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EUCAST and CLSI: Working Together Towards a Harmonized Method for Antifungal Susceptibility Testing

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Abstract The U.S. Clinical and Laboratory Standards Institute (CLSI) and the European Committee of Antimicrobial Susceptibility Testing (AFST-EUCAST) have developed broth microdilution methodologies for testing yeasts and filamentous fungi (molds). The mission of these methodologies is to identify in vitro antifungal resistance, which is accomplished by the use of either clinical breakpoints (CBPs), or to a lesser degree, epidemiologic cutoff values (ECVs). The newly adjusted and species-specific CLSI CBPs for *Candida* spp. versus fluconazole and voriconazole have ameliorated some of the differences between the two methodologies. In the absence of CBPs for mold testing, CLSI ECVs are available for six *Aspergillus* species versus the triazoles, caspofungin and amphotericin B. Recently, breakpoints were developed by the EUCAST for certain *Aspergillus* spp. versus amphotercin B, itraconazole and posaconazole, which to some extent are comparable to ECVs. We summarize these latest accomplishments, which have made possible the harmonization of some susceptibility cutoffs, if not methodologies for some agent/species combinations.

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Introduction

The incidence and prevalence of invasive fungal infections continue to increase, especially among chemically induced immunosuppressed patients [1–3, 4•]. Such increase prompted the introduction of new systemic antifungal agents, and the development of standardized broth microdilution methods for the in vitro susceptibility testing of yeasts and filamentous fungi (molds) by both the U.S. Clinical and Laboratory Standards Institute (CLSI) [5–7] and the European Committee of Antimicrobial Susceptibility Testing (AFST-EUCAS) [8, 9]. The availability of reliable methodologies made possible the study of mechanisms of resistance and cross-resistance in *Candida* spp., and more recently in *Aspergillus* spp. [10•, 11•, 12•, 13•, 14, 15•, 16, 17, 18•, 19]. The association of genetic mutations, high minimum inhibitory concentrations (MICs) and clinical outcome has been also elucidated. Standardization also made possible the definition of clinical breakpoints (CBPs) for the more common *Candida* spp. (by both CLSI and EUCAST) [20•, 21•, 22•, 23•, 24•, 25•] and some *Aspergillus* spp. (by EUCAST) [26•], as well as epidemiologic cutoffs (ECVs) for a variety of *Aspergillus* (by CLSI and EUCAST), *Cryptococcus* and *Candida* species (by CLSI) [27•, 28•, 29•, 30•, 31, 32•, 33•, 34•, 35•], and the important antifungal agents (triazoles, echinocandins, amphotericin B and flucytosine) that are currently available for the treatment/prevention of invasive fungal infections [36–38]. This review will focus on the published events that have

made possible the comparison not only of testing conditions, but of susceptibility endpoints developed by both CLSI and EUCAST. Because of this, we only discuss testing parameters and cutoffs values when they are available by both organizations.

Antifungal Susceptibility Methods

CLSI and EUCAST Parameters for Testing *Candida* spp.

The CLSI and EUCAST broth microdilution standardized methodologies have been discussed in detail elsewhere [10•, 11•, 39, 40], as well in the CLSI (M27-A3) and EUCAST (EDef 7.2) documents [5–9]. The similar testing conditions are (Table 1): RPMI-1640 (RPMI) broth, incubation temperatures (35° and 37° C) and time (24 h), a prominent inhibition of growth (≥50 %) for testing the susceptibilities of *Candida* spp. to triazoles and echinocandins. The differences are: inoculum densities (0.5×10^3 to 2.5×10^3 CFU/ml and 0.5×10^5 to 2.5×10^5 CFU/ml), the RPMI broth glucose content (0.2 % and 2.0 %), the microdilution wells (round and flat-bottom), and MIC determination (visual and spectrophotometric). Despite these differences, early and recent comparisons of EUCAST and the more practical CLSI 24 h triazole MICs [39–45], as well as of amphotericin B and flucytosine [45], have yielded comparable results regarding both essential, and more importantly, categorical agreement [42, 44].

CLSI and EUCAST Parameters for Testing *Cryptococcus* spp.

Another area where testing conditions differ between both organizations is for the *Cryptoccocus neoformans–C. gattii* species complex. As early as the 1990s, the CLSI established that, except for the incubation time (72 h), the standard parameters for this group of fungi were the same as those for *Candida* spp. [5, 6]. Based on large numbers of isolates of these species (from numerous laboratories) for which MICs were collected during the last 10 to 15 years, ECVs were defined by the CLSI for the *Cryptoccocus neoformans–C. gattii* species complex versus amphotericin B, flucytosine and the triazoles [33•, 34•]. In 2012, the EUCAST methodology was adopted for the testing of *Cryptococcus* species, and the plates read when the OD value is above 0.2. In cases with insufficient growth, it is suggested that the test be repeated, but that the trays be incubated at 30 °C [8, 46•]. The comparison with CLSI methodology was complicated, since growth by the CLSI method was insufficient to allow spectrophotometric MIC determination.

CLSI and EUCAST Parameters for Testing Molds

The CLSI M38-A2 document provides guidelines for non-dermatophyte molds with amphotericin B, triazoles and echinocandins [7]. The EUCAST has a similar broth

Table 1 Parameters for the performance of CLSI and EUCAST methods

	CLSI M27-A3 document for yeasts	EUCAST EDef. 7.2 document for yeasts*	CLSI M38-A2 document for molds	EUCAST EDef 9.1 document for molds*
Microplate well shape	Round bottom	Flat bottom	Round bottom	Flat bottom
Test medium	RPMI 1640 with 0.2 % glucose	RPMI 1640 with 2 % glucose	RPMI 1640 with 0.2 % glucose	RPMI 1640 with 2 % glucose
Inoculum size	0.5 to 2.5×10^3 CFU/ml	0.5 to 2.5×10^5 CFU/ml	0.4×10^4 to 5×10^4 CFU/ml	2×10^5 to 5×10^5 CFU/ml
Incubation time	24–48 h: *Candida* spp. 72 h: *Cryptococcus neoformans*	Same When OD>0.2: *C. neoformans*	24–48 h (echinocandins); 48 h other agents	Same
Temperature	35 °C	35 °C	35 °C	35 °C
Reading	Visual	Spectrophotometric	Visual	Visual
MIC criteria	Amphotericin B: the lowest drug concentration that prevents any discernible growth (clear wells).	Same	Amphotericin B, triazole MIC: the lowest drug concentration that prevents any discernible growth.	Same
	Azoles, echinocandins, flucytosine: the lowest drug concentration that inhibits ≥ 50 % of growth as compared to control.	Same	Echinocandin MEC: the lowest drug concentration that shows morphological changes.	Same

CLSI U.S. Clinical and Laboratory Standards Institute, *EUCAST* European Committee of Antimicrobial Susceptibility Testing, *MIC* minimal inhibitory concentration, *MEC* minimal effective concentrations

* http://www.eucast.org [5–9]

microdilution standard, again except for the sugar content of the RPMI broth (2 % and 0.2 % dextrose), the inoculum concentrations (~10^5 CFU/ml adjusted by conidial counting and ~10^4 CFU/ml adjusted by spectrophotometer), and the final DMSO concentration (0.5 % and 1 %) [7, 9]. Both methods recommend the determination of minimal effective concentrations (MECs) when testing echinocandins, instead of the traditional MIC when evaluating other agents [7, 9]. An early comparison of testing conditions for molds demonstrated that the higher EUCAST inoculum size and glucose RPMI concentration did not significantly elevated MICs of amphotericin B and itraconazole [47]. But a recent study reported that voriconazole EUCAST MICs are usually one dilution higher that CLSI values [32•].

Interpretive Guidelines for *Candida* and *Aspergillus* Species

CLSI and EUCAST Clinical Breakpoints for *Candida* spp.

As for any antimicrobial agent, detection of in vitro resistance is an important factor in the treatment of invasive fungal infections (e.g., *Candida* infections) for which agents have been licensed. For in vitro results to be meaningful, CBPs or ECVs should be available and established using data obtained by standardized methods. During the last two years, CBPs that serve to differentiate an organism as treatable or nontreatable [48•] have been adjusted (lowered) by the CLSI for most of the common *Candida* spp. versus fluconazole, voriconazole and the three echinocandins (Table 2) [20•, 21•, 22•]. Also, EUCAST breakpoints are available for amphotericin B, anidulafungin, fluconazole, posaconazole, and voriconazole [23•, 24•, 25•].

CBPs for Candida spp. and Fluconazole and Voriconazole

Between 1997 and 2006, the CLSI established susceptible (≤8 μg/ml for fluconazole and 1 μg/ml for voriconazole) and resistant (≥64 μg/ml and ≥4 μg/ml, respectively) CBPs to encompass all *Candida* spp. During the last three years, the following important factors demanded the adjustment of these CBPs: the CLSI validated the 24 h (when growth permits it) incubation time for testing these agents [41], the need to have species-specific CBPs, and the perception that the original CBPs were not sensitive enough to identify in vitro resistance to these agents, especially when testing *C. albicans, C. parapsilosis* and *C. tropicalis*. Therefore, using mutant strains (e.g., due to either target enzyme modifications or reduction of access of the triazole to the target), new pharmacokinetic (PK) and pharmacodynamic (PD) information, MIC distributions, and clinical experience with either mucosal and invasive candidiasis (fluconazole) or with non-neutropenic candidemia patients (voriconazole), CLSI CBPs were adjusted: susceptible (≤ 2 μg/ml for fluconazole and ≤0.125 μg/ml for voriconazole) and resistant (≥ 8 μg/ml and ≥1 μg/ml, respectively) for *C. albicans, C. parapsilosis* and *C. tropicalis* [20•, 21•]. No susceptible endpoint was established for *C. glabrata* and fluconazole. For the combination of voriconazole and *C. glabrata*, insufficient data that demonstrates a correlation between CLSI MICs and response to therapy precluded the definition of CBPs. These species-specific CBPs would better recognize non-treatable or resistant isolates. Therefore, although both organizations disagree with respect to the resistant endpoint of voriconazole and *C. albicans*, *C. parapsilosis* and *C. tropicalis* (resistant endpoints: EUCAST≥0.25 μg/ml and CLSI≥1 μg/ml), there is complete agreement regarding fluconazole resistant endpoint for these three species (≥8 μg/ml) (Table 2).

CBPs for Candida spp. and the Echinocandins

In 2008, the CLSI established a susceptible CBP (≤2 μg/ml) for echinocandins and all *Candida* spp. However, using *fks1* mutant strains, Garcia-Effron et al. [49, 50] demonstrated that although caspofungin MICs>2 μg/ml captured almost 100 % of mutant strains, the MICs of anidulafungin and micafungin that captured 95 % of these mutants were lower for *C. albicans* (>0.5 μg/ml) and *C. glabrata* (>0.25 μg/ml). More recently, the single susceptible echinocandin CBP was adjusted to species-specific CBPs; susceptible ≤0.25 μg/ml and resistant ≥1 μg/ml for *C. albicans*, *C. krusei* and *C. tropicalis*; lower interpretive endpoints were established for *C. glabrata*, which also were echinocandin-dependent (Table 2). Since the EUCAST has not established interpretive breakpoints for caspofungin and micafungin, due to insufficient evidence of correlations between in vitro and clinical response, we focus on anidulafungin CBPs (Table 2). EUCAST breakpoints for anidulafungin are based on ECVs, clinical experience and as pharmacokinetic information [24•]. Although both organizations are in agreement regarding the resistant endpoint of anidulafungin and *C. parapsilosis* (≥8 μg/ml), once more, EUCAST resistant endpoints are lower for *C. albicans* (≥0.06 μg/ml versus ≥1 μg/ml), and *C. glabrata*, *C. krusei*, and *C tropicalis* (≥0.12 μg/ml versus ≥0.5 μg/ml and ≥1 μg/ml); differences among susceptible endpoints are depicted in Table 2. The reason for these discrepancies could be that although clinical data from three clinical trials were used for the definition of anidulafungin EUCAST endpoints, correlation of EUCAST MICs and clinical response was not possible, since EUCAST in vitro data were not obtained at the time [24•].

Table 2 Breakpoints or ECVs of antifungal agents approved by European Committee of Antimicrobial Susceptibility Testing, (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) for susceptibility testing of *Candida*. Data in μg/ml

Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible or WT (ECV)*	S-DD	Intermediate	Resistant or non-WT*
Fluconazole	*C. albicans*	≤2	4	>4	≤2	4	–	>4
	C. glabrata	–	≤32	>32	–	≥32	–	≥64
	C. krusei	PT	PT	PT	PT	PT	PT	PT
	C. parapsilosis	≥2	4	>4	<2	4	–	≥8
	C. tropicalis	≥2	4	>4	≤2	4	–	≥8
Voriconazole	*C. albicans*	0.125	–	>0.125	≤0.125	–	0.25–0.5	≥1
	C. glabrata	IE	IE	IE	IE	IE	IE	IE
	C. parapsilosis	0.125	–	>0.125	≤0.125	–	0.25–0.5	≥1
	C. tropicalis	0.125	–	>0.125	≤0.125	–	0.25–0.5	≥1
Anidulafungin	*C. albicans*	≤0.03	–	>0.03	0.25	–	0.5	≥1
	C. glabrata	≤0.06	–	>0.06	0.125	–	0.25	≥0.5
	C. krusei	≤0.06	–	>0.06	0.25	–	0.5	≥1
	C. parapsilosis.	–	≤2	>2	≤2	–	4	≥8
	C. tropicalis	≤0.06	–	>0.06	0.25	–	0.5	≥1
Amphotericin B	*C. albicans*	≤1	–	>1	≤2*	–	–	>2*
	C. glabrata	≤1	–	>1	≤2*	–	–	>2*
	C. krusei	≤1	–	>1	≤2*	–	–	>2*
	C. parapsilosis	≤1	–	>1	≤2*	–	–	>2*
	C. tropicalis	≤1	–	>1	≤2*	–	–	>2*

***ECV**: Epidemiological cutoff (also known as CO_{WT}); it applies only to amphotericin B and posaconazole because CLSI has not approved breakpoints for these two agents and any fungal species.

PT: Susceptibility testing not recommended, as the species is a poor target for therapy with the drug or intrinsically resistant to fluconazole and MICs for this species should not be interpreted using these cutoffs

IE: Insufficient evidence to set breakpoints

S-DD: susceptible dose dependent

Epidemiological Cutoff Values (ECVs) for *Candida* spp.

In contrast to CBPs, the ECV is the susceptibility endpoint that differentiates between wild type (WT, isolates not harboring resistant mechanisms) from non-WT isolates (harboring one or more resistant markers) [48•, 51]. Among *Candida* spp., ECVs are usually lower than the CBP. Although an organism with an MIC above the drug ECV (non-WT) shows reduced susceptibility and is less likely to respond to the agent being evaluated as compared to the WT population, it may respond if the MIC is below the breakpoint. CLSI CBPs for *Candida* spp. versus itraconazole, amphotericin B and flucytosine are not available, due the lack of sufficient clinical and in vitro data to demonstrate correlations. Instead, the CLSI has recently defined ECVs that are based on CLSI MIC data from multiple laboratories [35•]. We focus on the comparison of CLSI amphotericin B ECVs and EUCAST breakpoints for *Candida* spp. (Table 2), since the latter organization has no interpretive values for either itraconazole or flucytosine; CLSI ECVs for those two latter agents can be found elsewhere [35•, 40].

ECVs and Breakpoints for Candida spp. and Amphotericin B

The EUCAST resistant amphotericin B breakpoints are lower (>1 μg/ml) than the CLSI non-WT endpoints (or isolates with reduced susceptibility to amphotericin B; >2 μg/ml) for the common *Candida* spp. (Table 2). These differences must be due to testing conditions. Based on peak attainable serum concentrations of 2 μg/ml and the pharmacodynamic correlate of the peak serum concentration-to-MIC ratio of 2 as the predictor of maximal activity, the cutoff of amphotericin B resistance traditionally has been an MIC of ≥1 μg/ml [40]. But so far, attempts to correlate this vitro result (≥1 μg/ml) with clinical outcome in *Candida* infections have failed [52]. One of the problems is the scarcity of *Candida* isolates

for which the MIC is >2 μg/ml. Therefore, a *Candida* isolate with such a high MIC should be considered unusual and less likely to respond to amphotericin B therapy.

CLSI and EUCAST ECVs and Breakpoints for *Aspergillus* spp.

Because recent clinical studies (not designed for correlation purposes) have only provided some insights regarding the potential value of MICs in certain mold–drug combinations, CLSI CBPs are not available for mold testing. For example, during voriconazole clinical trials for aspergillosis, its efficacy data is based on response to treatment of patients infected mostly with WT isolates. However, CLSI ECVs are available for six species of *Aspergillus* (*Aspergillus fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. versicolor*) and five antifungal agents (amphotericin B, three triazoles and caspofungin) [28•, 29•, 30•]; Table 3 depicts CLSI ECVs and available EUCAST breakpoints [26•]. As for *Candida* spp., ECVs could characterize the susceptibility of *Aspergillus* spp. to antifungal agents and monitor the emergence of strains with mutations and reduced antifungal activity.

ECVs and breakpoints for Aspergillus spp. and Amphotericin B

Although there are available amphotericin B CLSI ECVs for six *Aspergillus* spp., EUCAST has defined breakpoints only for *A. fumigatus* and *A. niger*, and both values are one dilution lower than CLSI ECVs (Table 3) [26•, 30•]. Information is scarce regarding the relationship between resistance mechanisms, high amphotericin MICs and clinical response to therapy; it is mostly available for *A. terreus* (intrinsically resistant to this agent) and *A. flavus* [10•, 11•]. Alteration of the cell wall composition of *A. flavus* has been responsible for amphotericin B resistance (MICs>1 μg/ml), while catalase production instead of ergosterol content has played a role in *A. terreus* resistance to this agent [12•].

ECVs and Breakpoints for Aspergillus spp. and Triazoles

The clinical relevance of mold testing remains uncertain, but both CLSI and EUCAST methodologies have elucidated the complexity of cross-resistance among triazoles, especially in *A. fumigatus*. Triazole resistance (MICs>2 μg/ml) has been associated with single or multiple point mutations of the *cyp51A* gene in *A. fumigatus* and *A. flavus,* as well as the incidence of multiazole, cross-resistance and patient failure to triazole treatment [13•, 14, 15•, 16, 27•, 28•]. However, other host and drug factors cannot be ignored. Triazole molecular resistance mechanisms have not been identified for the other non-*A. fumigatus* spp., but the presence of the two *cyp51* genes was reported in *A. terreus*, and azole resistance has been associated with multiplication of *cyp51A* in an engineered laboratory strain of *A. niger* [11•].

The CLSI and EUCAST have defined itraconazole and voriconazole ECVs of ≤1 μg/ml for *A. fumigatus* (non-WT MICs≥2 μg/ml) [27•, 28•]; these values were corroborated in a recent study [31]. With the exception of ECVs for *A. nidulans* and itraconazole (≤2 μg/ml by the CLSI and ≤1 μg/ml by the EUCAST), ECVs and breakpoints for *A. flavus* and *A. terreus* versus itraconazole by both organizations are the same (Table 3) [26•, 28•]. More recently, two potential susceptible breakpoints for voriconazole and *A. fumigatus* using either CLSI (susceptible, ≤0.5 μg/ml; resistant, >1 μg/ml) or the EUCAST (susceptible, ≤1 μg/ml; resistant, >2 μg/ml) methodologies also were suggested; these breakpoints were based on the fact that as the voriconazole MIC increased, a higher area under the concentration time curve (AUCs) was needed to achieve suppression of galactomannan in an in vitro dynamic model of invasive pulmonary aspergillosis [32•]. Again, the voriconazole ECV of 1 μg/ml was corroborated, but the EUCAST ECV was one dilution higher in that recent report (WT [no mutations] range by the CLSI 0.5–1 μg/ml and by the EUCAST 0.5–2 μg/ml). The EUCAST has not yet made decisions regarding this agent and *Aspergillus* spp.

Posaconazole ECVs for *A. fumigatus* in three different studies have been either 0.12 μg/ml or 0.25 μg/ml [27•, 28•, 31]. In 2011, the EUCAST established ≤0.12 μg/ml as the susceptible breakpoint for posaconazole versus *A. fumigatus* and *A. terreus*; both values are lower than the ECVs for these two species by the CLSI (Table 3) [26•].

In a recent study, 64 % of patients infected with a resistant *A. fumigatus* isolate were azole naïve and the case-fatality rate of azole-resistant invasive aspergillosis was 88 %. In addition, patients suffering a hematologic or oncologic disease were more likely to harbor an azole-resistant isolate than were other patient groups ($p<0.05$) [14]. Verweij et al. [53] recommended dose escalation of voriconazole and posaconazole with isolates for which MICs are above the ECV. The other consideration is bioavailability, e.g., the limited ability to increase posaconazole exposure with the oral solution and the variability of triazole serum levels. In an animal model of *A. fumigatus* (*cyp51A* mutations), the standard dosing regimen needed to be increased when the posaconazole MIC for the infecting isolate was 0.5 μg/ml to avoid treatment failure [15•]. All these results indicate that these endpoints could be useful in the monitoring and selection of antifungal therapy in aspergillosis, but more data needs to be gathered for them to be clinically relevant. This is important, since itraconazole, voriconazole and posaconazole have a role in the prevention and treatment of chronic, allergic or invasive aspergillosis.

Table 3 CLSI (ECVs) and EUCAST (breakpoints) susceptibility endpoints for six *Aspergillus* spp. at 48 h. Data in μg/ml

Antifungal agent and species (CLSI no. Isolates)[a]	CLSI MIC or MEC ECV-WT (μg/ml)[b]	CLSI MIC or MEC Non-WT (μg/ml)	CLSI Mode (μg/ml)[c]	S-BP (μg/ml)[d,e]	EUCAST R-BP (μg/ml)[d]
Amphotericin B					
Aspergillus fumigatus (3,988)	≤2	≥4	0.5	≤1	>2
Aspergillus flavus (793)	≤2	≥4	1	NA	NA
Aspergillus nidulans (184)	≤4	≥8	1	NA	NA
Aspergillus niger (673)	≤2	≥4	0.5	≤1	>2
Aspergillus terreus (545)	≤4	≥8	2	NA	NA
Aspergillus versicolor (135)	≤2	≥4	1	NA	NA
Itraconazole					
Aspergillus fumigatus (2,544)	≤1	≥2	0.5	≤1	>2
Aspergillus flavus (536)	≤1	≥2	0.5	≤1	>2
Aspergillus nidulans (141)	≤2	≥4	0.5	≤1	>2
Aspergillus niger (427)	≤2	≥4	1	NA	NA
Aspergillus terreus (369)	≤1	≥2	0.25	≤1	>2
Aspergillus versicolor (68)	≤2	≥4	1	NA	NA
Posaconazole					
Aspergillus fumigatus(1,647)	≤0.25	≥0.5	0.06	≤0.12	>0.25
Aspergillus flavus (321)	≤0.25	≥0.5	0.06	NA	NA
Aspergillus nidulans (129)	≤1	≥2	0.25	NA	NA
Aspergillus niger (325)	≤0.5	≥1	0.5	NA	NA
Aspergillus terreus (330)	≤0.5	≥1	0.25	≤0.12	>0.25
Aspergillus versicolor (41)	≤1	≥2	0.5	NA	NA
Voriconazole					
Aspergillus fumigatus (2,778)	≤1	≥2	0.25	≤1[e]	≥2[e]
Aspergillus flavus (590)	≤1	≥2	0.5	NA	NA
Aspergillus nidulans (139)	≤0.5	≥1	0.125	NA	NA
Aspergillus niger (479)	≤2	≥4	0.5	NA	NA
Aspergillus terreus (462)	≤1	≥2	0.5	NA	NA
Aspergillus versicolor (80)	≤2	≥4	0.25	NA	NA

CLSI U.S. Clinical and Laboratory Standards Institute, *EUCAST* European Committee of Antimicrobial Susceptibility Testing, *ECV* epidemiologic cutoff values, *MIC* minimum inhibitory concentration, *MEC* minimal effective concentrations, *NA* not available

[a] Number of MICs/isolates used for the establishment of CLSI ECVs [28•, 29•, 30•]

[b] Calculated ECVs comprising ≥95 % of the statistically modeled population; WT=Wild-type [28•, 29•, 30•]

[c] Most frequent minimum effective concentration (MEC) as determined in CLSI studies [28•, 29•, 30•].

[d] S-BP=susceptible-breakpoint; R=resistant-breakpoint

[e] The ECV and not the BP is listed for *A. fumigatus* and voriconazole as reported in ref. [27•]. However, the EUCAST has recently proposed this value as the BP for this agent/species combination.

ECVs for Aspergillus spp. and Caspofungin

Caspofungin ECVs of 0.25 and 0.5 μg/ml are also available for six *Aspergillus* spp. by the CLSI; these values are listed elsewhere [29•]. But, ECVs have not been defined for any other echinocandins or by the EUCAST for any species and echinocandin. Scarce information is available regarding high caspofungin MECs and clinical failure and the presence of *FKS1* resistant mutations [18•]. Yet, caspofungin MECs≥1 μg/ml for three of four isolates of *A. fumigatus*, and breakthrough infections were observed in patients receiving either empirical or prophylactic caspofungin therapy [54], but genetic studies were not performed. The latter results are in agreement with those reported for a laboratory mutant of *A. fumigatus* (caspofungin MEC≥16 μg/ml with a S678P amino acid change) [17].

Conclusions

Standardized broth microdilution (CLSI and EUCAST) and disk diffusion (CLSI) methods are available to test licensed

antifungal agents against both *Candida* spp. and *Aspergillus* spp. Standardized broth microdilution methodologies have allowed the establishment of species-specific breakpoints for most of the common *Candida* spp. versus fluconazole and voriconazole (CLSI and EUCAST), posaconazole and anidulafungin (EUCAST) and the three echinocandins (CLSI). In the absence of breakpoints, species-specific ECVs have been defined for amphotericin B, itraconazole and flucytosine versus most common *Candida* spp.; for amphotericin B, triazoles and caspofungin versus six *Aspergillus* spp.; and for amphotericin B, triazoles and flucytosine versus the *Cryptococcus neoformans-Cryptococcus gattii* species complex (CLSI). On the other hand, the EUCAST has recently established breakpoints for some *Aspergillus* spp. versus amphotericin B, posaconazole (two species), and itraconazole (four species). It is expected that ECVs may serve as sensitive markers for the emergence of isolates with decreased susceptibility to the agent being evaluated, or to separate WT from non-WT isolates. The association of non-WT with mechanisms of resistance has been elucidated for some of these species/agent combinations, but more related information is needed.

The question is, how harmonized are CLSI and EUCAST standards? It is clear that there are basic differences in some of the testing conditions (e.g., inoculum size), but the shortening of the CLSI incubation time to 24 h for testing *Candida* spp. and the adjustment to species-specific and more sensitive CBPs indicates that fluconazole, and to some extent voriconazole, CBPs are similar, and that both methods are useful to identify and monitor in vitro resistance to these agents. The same applies to the comparison of available ECVs and breakpoints by both organizations. However, further efforts are needed, since there are several gaps in the development of susceptibility endpoints, as well as the relevance of the available ones beyond in vitro assays.

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Papers of particular interest, published recently, have been highlighted as:
• Of importance

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