Classification of an isolate as a non-WT indicates that it could harbor acquired resistance mechanisms 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 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 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 1-4) (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* SS *cyp51A* (5 laboratories), Etest MICs for 81 *A. fumigatus cyp51A* (7 laboratories and one published study) (20) (Tables 1, 2 and 5). SYO MICs were received from single laboratories for the following mutants: 13 *C. glabrata* and 2 *C. albicans* with overexpression of the *CDR1* and *CDR2* genes, respectively, 78 *C. parapsilosis* (49 *ERG11* and 29 *MRR1*, respectively); and Etest MICs for 10 *C. albicans* (*ERG11*) (not listed in Tables 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 proposed ECVs 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 of *Candida* and *Aspergillus* were not included in the ECV analysis due to: aberrant or not defined modes, bimodal, or when the particular mode for a distribution was more than 1 to 2 dilutions from the global mode, or when there were less than five isolates in the distribution. MIC distributions were also excluded when the MIC data for the QC isolates were outside the recommended range (14,16). The total of SYO MICs for the 12 *Candida* species and the four triazoles pooled for ECV definition from 3 to 30 independent laboratories ranged from 11,171 to 17 isolates, including the data points for *C. parapsilosis SS* and *C. parapsilosis* SC, *C. metapsilosis*, *C. orthopsilosis*, *C. famata, C. kefyr,* and *S. cerevisiae*. The SYO MIC distributions for the 59 *C. albicans* and 39 *A. fumigatus SS* mutants from multiple laboratories were 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 only proposed for voriconazole (4 of 5 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 *sensu stricto* and 29% (263 data points) as species complex (identification by morphological methods, MALDI-TOF mass spectrometry, or by molecular methods [e.g., β-tubulin and calmodulin sequencing]) (30). *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 SC (Table 1).

Table 1 also depicts the SYO modes for *Candida* and *Aspergillus* species. The lowest SYO fluconazole modes (0.25 µg/ml) were for *C. albicans*, *C. dubliniensis* and *C. kefyr* and the highest mode for *C. krusei* (64 µg/ml). Similar modal diversity was noted among posaconazole MICs (modes of 0.01 µg/ml for *C. albicans* and 1 µg/ml for *C. glabrata*). However, itraconazole and voriconazole modes were mostly 0.06 µg/ml to 0.12 µg/ml or 0.008 to 0.03 µg/ml, respectively. The exceptions were itraconazole modes for *C. glabrata* (0.5 µg/ml), *C. guilliermondii* and *C. krusei* (0.25 µg/ml) and voriconazole modes for *C. guilliermondii*, *C. tropicalis* (0.06 µg/ml), *C. glabrata* and *C. krusei* (0.25 µg/ml). Most SYO modes for the *C. parapsilosis* complex were +/-1 double dilution, but all posaconazole modes for the four species in the complex were 0.03 µg/ml. SYO voriconazole modes for *Aspergillus* spp. and the itraconazole mode for *A. niger* ranged from 0.12 to 0.5 µg/ml. As expected, SYO modes for the *C. albicans* and *A. fumigatus* mutants were much higher than those for the non-mutant 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 *Candida* spp., *C. krusei*, and *Aspergillus* spp. Eight laboratories, including a published study (20), contributed Etest voriconazole and itraconazole data for the 75 and 81 *A. fumigatus SS* mutants, respectively (Tables 2 and 5). A total of 64% (712 of the 1,112 itraconazole MICs) of *A. fumigatus* isolates and most *Candida* isolates were identified at the species level (31,32), but *C. glabrata* and *C. parapsilosis* mainly as species complex. Therefore, we were unable to provide the potential antifungal susceptibility differences among the species in the *C. parapsilosis SC*, as we did by the SYO method (Table 1). 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. was superior. The lowest Etest modes were for *C. parapsilosis* versus fluconazole and posaconazole (0.5 and 0.01 µg/ml, respectively), *C. tropicalis* versus itraconazole (0.03 µg/ml), and *C. albicans* versus voriconazole (0.008 µg/ml). All Etest modal values for *Aspergillus* spp.

ranged between 0.12 and 0.25 µg/ml, except for the itraconazole modes for *A. fumigatus* (0.5 µg/ml), and for *A. niger* (1 µg/ml). Etest modes for the *A. fumigatus* mutants also were much higher than those for the non-mutant isolates.

Tables 3 and 4 depict the proposed ECOFFinder SYO and Etest triazole ECVs, respectively, for 97.5% of the modelled 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 ≥50% of the total. In addition to SYO ECVs for the prevalent *Candida* spp., fluconazole ECVs were proposed for *C. orthopsilosis* (4 µg/ml) and *S. cerevisiae* (16 µg/ml) (Table 3). Although fluconazole ECVs for *C. parapsilosis* SS and SC were the same (2 µg/ml), the other ECVs for *C. parapsilosis* SS were one dilution higher. To our knowledge, ECVs for *C. parapsilosis* SS 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 µg/ml for five *Candida* spp. and ECVs of 0.12 to 64 µg/ml for the other three agents and 3 to 4 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 submitted from only two laboratories) precluded ECV definition 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 as listed in the new edition of the M59 document (26). In general, SYO ECVs were one to two 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 µg/ml and 2 and 0.25 µg/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 µg/ml recognized the highest percentage of mutants (55/59: 93%), followed by the itraconazole ECV of 0.12 µg/ml (53/59: 90%), the voriconazole ECV of 0.01 µg/ml (52/59: 88%) and the fluconazole SYO ECV of 1 µg/ml (48/59 (81%). These *C. albicans* mutants had the following *ERG11* substitutions: F145L, Y132H, S442F, S405F, G464S, A114S, G464S, F145T, T22OL, and P98A (alone or in combination). Although high CLSI triazole MICs have been documented for most of those substitutions (6,8,33-35), T22OL 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 µg/ml (Table 1), it seems that these strains also could harbor combined resistance mechanisms (e.g., the most common efflux pump overexpression +erg11 overexpression and/or mutation). These molecular combinations are due to aneuploidy (duplication of the 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 listed the *C. albicans* and *A. fumigatus* mutants that according to our method-dependent ECVs could be categorized as either WT (MICs =< each ECV) and/or non-WT (MICs >the ECV). Those substitutions have been reported as both "susceptible and resistant" isolates using CLSI methodologies and BPs (8,33-35). Regarding data from the single laboratories, SYO MICs of the four agents for the two *C. albicans* and 11 of the 13 *C. glabrata* strains from single laboratories with overexpression of the *CDR2* and *CDR1* 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 µg/ml, respectively) recognized >96% of the 78 *C. parapsilosis* mutants. Therefore, the potential ability of our SYO ECVs in recognizing ≥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* spp. 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 to 81 Etest (voriconazole and itraconazole, respectively) and 39 SYO (voriconazole) MICs for *A. fumigatus* SS *cyp51A* mutants, were evaluated. Our proposed Etest itraconazole ECV of 2 µg/ml for *A. fumigatus* had a superior performance in recognizing the *cyp51A* mutants (78/81: 96%) than the voriconazole Etest ECV of 0.5 µg/ml (50/75: 67%) and the SYO ECV of 1 µg/ml (26/39: 67%) (Table 5). Etest

itraconazole MICs were above the ECV for the following mutations: 48 TR34/L98 (59%), 12 G54 (15%), 9 M220 (11%), 5 G448S (6%) and 7 (9%) miscellaneous mutations, including two TR46/Y121F (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 M220 with either high or low triazole MICs (36). An overlap between posaconazole MICs for non-mutants and a much larger number of mutants of *A. fumigatus* by three antifungal susceptibility methods (CLSI, EUCAST and Etest) also has 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., further collaborative studies should evaluate the endpoint determination; both color change and growth inhibition have been have 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 yeasts species, including MIC distributions for the *C. parapsilosis* complex (*C. parapsilosis* SS, *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, as well as for *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 was better by the Etest 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 µg/ml for *C. albicans* and the Etest itraconazole ECV of 2 µg/ml for *A. fumigatus* were the best predictors in recognizing the non-WT or mutants (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, results were unsatisfactory with posaconazole and itraconazole ECVs. Data for mutants for other *Candida* spp. would better assess the method-dependent proposed ECVs. The SYO method appears to yield more suitable MIC data for testing most *Candid*a spp. and the Etest for *Aspergillus* spp.

Materials and Methods

Isolates: The *Candida* and other yeast isolates evaluated were recovered mostly from blood and other normally sterile sites from patients with candidemia or other deep infections (>90%) as well as superficial, oral, vaginal and thrush. The *Aspergillus* isolates also were recovered from deep infections, sterile and other sites (mostly [>90%] bronchoalveolar lavage fluids, 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, Ontario, 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, S. 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 Microbilogía, 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, Pennsylvania, 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.

The total of submitted triazole MICs of the four triazoles by both SYO and/or Etest methods from 3 to 30 laboratories for yeast species were as follows (method-agent dependent) (Tables 1 and 2): 11,171 *C. albicans*, 215 *C. dubliniensis*, 4,418 *C. glabrata* SC (including 349 *C. glabrata* SS), 157 *C. guilliermondii*, 676 *C. krusei*, 298 *C. lusitaniae*, 3,691 *C. parapsilosis* SC, 922 *C. parapsilosis* SS, and 1,854 *C. tropicalis* isolates were evaluated for ECV definition (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* as well as SYO data for 244 isolates of *S. cerevisiae*. In addition, we pooled SYO and mostly Etest data for the four most prevalent *Aspergillus* complexes as follows (method-agent dependent): 1,409 *A. fumigatus*, 389 *A. flavus*, 103 *A. nidulans*, 233 *A. niger*, and 302 *A. terreus* isolates originating from 3 to 11 independent laboratories.

We also received a total of 282 MICs for mutants: 59 SYO and 10 Etest MICs, respectively, for *C. albicans* (*Erg11* gene mutations), 2 *C. albicans* and 13 *C. glabrata*, respectively, (overexpression of CDR2 or CDR1 efflux pumps, respectively), and 78 *C. parapsilosis* (*Erg11* and *MRR1*). SYO and Etest MICs were gathered for 39 and 81 strains for *A. fumigatus* SS 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 Table 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 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 non-mutant 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: *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258 and *Paecilomyces variottii* ATCC MYA-3630 and/or reference isolates *A. fumigatus* ATCC MYA-3626 and *A. flavus* ATCC MYA-204304 were evaluated by the two methods in each of the participant laboratories (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 manufacturer's guidelines (14-16). The SYO MIC was the first blue or purple well after 24 h (*Candida*) or mostly 48 h (*Aspergillus*) of incubation and isolates, respectively). 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 as well as above (27,28). Briefly, the ECV is the highest MIC/MEC distribution of 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 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 one doubling dilution), and 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 it has been shown in our study (Table 6).

Data collation and analyses. Triazole MICs were submitted from 39 independent worldwide laboratories (method/agent/species dependent) in order: (i) to define 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* also were reported when they originated from at least three laboratories and had comparable modes.

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References

1. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2016. Clinical practice guidelines for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 62:1–50. https://doi.org/10.1093/cid/civ747.

2. Patterson TF, Thompson GR, III, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, Segal BH, Steinbach WJ, Stevens DA, Walsh TJ, Wingard JR, Young JA, Bennett JE. 2016. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 63:e1-e60. https://doi.org/10.1093/cid/ciw326.

3. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M,

509 **Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikkos G,**
510 **Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ**, **ESCMID Fungal Infection**
511 **Study Group**. 2012. ESCMID guidelines for the diagnosis and management of *Candida*
512 diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 18:19-37.
513 https://doi.org/10.1111/1469-0691.12039.
514 **4. Klingspor L, Saaedi B, Ljungman P, Szakos A.** 2015. Epidemiology and outcomes of
515 patients with invasive mould infections: a retrospective observational study from a single centre
516 (2005-2009). Mycoses 58:470-477. https://doi.org/10.1111/myc.12344.
517 **5. Kosmidis C, Denning DW**. 2015. The clinical spectrum of pulmonary aspergillosis. Thorax
518 70:270-277. https://doi.org/10.1136/thoraxjnl-2014-206291.
519 **6. Berkow EL, Lockhart SR.** 2017. Fluconazole resistance in *Candida* species: a current
520 perspective. Infect Drug Resist 10:237-245.
521 **7**. **Vale-Silva LA, Moeckli B, Torelli R, Posteraro B, Sanguinetti M, Sanglard D.** 2016.
522 Upregulation of the adhesin gene *EPA1* mediated by *PDR1* in *Candida glabrata* leads to
523 enhanced host colonization. mSphere 1(2):e00065-15. doi:10.1128/mSphere.00065-15.
524 **8. Sanglard D, Coste AT.** 2016. Activity of isavuconazole and other azoles against *Candida*
525 clinical isolates and yeast model systems with known azole resistance mechanisms. Antimicrob
526 Agents Chemother **60**:229-238. doi:10.1128/AAC.02157-15.
527 **9. Forastiero A, Mesa-Arango AC, Alastruey-Izquierdo A, Alcazar-Fuoli L, Bernal-Martinez**
528 **L, Pelaez T, Lopez JF, Grimalt JO, Gomez-Lopez A, Cuesta I, Zaragoza O, Mellado E.** 2013.
529 *Candida tropicalis* antifungal cross-resistance is related to different azole target (Erg11p)
530 modifications. Antimicrob Agents Chemother 57:4769-4781.
531 http://dx.doi.org/10.1128/AAC.00477-13.
532 **10. Bernal-Martínez L, Gil H, Rivero-Menéndez O, Gago S, Cuenca-Estrella M, Mellado E,**
533 **Alastruey-Izquierdo A**. 2017. Development and validation of a high resolution melting assay to
534 detect azole resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 61:e01083-17.
535 https://doi.org/10.1128/AAC.01083-17.
536 **11. Clinical and Laboratory Standards Institute.** 2017. Reference method for broth dilution
537 antifungal susceptibility testing of yeasts. 4th ed. CLSI standard M27. Clinical and Laboratory
538 Standards Institute, Wayne, PA**.**
539 **12. Clinical and Laboratory Standards Institute. 2017.** Reference method for broth dilution
540 antifungal susceptibility testing of filamentous fungi, 3rd ed. CLSI standard M38. Clinical and
541 Laboratory Standards Institute, Wayne, PA.

13. Arendrup MC, Meletiadis J, Mouton JW, Guinea J, Cuenca-Estrella M, Lagrou K, Howard SJ, Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing. 2016. EUCAST technical note on isavuconazole breakpoints for *Aspergillus*, itraconazole breakpoints for *Candida* and updates for the antifungal susceptibility testing method documents. Clin Infect Dis 22:571.e1-571.e4. https://doi.org/10.1016/j.cmi.2016.01.017.

14. Trek Diagnostic Systems. 2012. Sensititre Yeast One: Yeast One susceptibility, v1.8. Trek Diagnostic Systems, Cleveland, OH.

15. **bioMérieux SA**. 2013. Etest antifungal susceptibility testing package insert. bioMérieux SA, Marcy-l'Etoile, France.

16. bioMérieux SA. 2013. Etest performance, interpretative criteria and quality control ranges Table. bioMérieux SA, Marcy-l'Etoile, France.

17. Pfaller MA, Espinel-Ingroff A, Jones RN. 2004. Clinical evaluation of Sensititre Yeastone colorimetric antifungal plate for antifungal susceptibility testing of the new triazoles voriconazole, posaconazole, and ravuconazole. J Clin Microbiol 42:4577-4580**.**

18. Espinel-Ingroff A, Pfaller M, Erwin ME, Jones RN. 1996. Interlaboratory evaluation of Etest method for testing antifungal susceptibilities of pathogenic yeasts to five antifungal agents using a casitone agar and solidified RPMI 1640 medium with 2% glucose. J Clin Microbiol 34:848-85.

19. Espinel-Ingroff A, Rezuzta A. 2002. E-Test method for testing susceptibilities of *Aspergillus* spp. to the new triazoles voriconazole and posaconazole and to established antifungal agents: comparison with NCCLS broth microdilution method. J Clin Microbiol 40:2001-2107.

20. Burgel P-R, Baixench M-T, Amsellem M, Audureau E, Chapron J, Kanaan R, Honoré I, Dupouy-Camet J, Dusser D, Klaassen CH, Meis JF, Hubert D, Paugam A. 2012. High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. Antimicrob Agents Chemother 56**:**869-874. https://doi:10.1128/AAC.05077-11.

21. Wu C-J, Wang H-C, Lee J-C, Lo H-J, Dai C-T, Chou P-H, Ko W-C, Chen Y-C. 2015. Azole-resistant *Aspergillus fumigatus* isolates carrying TR34/L98H mutations in Taiwan. Mycoses 58:544-549. https://doi:10.1111/myc.12354.

22. Mello E, Posteraro B, Vella A, De Carolis E, Torelli R, D'Inzeo T, Verweij PE, Sanguinetti M. 2017. Susceptibility testing of common and uncommon *Aspergillus* species against posaconazole and other mold-active antifungal azoles using the Sensititre method. Antimicrob Agents Chemother. 61:e00168-17. https://doi.org/10.1128/AAC.00168-17.

23. Espinel-Ingroff A, Turnidge J, Alastruey-Izquierdo A, Dannaoui E, Garcia-Effron G, Guinea J, Kidd S, Pelaez T, Sanguinetti M, Meletiadis J, Botterel F, Bustamante B, Chen Y-C, Chakrabarti A, Chowdhary A, Chryssanthou E, Córdoba S, Gonzalez GM, Guarro J, Johnson EM, Kus JV, Lass-Flörl C, Linares-Sicilia MJ, Martín-Mazuelos E, Negri CE, Pfaller MA, Tortorano AM. 2018. Posaconazole MIC distributions for *Aspergillus fumigatus* species complex by four methods: impact of *cyp51A* mutations on estimation of epidemiological cutoff values. Antimicrob Agents Chemother. 62:e01916-17. https://doi.org/10.1128/AAC .01916-17.

24. Szweda P, Gucwa K, Romanowska E, Dzierz˙anowska-Fangrat K, Naumiuk Ł, Brillowska-Da˛browska A, Wojciechowska-Koszko I, Milewski S. 2015. Mechanisms of azole resistance among clinical isolates of *Candida glabrata* in Poland. J Med Microbiol 64:610-619. DOI 10.1099/jmm.0.000062.

25. Espinel-Ingroff A, Arendrup M, Cantón E, Cordoba S, Dannaoui E, García-Rodríguez J, Gonzalez GM, Govender NP, Martin-Mazuelos E, Lackner M, Lass-Flörl C, Linares Sicilia MJ, Rodriguez-Iglesias MA, Pelaez T, Shields RK, Garcia-Effron G, Guinea J, Sanguinetti M, Turnidge J. 2017. Multicenter study of method-dependent epidemiological cutoff values for detection of resistance in *Candida* spp. and *Aspergillus* spp. to amphotericin B and echinocandins for the Etest agar diffusion method. Antimicrob Agents Chemother 61:e01792-16. https://doi.org/10.1128/AAC .

26. Clinical and Laboratory Standards Institute. 2018. Epidemiological cutoff values for antifungal susceptibility testing. CLSI supplement M59, 2nd ed. Clinical and Laboratory Standards Institute, Wayne, PA.

27. Clinical and Laboratory Standards Institute. 2016. Principles and procedures for the development of epidemiological cutoff values for antifungal susceptibility testing. CLSI M57 document, 1st ed. Clinical and Laboratory Standards Institute, Wayne, PA.

28. Espinel-Ingroff A, Turnidge J. 2016. The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds. Rev Iberoam Micol 33:63-75.

29. Turnidge J, Kahmeter G, Kronvall G. 2006. Statistical characterization of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect 12:418-425. https:// doi.org/10.1111/j.1469-0691.2006.01377.x.

30. Meletiadis J, Curfs-Breuker I, Meis JF, Mouton JW. In Vitro Antifungal Susceptibility Testing of *Candida* Isolates with the EUCAST Methodology, a New Method for ECOFF

608 Determination. Antimicrob Agents Chemother. 2017; 61:e02372-16. doi: 10.1128/AAC.02372-
609 16.
610 **31. Alastruey-Izquierdo A, Alcazar-Fuoli L, Cuenca-Estrella M**. 2014. Antifungal
611 susceptibility profile of cryptic species of *Aspergillus*. Mycopathologia 178:427-433.
612 https://doi.org/10.1007/s11046-014-9775-z.
613 **32. Howell S, Hazen KC**. 2011. *Candida*, *Cryptococcus* and other yeasts of medical
614 importance. 2011.In: Manual of Clinical Microbiology. 10th ed. J. Versalovic J, Carroll KC, Funke
615 G, Jorgensen JH, Landry ML, Warnock DW. Eds. ASM Press, Washington DC.
616 **33. Chau AS, Mendrick CA, Sabatelli FL, Loebenberg D, McNicholas PM**. 2004. Application
617 of real-time quantitative PCR to molecular analysis of *Candida albicans* strains exhibiting
618 reduced susceptibility to azoles. Antimicrob Agents Chemother 48:2124-2131.
619 **34. Morio F, Loge C, Besse B, Hennequin C, Le Pape P.** 2009. Screening for amino acid
620 substitutions in the *Candida albicans* Erg11 protein of azole-susceptible and azole-resistant
621 clinical isolates: new substitutions and a review of the literature. Diag Microbiol Infect Dis
622 66:373-384. DOI: https://doi.org/10.1016/j.diagmicrobio.2009.11.006
623 **35. Flowers SA, Brendan CB, Whaley SG, Schuler MA. Rogers D.** 2015. Contribution of
624 clinically derived mutations in ERG11 to azole resistance in *Candida albicans*. Antimicrob.
625 Chemother 59:450-460. doi:10.1128/AAC.03470-14.
626 **36. Rivero-Menendez O, Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M.** 2016.
627 Triazole resistance in *Aspergillus* spp.: a worldwide problem? J Fungi (Basel) 2:1-21.
628 https://doi.org/10.3390/jof2030021.
629 **37. Garcia-Effron G, Dilger A, Alcazar-Fuoli L, Park S, Mellado E, Perlin DS**. 2008**.** Rapid
630 detection of triazole antifungal resistance in *Aspergillus fumigatus*. J Clin Microbiol. 46:1200-
631 1206.
632 **38. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G**. 2005. Mechanisms of
633 azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of
634 antifungal resistance. Antimicrob Agents Chemother 49: 668–679.
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Table 1. SYO pooled triazole MIC distributions for species of *Candida*, *Saccharomyces* and *Aspergillus*[a]

Agent and species	No. isolates	No. labs used/ Total[b]	Number of isolates with MIC (µg/ml) of:[c]											
Fluconazole			**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**
C. albicans	11,171	28/30	12	1,016	**4,252**	4,152	978	238	122	82	78	33	49	159
Confirmed *ERG11* *mutants*	59	4/4		3	1	4	3		1	2	2		**43**	
C. dubliniensis	195	7/10		48	**64**	57	13	6	3	2		1	1	
C. famata	23	3/6			1	2	**11**	5	3	1				
C. glabrata	4,418	30/30		13	9	23	64	152	375	1,049	**1,330**	691	216	496
C. guilliermondii	153	8/13		2	1	6	20	36	**46**	19	10	6	4	3
C. kefyr	55	3/4		13	**25**	15	2							
C. krusei	537	15/16		1	1	1			3	8	43	193	**220**	67
C. lusitaniae	298	12/12		16	41	75	**99**	43	12	5	1	4	1	1
C. parapsilosis	3,691	28/30		89	502	**1,210**	958	421	221	151	82	27	19	11
C. parapsilosis SS	911	5/5		18	118	**282**	216	121	74	53	19	6	4	
C. metapsilosis	36	4/4			1	2	**17**	10	5		1			
C. orthopsilosis	110	5/5		3	4	29	**43**	15	8	4	2		1	1
C. tropicalis	1,854	24/28		20	82	270	**701**	482	129	53	19	24	14	60
S. cerevisiae	244	3/3		4	3	9	40	70	**76**	26	10	4	2	
Itraconazole			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. albicans	7,843	27/30	69	995	2,696	**2,754**	905	164	77	27	14	7	9	126
Confirmed *ERG11* *mutants*	59	4/4			2	2	2	5	11	3	7	4		**23**

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C. dubliniensis	125	6/8		13	21	**47**	27	7	2	5	1			2
Itraconazole (Cont.)			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. famata	18	3/5		1	1	2	3	**7**	3	1				
C. glabrata	3,594	29/30		12	19	42	112	428	**1,335**	910	195	71	26	444
C. guilliermondii	149	9/13		3		8	31	**55**	37	10	2			3
C. kefyr	45	3/3		5	10	**17**	12	1						
C. krusei	574	13/16		4	3	14	69	**283**	156	33	2	1		9
C. lusitaniae	171	8/11		11	12	52	**60**	28	7	1				
C. parapsilosis	3,353	23/30		209	570	1,098	**1,150**	252	59	13	1	1		
C. parapsilosis SS	730	4/5		68	79	237	**254**	83	6	3				
C. metapsilosis	32	4/4		3	3	**12**	11	1	2					
C. orthopsilosis	88	3/4		2	13	**35**	26	12						
C. tropicalis	1,399	23/29		14	51	138	**513**	508	126	16	4	1	5	23
S. cerevisiae	41	3/3		1	1	3	**21**	11	2	2				
A. niger	233	6/7		18	23	48	**69**	44	17	6			1	7
Posaconazole			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. albicans	6,729	27/30	596	**2,768**	2,318	587	175	96	56	32	10	3	60	28
Confirmed *ERG11* mutants	59		1		3	1	4	9	8	7	1	1		**24**
C. dubliniensis	185	7/8	35	56	**63**	25	4			2				
C. glabrata	2,999	25/29	4	5	28	39	50	153	590	**1,145**	579	62	251	93
C. guilliermondii	111	9/12	3	1	9	15	27	**35**	18	3				
C. kefyr	40	3/3			7	**13**	13	5	2					
C. krusei	562	13/15	1	1	3	20	90	**264**	151	25	5		2	

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C. lusitaniae	172	11/11	17	49	**58**	36	10		1	1				
Posaconazole (Cont.)			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. parapsilosis	3,085	26/30	136	538	**1,091**	915	297	86	11	7	1	1		2
C. parapsilosis SS	670	5/5	40	127	**206**	193	69	31	2				2	
C. metapsilosis	17	3/4		3	**7**	4	3							
C. orthopsilosis	30	3/4		5	**14**	7	4							
C. tropicalis	1,366	23/29	16	50	107	250	**408**	336	147	22	6		17	7
S. cerevisiae	41	3/3				3	6	**20**	9	2	1			
Voriconazole			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. albicans	8,747	29/30	**5,947**	1,691	481	222	111	82	47	22	10	15	76	43
Confirmed *ERG11* mutants	59	4/4	3	4	4	1	4	1	7	6	3	5	2	**19**
C. dubliniensis	215	7/9	**182**	21	5	3	1	2			1			
C. famata	25	3/5	5	**10**	4	2	2	2						
C. glabrata	3,255	24/30	23	29	65	189	486	**911**	824	340	136	156	82	14
C. guilliermondii	157	11/12	8	10	32	**46**	34	10	11	4	1			1
C. kefyr	55	3/3	**46**	8	1									
C. krusei	676	14/16	2	1	1	16	108	**291**	199	42	11	3	1	1
C. lusitaniae	248	11/12	**120**	70	32	15	4	1	2	4				
C. parapsilosis	2,670	26/30	**1,213**	695	364	210	103	50	16	10	9			
C. parapsilosis SS	718	5/5	**261**	185	122	80	47	12	5	4	2			
C. metapsilosis	30	3/4	2	10	**11**	4	2	1						
C. orthopsilosis	20	3/4	1	**8**	3	6	2							
C. tropicalis	1,637	19/28	45	92	227	**466**	443	200	70	25	20	9	23	17
S. cerevisiae	41	3/3	1	3	**17**	15	2	2	1					

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A. fumigatus	903	8/8	2	7	35	64	157	**396**	179	33	8	7	7	8
Confirmed *Cyp51A mutants*	39	5/5					3	4	8	3	4	5	2	10
Voriconazole (Cont.)			**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
A. flavus	389	6/7	5	1	14	32	89	**139**	59	29	16	1	0	4
A. niger	74	3/6				1	9	19	**33**	12				
A. terreus	302	5/6	6	5	16	19	48	**122**	69	15	2			

[a]Including the complexes of *C. glabrata, C. parapsilosis* and *Aspergillus* spp.; the *cyp51A* mutants are *A. fumigatus* SS; *C. famata* (*D. hansenii*), *C. guilliermondii* (*M. guilliermondii*), *C. kefyr* (*K. marxianus*), *C. krusei* (*P. kudriavzevii*) and *C. lusitaniae* (*Clavispora lusitaniae*).

[b]Total number of laboratories included in the ECV definition pool/total number of laboratories that submitted data.

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

Table 2. Etest Triazole pooled MIC distributions for species of *Candida* and *Aspergillus*[a]

Agent and species	No. isolates	No. labs used/ total[b]	Number of isolates with MIC (µg/ml) of:[c]												
Fluconazole			**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**
C. glabrata	356	7/10			1	4	13	34	50	79	**88**	36	13	28	10
C. parapsilosis	639	9/9	3	19	68	131	**153**	138	66	21	9	7	20		4
C. tropicalis	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**
C. albicans	975	8/9	7	55	145	237	**295**	150	27	19	16	6	7	5	6
C. krusei	101	3/3				1		2	9	**36**	35	7	7	1	3
C. tropicalis	165	5/8	2	12	23	**39**	30	22	15	11	7	2	1	1	
A. fumigatus	1,112	10/10		4	1	3	30	56	157	**483**	268	73	12	5	20
Confirmed *Cyp 51A* mutants	81	8/8					1			1		1	7	4	**67**
A. flavus	250	7/8			1	4	19	37	**103**	69	16	1			
A. nidulans	130	4/4			1	1	13	**39**	34	23	11	7		1	
A. niger	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**
C. albicans	305	4/6	6	29	94	**102**	44	17	9	3	1				
C. krusei	48	3/3					1	5	**17**	16	7				2
C. parapsilosis	162	4/5	8	26	**51**	37	23	9	3	2			1		2
C. tropicalis	101	4/5		9	21	**32**	21	4	8	3	1		1	1	
A. flavus	204	7/7			1	4	14	70	**96**	17	2				
A. niger	168	4/5				5	16	58	**73**	15	1				
A. terreus	194	5/5				8	47	**105**	27	4	2		1		

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Voriconazole (Cont. Table 2)			**≤0.004**	**0.008**	**0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**≥16**
C. albicans	2,159	8/11	485	**803**	491	158	104	42	22	20	11	5	7	1	**10**
C. glabrata	551	8/9		7	11	20	37	105	**143**	96	63	30	15	14	10
C. krusei	130	6/6			1	2	3	20	30	**45**	24	5		2	
C. parapsilosis	506	7/9	4	20	46	97	**167**	100	39	15	4	4	8	1	1
C. tropicalis	260	6/10	4	4	12	28	82	**88**	22	14	5			1	
A. fumigatus	1,409	11/11	1	6	2	30	132	**633**	473	100	19	7	3	2	1
Confirmed *Cyp 51A* mutants	75	8/8					5	6	7	8	13	15	3	1	**17**
A. flavus	257	7/7			1		18	84	**103**	39	10	1			1
A. niger	173	4/5			2	3	22	37	**81**	25	1	1			1

[a]Including the complexes of *C. parapsilosis*, *C. glabrata* and *Aspergillus* species; the *Cyp 51A* mutants are *A. fumigatus* SS; *C. krusei* (*P. kudriazveii*).

[b]Total number of laboratories included in the ECV definition pool/total number of study laboratories and one published study (20) that submitted data.

[c]Data 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 MIC or the mode) is in bold.

Table 3. Method-dependent SYO ECOFFinder ECVs of four triazoles for species of *Candida, Saccharomyces*, and *Aspergillus*[a]

Agent and species	No. isolates	No. labs used/total	MIC (µg/ml)		ECVs (µg/ml)[b]
			Range	Mode	
Fluconazole					
C. albicans	11,171	28/30	0.06-≥128	0.25	1
C. dubliniensis	195	7/10	0.12-64	0.25	1
C. glabrata	4,418	30/30	0.12-≥128	16	64
C. guilliermondii	153	8/13	0.12-≥128	4	16
C. krusei	537	15/16	0.12-≥128	64	128
C. lusitaniae	298	12/12	0.12-≥128	1	4
C. parapsilosis	3,691	28/30	0.12-≥128	0.5	2
C. parapsilosis SS	911	5/5	0.12-64	0.5	2
C. orthopsilosis	110	5/5	0.12-≥128	1	4
C. tropicalis	1,854	24/28	0.12-≥128	1	4
S. cerevisiae	244	3/3	0.12-64	4	16
Itraconazole					
C. albicans	7,843	27/30	0.008-≥16	0.06	0.12
C. dubliniensis	125	6/8	0.01-≥16	0.06	0.25
C. glabrata	3,594	29/30	0.01-≥16	0.5	2
C. guilliermondii	149	9/13	0.01-≥16	0.25	1
C. krusei	574	13/16	0.01-≥16	0.25	1
C. lusitaniae	171	8/11	0.01-1	0.12	0.5
C. parapsilosis	3,353	23/30	0.01-4	0.12	0.25
C. parapsilosis SS	730	4/5	0.01-1	0.12	0.5
C. tropicalis	1,399	23/29	0.01-≥16	0.12	0.5
A. niger	233	6/7	0.01-≥16	0.12	1
Posaconazole					
C. albicans	6,729	27/30	0.008-≥16	0.01	0.06
C. dubliniensis	185	7/8	0.008-1	0.03	0.12
C. glabrata	2,999	25/29	0.008-≥16	1	4
C. guilliermondii	111	9/12	0.008-1	0.25	1
C. krusei	562	13/15	0.008-8	0.25	1
C. lusitaniae	172	11/11	0.008-1	0.03	0.12
C. parapsilosis	3,085	26/30	0.008-≥16	0.03	0.12
C. parapsilosis SS	670	5/5	0.008-8	0.03	0.25
C. tropicalis	1,366	23/29	0.008-≥16	0.12	1
Voriconazole (Cont. Table 3)					

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C. albicans	8,747	29/30	0.008-≥16	0.008	0.01[c]
C. dubliniensis	215	7/9	0.008-2	0.008	0.01[c]
C. glabrata	3,255	24/30	0.008-≥16	0.25	2
C. guilliermondii	157	11/12	0.008-≥16	0.06	0.5
C. krusei	676	14/16	0.008-≥16	0.25	1
C. lusitaniae	248	11/12	0.008-1	0.008	0.03[c]
C. parapsilosis	2670	26/30	0.008-2	0.008	0.01[c]
C. parapsilosis SS	718	4/5	0.008-2	0.008	0.03[c]
C. tropicalis	1,637	19/28	0.008-≥16	0.06	0.5
A. fumigatus	903	8/8	0.008-≥16	0.25	1
A. flavus	389	6/7	0.008-≥16	0.25	1
A. terreus	302	5/6	0.008-2	0.25	1

[a]Including the complexes of *C. parapsilosis*, *C. glabrata* and *Aspergillus* species; *C. guilliermondii* (*M. guilliermondii*), *C. krusei* (*P. kudriavzevii*) and *C. lusitaniae* (*Clavispora lusitaniae*. Modal variability or insufficient data precluded the proposal of ECVs for some species of both *Candida* and *Aspergillus*.

[b]ECOFFinder ECVs for 97.5% of the statistically modelled population based on MICs by the colorimetric broth microdilution SYO method (14,29) except where indicated by superscript c, referring to footnote c. Proposed method-dependent SYO ECV for *A. fumigatus* and posaconazole is 0.06 µg/ml, as reported elsewhere (23). *C. krusei* is intrinsically resistant to fluconazole regardless of the MIC.

[c]ECV as estimated using the second derivative method (30).

Table 4. Method-dependent Etest ECOFFinder ECVs of four triazoles for species of *Candida* and *Aspergillus*[a]

Agent and species	No. isolates	No. labs used/total[b]	MIC (µg/ml)		ECVs (µg/ml)[c]
			Range	Mode	
Fluconazole					
C. glabrata	356	7/10	0.12-≥128	8	64
C. parapsilosis	639	9/9	0.03-≥128	0.5	4
C. tropicalis	368	9/10	0.03-≥128	1	4
Itraconazole					
C. albicans	975	8/9	≤0.004-≥16	0.06	0.25
C. krusei	101	3/3	0.03-≥16	0.5	2
C. tropicalis	165	5/8	≤0.004-8	0.03	0.5
A. fumigatus	1,112	10/10	0.008-≥16	0.5	2
A. flavus SC	250	7/8	0.01-2	0.25	1
A. nidulans	130	4/4	0.01-8	0.12	1
A. niger	176	4/5	0.03-≥16	1	4
Posaconazole[c]					
C. albicans	305	4/6	≤0.004-1	0.03	0.12
C. parapsilosis	162	4/5	≤0.004-≥16	0.01	0.12
C. tropicalis	101	4/5	0.008-8	0.03	0.12
A. flavus	204	7/7	0.01-1	0.25	0.5
A. niger	168	4/5	0.03-1	0.25	0.5
A. terreus	194	5/5	0.03-4	0.12	0.25
Voriconazole					
C. albicans	2,159	8/11	≤0.004-≥16	0.008	0.03
C. glabrata	551	8/9	0.008-≥16	0.25	2
C. krusei	130	6/6	0.01-8	0.5	2
C. parapsilosis	506	7/9	≤0.004-≥16	0.06	0.25
C. tropicalis	260	6/10	≤0.004-8	0.12	0.5
A. fumigatus	1,409	7/7	≤0.004-≥16	0.12	0.5
A. flavus	257	7/7	0.01-≥16	0.25	0.5
A. niger	173	4/5	0.01-≥16	0.25	1

[a]Including the complexes of *C. parapsilosis*, *C. glabrata* and *Aspergillus* spp.; *C. krusei* (*P. kudriazveii*). Variability or insufficient data precluded the proposal of ECVs for some species of both *Candida* and *Aspergillus*.

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

[c]ECVs for 97.5% of the statistically modelled population by ECOFFinder calculations and based on MICs by the commercial agar diffusion Etest method (15,29). Proposed method-dependent Etest ECV for *A. fumigatus* and posaconazole was 0.25 µg/ml, as reported elsewhere (23).

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

Species/agent	Mutation/ Method	No. of mutants with MIC (µg/ml) of:[b]							Total mutants ≤ECV[b]
Fluconazole	**SYO**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**≥8**	
C. albicans	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	**SYO**	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**≥8**	
C. albicans	E266D		1					7	2
	E266D/V4881		1					7	1
	V112I/G450R	1			4			1	1
	D116E/K128T/ V159I	3						1	3
									6/59
Posaconazole	**SYO**	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**≥8**	
C. albicans	V112I/G450R	1			2	1			1
	D116E/K128T/ V159I	3						1	3
									4/59
Voriconazole	**SYO**	**≤0.01**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**≥1**	
C. albicans	E266D	1	1		2			6	1
	E266D/V4881	1						7	1
	K128T	1	1					1	1
	D116E/K128T	4						4	4
									7/59
Itraconazole	**Etest**	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**≥8**	
A. fumigatus	G448S				1			4	1
	M220K						1		1
	I301T	1						1	1
									3/81
Voriconazole	**Etest**	**<0.06**	**0.12**	**0.2**	**0.5**	**1**	**2**	**≥4**	
A. fumigatus	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

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(Cont. Table 5)

Voriconazole	SYO	<0.06	0.12	0.25	0.5	1	2	≥4	
A. fumigatus	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

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

[b]The proposed SYO ECVs were: *C. albicans* versus fluconazole (1 µg/ml), itraconazole (0.12 µg/ml), posaconazole (0.06 µg/ml) and voriconazole (0.01 µg/ml); and for *A. fumigatus* and voriconazole (1 µg/ml). Etest ECVs were: *A. fumigatus* versus itraconazole (2 µg/ml) and voriconazole (0.5 µg/ml).

Table 6. Method-dependent ECVs of four triazoles for species of *Candida*, *Saccharomyces*, and *Aspergillus* by three susceptibility testing methods[a]

Species	Agent/ Method-dependent ECVs (µg/ml)											
	FLU			ITR			POS			VOR		
	SYO	Etest	CLSI	SYO	Etest	CLSI	SYO	Etest	CLSI	SYO	Etest	CLSI
C. albicans	1	AM	0.5	0.12	0.25	NA	0.06	0.12	0.06	0.01	0.03	0.03
C. dubliniensis	1	ID	0.5	0.25	ID	NA	0.12	ID	0.25	0.01	ID	0.03
C. glabrata	64	64	8	2	8	4	4	ID	1	2	2	0.25
C. guilliermondii	16	ID	8	1	ID	NA	1	ID	0.5	0.5	ID	0.12
C. krusei	128	ID	32	1	2	1	1	ID	0.5	1	2	0.5
C. lusitaniae	4	ID	1	0.5	ID	0.5	0.12	ID	0.06	0.03	ID	0.06
C. parapsilosis SC	2	4	1	0.25	AM	NA	0.12	0.12	0.25	0.01	0.25	0.03
C. parapsilosis SS	2	NA	NA	0.5	NA	NA	0.25	NA	NA	0.03	NA	NA
C. tropicalis	4	4	1	0.5	0.5	0.5	1	0.12	0.12	0.5	0.5	0.12
S. cerevisiae	16	ID	NA	ID	ID	NA	ID	ID	NA	ID	ID	NA
A. fumigatus	NA	NA	NA	AM	2	1	0.06[b]	0.25[b]	0.25[b]	1	0.5	1
A. flavus	NA	NA	NA	AM	1	1	NA	0.5	0.5	1	0.5	2
A. niger	NA	NA	NA	1	4	4	NA	0.5	2	ID	1	2
A. terreus	NA	NA	NA	AM	AM	2	NA	0.25	1	1	AM	2

[a]SYO/Etest proposed ECVs in the present study, based on MICs determined by both commercial, respectively, and CLSI broth microdilution (M27 and M38) methods (11,12,14,15). *C. guilliermondii* (*M. guilliermondii*), *C. krusei* (*P. kudriavzevii*) and *C. lusitaniae* (*Clavispora lusitaniae*).

[b]Posaconazole ECVs for *A. fumigatus* as reported elsewhere (23,26); the SYO ECV for *C. orthopsilosis* was 4 µg/ml.

AM: aberrant modes, modal variability; ID, insufficient number of laboratories/isolates entering the ECV definition pool.

NA: not available or applicable.

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