EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Comments on proposed micafungin breakpoints for *Candida* spp.

In December 2012 the EUCAST rationale document for micafungin and *Candida* spp. was released for consultation. Comments received and responses from the EUCAST Antifungal Susceptibility Testing Subcommittee (EUCAST AFST) are listed below:

Comment	EUCAST AFST response
Australian Society for Antimicrobials, National AST Committee	
Introduction, paragraph 3, 4th sentence: Although obvious, we suggest adding “”before reporting micafungin susceptibility” to the end of the sentence	As stated we regard species identification mandatory and as the BPs are species specific, susceptibility classification cannot be done without it. Yet an MIC may be helpful even before the species ID is reached particularly in cases where this is delayed for whatever reason as a rough marker of the likelihood of this isolate being susceptible or not. Hence we have not revised the text as suggested, but as follows: “Therefore, and as the breakpoints are species specific, species identification is important and every attempt should be made to identify Candida to species level.”
2. MIC distributions It doesn’t seem that ECOFFs were calculated statistically. We would recommend statistical analysis. Attached are the statistical calculations using the Turnidge et al., method. We recommend the 97.5% value for ECOFFs – 0.03, 0.03, 0.25, 4, 0.06 respectively in the Table. We agree that there are too few data points to estimate an *C. guilliermondii* ECOFF	We agree that statistical calculation of ECOFFs is an unbiased and objective way forward. For micafungin the ECOFFs obtained by this approach are identical to the ones selected by the eyeball method for micafungin and candida except for *C. albicans* (one step higher), and *C. parapsilosis* (one step higher), which overall is reassuring. In the combined MIC data set presented in the RD table we have the un-truncated data from 3 laboratories, 98.9% of the isolates are below the selected ECOFF of 0.016 mg/l. Three more dataset were available. The first one was truncated at 0.03 mg/l but 100% of the isolates had MICs of <0.03mg/l indirectly suggesting (but not demonstrating) the ECOFF for this dataset would have been lower. The second dataset was truncated at 0.04 mg/l and 100% of the isolates were below our ECOFF of 0.016 mg/l. The third dataset was truncated at 0.016 mg/l and the MIC distribution for this dataset was clearly one dilution step higher than for the other 5 datasets, contributing 35 of the 39 isolates with an MIC of 0.03 mg/l. An ECOFF for *C. albicans* of 0.016 mg/l includes 97.2% of the *C. albicans* MICs and 99.4% if the dataset with the MICs elevated by one step were excluded. On this background we have not changed the ECOFF for *C. albicans.*

	For *C. parapsilosis* the same issue was observed. Data from 7 sources were included. For 14 isolates an MIC of 4 mg/l was found. 8/14 come from one specific laboratory and, despite including this dataset that apparently showed MICs one step higher than the other six, we still only have 1.9% of the isolates above our ECOFF of 2 mg/l.
5. Pharmacodynamics Are the values presented fAUC/MIC or total AUC/MIC? The column label states the former but the Comments section suggests the latter. It obviously reflects on the Monte Carlo simulation that follows.	The AUC/MIC targets are total drug targets, and the document has been amended accordingly
We note that the stated (f?)AUC/MIC value for stasis quoted for *C. albicans* is not found directly in Slater *et al*. Although the EUCAST MICs appear in the reference, the AUC_{24}s (plasma or kidney) of the different doses do not. Hence we believe it would be of value to provide more detail in the comments about what assumptions were made and how this (f?)AUC/MIC value was obtained– we assume that other data from that study were available to the AFST.	We agree with much of the discussion about the pharmacodynamic targets for *Candida albicans* and *Candida glabrata*. Some discussion to acknowledge similarities and differences with pharmacodynamic targets determined using CLSI methodology has been included in the rationale document. There are differences in experimental design between laboratories that may account for at least some of these differences. To date, there have not been head-to-head pharmacodynamic studies that have used both CLSI and EUCAST methods. The EUCAST AFST has always been extremely cautious about extrapolating pharmacodynamic data acquired using CLSI methodology to inform EUCAST breakpoints. Nevertheless, we agree that it is quite proper to discuss these results in the rationale document. Consequently, the document has been amended accordingly.
Similarly, we also note that the (f?)AUC/MIC value for stasis quoted for *C. glabrata* is not mentioned directly in Howard *et al*. Figure 6 of that publication suggests that stasis total AUC/MIC value of ~1000 may be correct. Again more detail in the comments about what assumptions were made and how this fAUC/MIC value was obtained are required.	The definition of stasis used in the rationale document is standard -i.e.- the drug exposure that is required to "hold" fungal burden steady. Thus the targets in the rationale document were determined by estimating the drug exposure required to achieve the same fungal burden as was present at the time therapy was initiated.
For both of these two species we suspect that the values quoted are for total AUC/MIC	Yes, as stated above.
We note that there are differences in MICs as determined by the CLSI versus EUCAST methods in Arendrup et al. AAC 2010; 54:426 (CLSI	Commented on above

about 2.8 fold higher than EUCAST – see attached spreadsheet). As such we would expect that AUC/MIC ratios using CSLI-generated values in the denominator would be lower. We found the following total AUC/MIC ratios for a static effect in mouse models: >Andes et al., AAC 2008; 52:3497 (CLSI MICs) *C. albicans* – mean AUC/MIC = 5148 *C. glabrata* – mean AUC/MIC = 2081 >Andes et al. AAC 2010; 54:2497 (CLSI MICs) *C. albicans* – mean AUC/MIC = 5299 *C. glabrata* – mean AUC/MIC = 1542 *C. parapsilosis* – mean AUC/MIC = 1981 >Slater et al., AAC 2011; 55:3075 (EUCATST MICs) *C. albicans* – stated in this rationale document to be 2420 >Howard et al., AAC 2011; 55:4880 *C. glabrata* – stated in this rationale document to be 1283 Thus the opposite seems to be true, i.e. AUC/MIC ratios defining a static effect in the mouse model seem to be higher. This needs some discussion. We wonder about the reporting of the CLSI MIC values in Arendrup et al. AAC 2010; 54:426, as they seem to be substantially higher in the wild type than those reported in Andes et al, AAC 2011: 55:2113. Overall, we are not sure why these other animal models are not discussed in the document, and any potential conflicting information dealt with. Otherwise, the choice of PD targets will be disputed. We note that the PK-PD analysis	

of clinical trial data (Andes et al, AAC 2011: 55:2113) suggested a target total AUC/MIC ratio of 5000 for *C. albicans* mainly and a lower target (285) for *C. parapsilosis*. As these were estimated based on CLSI MICs (at least stated explicitly for one of the two published studies which formed the basis of the analysis, this also needs discussion.	
6. Monte Carlo simulations We note that Andes et al. referred to in the last sentence did not specifically address *C. glabrata*, rather it just dealt with *C. parapsilosis* and "all" others.	It is correct that the Andes paper does not specifically address either *C. albicans* or *C. glabrata*, but "non-*C. parapsilosis"* which is dominated by *C. albicans.* Yet the target for this group is MIC ≤ 0.016 mg/l, so we feel the statement is valid. A summary of this study has been included in section 7 as suggested and as described below. We have revised the last sentence which refers to the Andes paper as follows: "Similar estimates have been obtained using clinical data and the CLSI MIC method as summarised below (7. Clinical data, last paragraph) (Andes et al, Antimicrobial Agents Chemother 2011; 55: 2113–2121)."
7. Clinical data Given that Andes et al, AAC 2011: 55:2113 actually addresses clinical data, a summary of its contents might be useful here, especially the PK-PD targets and cutoffs which align with the EUCAST Monte Carlo analyses.	As suggested we have included a summary of the contents as follows: **Target attainment analysis for invasive candidiasis:** The Micafungin PK/PD MIC cut off values were determined based on the clinical data from two clinical studies (Kuse et al, Lancet 2007; 369: 1519–27 and Pappas et al, Clin. Inf. Dis. 2007; 45: 883–93). The approved micafungin regimen for treatment of invasive candidiasis or candidemia (100 mg/day) was used. MIC determination according to CLSI was used. Micafungin exposures were estimated using a population pharmacokinetic model, and univariable and multivariable logistic regressions were used to identify factors associated with outcome, including the micafungin area under the concentration-time curve (AUC)/MIC ratio. Monte Carlo simulation was used to evaluate the probability of achieving AUC/MIC ratios associated with efficacy. If one considered the AUC/MIC ratio target for efficacy identified for the *C. parapsilosis* cohort (AUC/MIC ratio > 285), the target would be expected to be attained for organisms with MIC values ≤ 0.25 mg/l. If one considered the AUC/MIC ratio target for efficacy identified for the non-*C. parapsilosis* cohort (mainly *C. albicans,* AUC/MIC ratio > 5000), the target would be expected to be attained for organisms with MIC values ≤ 0.016 mg/l. (Andes et al, Antimicrobial Agents Chemother, 2011; 55: 2113-2121).
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P2/12 consider changing "target protein" to Fksp to be more	Revised as suggested

specific and clear	
P3/12 consider adding a title to the table such as: 2. MIC Distributions and Epidemiological Cut-off (ECOFF) values (mg/L)' as in the Anidulafungin Rationale document	Revised as suggested
P3/12 consider changing order of species in Table either alphabetical order, or ideally group parapsilosis and guilliermondii together as they have inherent fks polymorphisms and therefore an elevated MIC distribution. Currently C. tropicalis seems oddly placed.	Historically we only had the five most common ones which were listed alphabetically. For anidulafungin and voriconazole *C. guilliermondii* was added but in a systematic way we agree as it was listed below the others in the anidulafungin RD but included in the alphabetic order for the voriconazole RD. We have revised the order in this and the anidulafungin RD to follow that of voriconazole.
P2/12 typo in dosage section. "Most common dose 100 mg mg/day" please remove first "mg"	Typographic error has been corrected.
P5/12 column header "children <5 years" space missing between children and <	Typographic error has been corrected.
P5/12 inconsistent use of "," and "." In total body clearance row.	Typographic error has been corrected.
P7/12 last sentence: "similar estimates have been obtained using CLSI" please add "methodology" after CLSI.	Sentence has been revised and now reads: "Similar estimates have been obtained using clinical data and the CLSI MIC method as summarised below (7. Clinical data, last paragraph)
P6/12 consider repeating "liposomal" when stating "amphotericin" throughout the paragraph.	Liposomal added throughout
P9/12 last sentence: please italicize Aspergillus. Also make p-value a lower case P instead of capitalized.	Revised as suggested
P 2/12, 10/12 and other places which describe Mycamine approved dosing, the cut-off for weight greater than **or equal** to 40 kg for the mg dosing for each indication and less than 40 kg for the mg/kg dosing.	Revised as suggested