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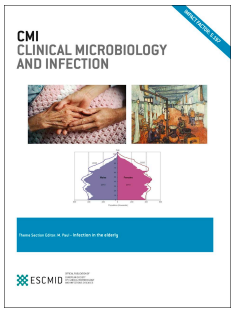

How to: interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST)

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How to: Interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Running title: Update on revised EUCAST antifungal breakpoints

Indented category: "How to" review

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Abstract

Background: EUCAST has revised the definition of the susceptibility category "I" from "Intermediate" to "Susceptible, Increased exposure". This implies that "I" can be used where the drug-concentration at the site of infection is high, either because of dose escalation or through other means to ensure efficacy. Consequently, "I" is no longer used as a buffer-zone to prevent technical factors from causing misclassifications and discrepancies in interpretations. Instead, an "Area of Technical Uncertainty" (ATU) has been introduced for MICs that cannot be categorised without additional information as a warning to the laboratory that decision on how to act has to be made. To implement these changes, the EUCAST-AFST (Subcommittee on Antifungal Susceptibility Testing) reviewed all, and revised some, clinical antifungal breakpoints.

Objectives: To present an overview of the current antifungal breakpoints and supporting evidence behind the changes.

Sources: This document is based on the 10 recently updated EUCAST rationale documents, clinical breakpoint and breakpoint-ECOFF documents.

Content: The following breakpoints (in mg/L) have been revised or established for *Candida* species: micafungin against *C. albicans* (ATU=0.03); amphotericin B (S≤/>R=1/1), fluconazole (S≤/>R=2/4), itraconazole (S≤/>R=0.06/0.06), posaconazole (S≤/>R=0.06/0.06) and voriconazole (S≤/>R=0.06/0.25) against *C. dubliniensis*; fluconazole against *C. glabrata* (I≤/>R=16/32); and anidulafungin (S≤/>R=4/4) and micafungin (S≤/>R=2/2) against *C. parapsilosis.* For *Aspergillus,* new or revised breakpoints include: itraconazole (ATU=2) and isavuconazole against *A. flavus* (S≤/>R=1/2, ATU=2); amphotericin B (S≤/>R=1/1), isavuconazole (S≤/>R=1/2, ATU=2), itraconazole (S≤/>R=1/1, ATU=2), posaconazole (ATU=0.25) and voriconazole (S≤/>R=1/1, ATU=2) against *A. fumigatus*; itraconazole (S≤/>R=1/1, ATU=2) and voriconazole (S≤/>R=1/1, ATU=2) against *A. nidulans*; amphotericin B against *A. niger* (S≤/>R=1/1); and itraconazole (S≤/>R=1/1, ATU=2) and posaconazole (ATU=0.25) against *A. terreus*.

Implications: EUCAST-AFST has released 10 new documents summarising existing and new breakpoints and MIC-ranges for control strains. A failure to adopt the breakpoint changes may lead to misclassifications and sub-optimal or inappropriate therapy of patients with fungal infections.

Introduction

The EUCAST recently revised the definition of the "I" category from "Intermediate" to "Susceptible, Increased exposure". Before this change, the I-category was used in two very different scenarios. First, when a level of antimicrobial activity was associated with uncertain therapeutic effect. This implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated (as is the case for some antibiotics in the urine) or when a high dosage of drug can be used (as is the case for fluconazole and *C. glabrata*). Second, intermediate was used as a buffer zone to prevent small, uncontrolled, technical factors from causing misclassifications and major discrepancies in interpretations, for example when the MICs for susceptible and resistant organisms overlap.

Obviously, the clinical implication of these two scenarios is very different. In the first, the organism is susceptible given the circumstances mentioned are met, whereas in the second scenario the MIC alone cannot inform whether the organism is susceptible or not. To separate these scenarios, EUCAST revised the definition of the I-category to "Susceptible, Increased exposure" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection. For the second scenario, an Area of Technical Uncertainty (ATU) was introduced as a warning to alert the laboratory to the uncertainty of the MIC result and that the laboratory needs to decide how to react to the warning before reporting a susceptibility classification to the clinician.

Consequently, MICs falling in the former Intermediate category had to be reviewed and categorised as one of the following

1. S (susceptible) when current evidence supports that there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
2. I (Susceptible, Increased exposure) when current evidence supports that there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
3. R (Resistant) when current evidence supports that there is a high likelihood of therapeutic failure even when there is increased exposure.
4. ATU (Area of Technical Uncertainty) to warn the laboratory staff that the value is in an area where there are interpretative difficulties. The reason is that a breakpoint is in a place where reproducible interpretation cannot be achieved. The ATU is not related to uncertainties in the testing procedures although the natural unavoidable variation in testing will influence the actions that may need to be taken. The ATU assumes that the susceptibility test is correctly performed and that the MIC value obtained is correct in itself.

For the antifungal agents, the revised "I" category is therefore only applicable in situations where increased antifungal drug exposure can be achieved either because a dose escalation option is approved (example: fluconazole), because specific drug formulations of the same compound are associated with higher exposure (example: posaconazole gastric tablet and i.v. formulations compared to the oral solution), because high exposure can be documented through therapeutic drug monitoring (TDM, example: mould-active azoles) or because the compound is physiologically concentrated at the site of infection (no good examples for antifungals (yet) but well known for some antibacterials and urinary tract infections). The latter is relevant for some antibacterials, for example those concentrated in the urine during urinary tract infections. It is, however, not a common scenario for the antifungal agents used for invasive infections, although it might be appropriate for some antifungals also used as topical agents when more data on MIC and outcome relationships for superficial infections emerge.

The EUCAST antifungal susceptibility testing committee (EUCAST-AFST) has reviewed all current antifungal BPs and recently released a revised breakpoint table v 10.0 BPs and eight revised rationale documents. The process has involved a consultation among the national representatives in the full AFST Sub-committee (with representation of twenty nations) and subsequently a public consultation at the EUCAST website. Finally, the EUCAST steering committee has reviewed and approved the revised breakpoints. The important changes affect the majority of the former BPs set for *Aspergillus* and *Candida* species and are summarised in Tables 1 and 2 together with the key recommendations for MIC results in the ATU area. Below follows a description of the revised and new breakpoints and the considerations and evidence upon which the decisions were made.

Amphotericin B

Updates: The breakpoints have been revised for amphotericin B against *A. fumigatus* and *A. niger*. Breakpoints have been established for *C. dubliniensis*.

Background: Amphotericin B is licensed for treatment of systemic or severe *Candida* and *Aspergillus* infections (and other fungal infections). Elevated MICs have been reported for some *Aspergillus* species including *Aspergillus flavus, Aspergillus terreus*, *Aspergillus nidulans*, *Aspergillus lentulus* and *Aspergillus fumigatiaffinis* [1]. In contrast, the *in vitro* activity of amphotericin B against species of *Candida* is mostly uniform. Amphotericin B has limited clinical activity against *Candida lusitaniae* although the MICs are comparable to those for the other *Candida* spp. This is due to a higher mutational rate and less fungicidal activity when exposed to amphotericin B [2].

Considerations related to breakpoints: The PK/PD relationship of different amphotericin B formulations is not well understood and the link between serum concentration profiles of different formulations with their efficacy is not well defined. Hence, the revised definition of the "I" does not apply for amphotericin B as no evidence exists that dose escalation is a valid option for isolates in the former Intermediate category. Consequently, the former Intermediate categories (for *A. fumigatus* and *A. niger*) have been reclassified as R. For *Candida*, the breakpoints have remained unchanged and for *C. dubliniensis* breakpoints have been established S ≤1/ R>1 mg/L (Tables 1 and 2). Epidemiological cut off values (ECOFFs) and tentative ECOFFs have been established for a range of organisms lacking amphotericin B breakpoints allowing classification of such isolates as wildtype or non-wildtype.

Echinocandins

Updates: The breakpoints have been revised for anidulafungin and micafungin against *C. parapsilosis*, and for micafungin against *C. albicans*.

Background: The *in vitro* activity of the echinocandins against *Candida* species is not uniform. The species more frequently associated with human infections include *C. albicans, C. dubliniensis, C. glabrata*, *C. parapsilosis, C. tropicalis* and *C. krusei*, of which all but *C. parapsilosis* (and its sibling species *C. metapsilosis* and *C. orthopsilosis*) exhibit low MIC values. The underlying reason for the higher MICs for *C. parapsilosis* (and *C. guilliermondii*) is the presence of a naturally occurring amino-acid substitution(s) in the hot spot region of the Fks1 target enzyme, known to confer resistance in other species. Therefore, species identification is important and every attempt should be made to identify *Candida* to species level. Susceptibility testing of caspofungin has been associated with a level of variation prohibitive for breakpoint setting [3,4]. As there is a high degree of cross-resistance between the three echinocandins, isolates categorised as anidulafungin and micafungin susceptible can be regarded as susceptible to caspofungin until drug specific breakpoints are available for caspofungin [5]. Isolates with discrepant classification to anidulafungin and micafungin (e.g. Anidulafungin S and Micafungin R), should be further analysed with target gene sequencing as such isolates may harbour "weak mutations" causing a discrete loss of susceptibility.

Considerations related to breakpoints:

i) Echinocandins and *C. parapsilosis*. The *C. parapsilosis* wildtype populations were classified as intermediate for anidulafungin and micafungin with the former breakpoints [6]. The reasons were, a) that the outcome was numerically better in the fluconazole arm than the anidulafungin arm in the randomized,

double-blind, non-inferiority trial of Reboli *et al* [7]; b) that echinocandin use has been associated with persistent candidaemia compared with both fluconazole and amphotericin B in subgroup analyses of randomized trials restricted to patients with *C. parapsilosis* [8]; and c) that an increase in *C. parapsilosis* was associated with caspofungin use at some centres [9,10]. An "increased exposure" option is not applicable for the echinocandins as no dose escalation option exists. *C. parapsilosis* was reclassified as susceptible for the following reasons: a) the echinocandins have been used for almost two decades as initial therapy (before the species identification is known) but also as continued therapy after the species ID is available because it is classified as susceptible by the CLSI [11]; b) in a recent retrospective observational cohort study, including 307 unique patients with *C. parapsilosis* candidaemia of whom 126 (41%) received fluconazole and 181 (59%) received an echinocandin, mortality was equal (fluconazole 9.5% vs echinocandin 9.9%, (OR 1.05, 95% CI 0.49–2.26)) [12]; c) fluconazole resistance is emerging in *C. parapsilosis* in some countries in which case echinocandins are a valid alternative considering the study above and the amphotericin B related toxicity [13–17]; and d) that treatment guidelines still emphasize that fluconazole is the preferred agent for *C. parapsilosis* when the isolate is susceptible thus limiting the risk of increased persistent candidaemia (Table 1) [18–21].

ii) Micafungin and *C. albicans*. The former susceptibility breakpoint for micafungin against *C. albicans* was stringent and only one dilution higher than the modal MIC (S: ≤0.016 mg/L, modal MIC 0.008 mg/L). EUCAST-AFST has been notified of frequent discrepant classifications of isolates as anidulafungin S and micafungin R in absence of Fks1 hot spot alterations [22,23]. EUCAST-AFST therefore collected Fks1 hot spot data for isolates with discrepant classification (micafungin of MIC 0.03 mg/L (R with former breakpoints) and anidulafungin MIC ≤ 0.03 mg/L (S with former and revised breakpoints)) and found no Fks1 alterations among 10 isolates (EUCAST-AFST, unpublished data). Additionally, reports of differential susceptibility to echinocandins confirmed in animal models are very limited and includes a *C. glabrata* where a the Fks1-S663F alteration conferred significant loss of efficacy to caspofungin (MIC 1 mg/L) and anidulafungin (MIC 0.5 mg/L) but not to the same extend to micafungin (MIC 0.06 mg/L) [24], and a case of *C. albicans* harbouring Fks1- R647R/G and P649P/L alterations conferring high level *in vitro* resistance to caspofungin and micafungin (MIC >1 mg/L) but not to anidulafungin (MIC = 0.03 mg/L) [25]. None of these cases involved isolates with the MIC combination of micafungin of MIC 0.03 mg/L and anidulafungin MIC ≤ 0.03 mg/L. Therefore, an ATU has been introduced for micafungin MIC of 0.03 mg/L against *C. albicans* with the advice that the MIC should be interpreted based upon the susceptibility to anidulafungin (Table 1).

Azoles

Updates: Breakpoints have been revised for fluconazole against *C. glabrata* and established for fluconazole, itraconazole, posaconazole and voriconazole against *C. dubliniensis*. Breakpoints have also been revised for isavuconazole, itraconazole, posaconazole and voriconazole against several *Aspergillus* species and established for isavuconazole against *A. flavus* and voriconazole against *A. nidulans*.

Background: The systemic azoles include fluconazole (spectrum includes *Candida* but not *Aspergillus*) and itraconazole, posaconazole, isavuconazole and voriconazole (spectrum includes both). The activity *in vitro* of fluconazole against species of *Candida* is not uniform. *C. albicans*, *C. dubliniensis, C. parapsilosis* and *C. tropicalis* tend to have relatively low MICs, whereas the MICs for *C. glabrata* tend to be higher. In addition, *C. krusei* is inherently resistant to fluconazole. The *in vitro* activity of the mould active azoles against the most prevalent species of *Aspergillus* is fairly uniform, although differences do occur even between the recently described and rarer "sibling" species belonging to the species complexes (e.g. *Aspergillus lentulus* belongs to the *A. fumigatus* complex and is multidrug resistant) [26]. Acquired resistance is reported with increasing frequency even among isolates obtained from azole-naive patients. The most commonly detected underlying mechanism is target gene alterations (*cyp51A*) with or without duplications in the promotor region of the target gene [27]. The degree of MIC elevation for isolates with Cyp51A alterations depend on the codon affected and the amino acid substitution, but in general confer a parallel MIC increase for itraconazole and posaconazole, and for voriconazole and isavuconazole, respectively [28–30]. Thus, correct species identification and susceptibility testing is of utmost importance.

Considerations related to breakpoints

i) Azoles and *Candida*: With the former breakpoints the entire wildtype population of *C. glabrata* was classified as intermediate for fluconazole [6]. This was in order to accommodate use in some clinical situations such as the treatment of urinary tract infections and mucosal infections managed in the primary health care setting, where alternatives are few. In cases where fluconazole is the only available antifungal agent for treating *C. glabrata* infections the use of a higher dosage may be required. However, with the revised definition of the "I" the concern was raised that an "I" category of ≤32 mg/L was too high with the new definition of the "I". The original ECOFF of 32 mg/L was set including EUCAST, Etest and CLSI MICs. Therefore EUCAST-AFST collected new datasets and included only those performed with the EUCAST E.Def 7.3 methodology [31]. Based on this dataset the ECOFF was revised to 16 mg/L. Consequently, the "I" category was maintained for *C. glabrata* but with a revised I breakpoint of ≤16 mg/L to acknowledge the use of fluconazole in some clinical situations provided a high dose (800 mg or 12 mg/kg) is prescribed (Table 3).

C. dubliniensis is closely related to *C. albicans*. The susceptibility pattern for the azoles is almost identical for wildtype isolates of the two species with *C. albicans* being <1 two-fold dilution more susceptible to azoles than *C. dubliniensis*. Hence, in the absence of species-specific MIC-outcome data and a sufficient number of MIC distributions to set final ECOFFs and breakpoints for *C. dubliniensis*, EUCAST-AFST adopted the breakpoints for *C. albicans* for *C. dubliniensis*.

ii) Azoles and *Aspergillus*: The former breakpoints included an intermediate category for itraconazole (2 mg/L), posaconazole (0.25 mg/L) and voriconazole (2 mg/L) against *Aspergillus* species. The Intermediate category served in part as a buffer zone between S and R. But it also reflected that the outcome for infections involving isolates with intermediate susceptibility depend on a number of other factors. These factors include: 1) the heterogeneity of *Aspergillus* infections (ranging from slow chronic infections to acute invasive infections); 2) the heterogeneity of the host's immune response (non-immunocompromised to severely neutropenic); 3) the variability in drug exposure (due to individual dosing, absorption and metabolism); and 4) the presence or absence of low grade resistance mechanisms (particularly in the setting of *A. fumigatus*) [31,32]. With the new definition, I requires a high likelihood of success with increased exposure. Increased exposure is in theory possible via TDM but concerns were raised because 1) evidence is lacking (apart from PK/PD data suggesting a relationship between exposure and outcome), 2) it takes time to increase exposure and TDM is not always available in a timely fashion and 3) invasive aspergillosis is a very severe infection with significant morbidity and mortality [33–35]. On the other hand, particularly for chronic and non-invasive infections, an MIC in the former intermediate range might be manageable and, with no other oral options, sometimes is the preferred option provided high levels can be obtained [36]. The revised breakpoints have been established to accommodate both aspects. Thus, an I-category has been omitted and the R breakpoint lowered 1 two-fold dilution to prevent risk of inappropriate therapy of invasive infections involving isolates with MICs 1 dilution above the original S breakpoint. However, in order not to deprive patients with milder infection and few other alternatives a treatment attempt an ATU has been introduced for the previous intermediate category. For itraconazole and voriconazole, MICs in the ATU should be reported as R with the following comment: "In some clinical situations (non-invasive infection forms) itraconazole/voriconazole can be used provided sufficient exposure is ensured" (Table 3). For isavuconazole and posaconazole the former S breakpoints cut into the wildtype distributions (isavuconazole S BP = 1 mg/L but ECOFF = 2 mg/L, and similarly posaconazole S BP is 0.125 mg/L but the ECOFF is 0.25 mg/L) because MIC distributions for wildtype and non-wildtype isolates overlap. The stringent breakpoints lead to many misclassifications of wildtype isolates as non-susceptible as noted in the rationale documents for these compounds [31,37]. Posaconazole resistance in the absence of itraconazole resistance and isavuconazole resistance in the absence of voriconazole resistance are rare and

not to our knowledge reported with robust supporting clinical evidence. Thus, isavuconazole MICs of 2 mg/L and posaconazole MICs of 0.25 mg/L are categorised as ATU with the recommendation to test voriconazole and itraconazole, respectively, and report as S or R depending of voriconazole and itraconazole susceptibility, respectively (Table 2).

ECOFFs and clinical breakpoints

Several factors are considered by EUCAST when clinical breakpoints are established, including dosing information, MIC distributions, ECOFFs, preclinical and clinical PK/PD, Monte Carlo simulations and PK/PD breakpoints and clinical data [32]. For ECOFF setting, at least five datasets, each consisting of at least 15 MICs, in total comprising at least 100 MICs, and with the modal MIC within ± 1 two-fold dilution from the most common modal MIC. This amount of data is often not available and then breakpoints are set with the available data when deemed appropriate. An example is the breakpoints set for *C. dubliniensis* because the close resemblance to *C. albicans* with respect to phylogeny, clinical infections and MICs.

For the species infrequently causing human infections sufficient data for breakpoint setting will not be available in the near future. For some of these species however, available MIC data allow setting tentative or final ECOFFs. ECOFFs are informative regarding the upper limit of the wildtype distribution, and when a microorganism has acquired resistance mechanisms, indicating that the clinical outcome may deviate from the general experience for that species. Moreover, ECOFFs allow a comparison with other species with respect to intrinsic susceptibility pattern. Therefore, an overview table of current EUCAST ECOFFs and breakpoints has been released this year and summarised as Tables 4 and 5. Until, species specific clinical breakpoints are established for the rarer species, a pragmatic approach is to prefer an antifungal agent for which the ECOFF does not exceed that for the most common species in that genus. The rationale behind this advice is that the most common species within a genus is in general the most virulent one and hence, what is appropriate to treat this organism is likely also appropriate for infections caused by other species with similar susceptibility patterns *in vitro* from that same genus. For *C. lusitaniae* for example the tentative amphotericin B ECOFF is equal to that for *C. albicans* whereas the fluconazole ECOFF is 32 times higher suggesting that amphotericin B should be preferred. EUCAST AFST is in the process of setting ECOFFs for a number of compounds and less common species. These ECOFFs will be released in due course.

Conclusion

The EUCAST AFST has reviewed all and revised many breakpoints for the antifungal agents to implement the revised EUCAST 2019 change in definitions of susceptibility categories S, I and R, especially relevant for the definition of "I" as "Susceptible, Increased exposure". "I" has been retained for fluconazole and voriconazole against all *Candida* species with advice on a dose escalation. An ATU has been introduced for micafungin against *C. albicans* and for isavuconazole and posaconazole against some *Aspergillus* species with the advice to use a "marker compound" to determine if the MIC in the ATU should be reported as S or R. ATU has also been introduced for itraconazole and voriconazole against several *Aspergillus* species with the recommendation to report as R but with the comment that the compounds may be considered for less severe non-invasive infections provided good drug exposure is achieved and ensured. We hope these changes will reduce confusion on how to act on S, I and R categories. **S** is for **S**usceptible, and for **S**imilar response as in other patients on **S**tandard dose. **I** is for susceptible **I**ncreased exposure, and for **I**ntelligence needed as **I**ncreased dosage is **I**mportant, and **R** is for **R**esistance, and for **R**isk because change of therapy is **R**equired.

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M.C.A has, over the past 5 years, received research grants/contract work (paid to the SSI) from Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics, Scynexis and T2Biosystems and speaker honoraria (personal fee) from Astellas, Gilead, MSD, and Seges Pfizer. She is the current chairman of the EUCAST-AFST.

N.F. has not, over the past 5 years, received any research grants nor contract work from any commercial/pharmaceutic company. She is a NAC representative in EUCAST-AFST.

M.M. has received funds for organizing educational activities on behalf of MSD.

G.K. has nothing to declare

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C.T.A. has nothing to declare

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E.C. has nothing to declare

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References

[1] Alastruey-Izquierdo A, Alcazar-Fuoli L, Cuenca-Estrella M. Antifungal Susceptibility Profile of Cryptic Species of Aspergillus. Mycopathologia 2014;178:427–33. https://doi.org/10.1007/s11046-014-9775-z.

[2] Atkinson BJ, Lewis RE, Kontoyiannis DP. Candida lusitaniae fungemia in cancer patients: risk factors for amphotericin B failure and outcome. Med Mycol 2008;46:541–6. https://doi.org/10.1080/13693780801968571.

[3] Arendrup MC, Rodriguez-Tudela J-L, Park S, Garcia-Effron G, Delmas G, Cuenca-Estrella M, et al. Echinocandin susceptibility testing of Candida spp. Using EUCAST EDef 7.1 and CLSI M27-A3 standard procedures: analysis of the influence of bovine serum albumin supplementation, storage time, and drug lots. Antimicrob Agents Chemother 2011;55:1580–7. https://doi.org/10.1128/AAC.01364-10.

[4] Espinel-Ingroff A, Arendrup MC, Pfaller MA, Bonfietti LX, Bustamante B, Canton E, et al. Interlaboratory variability of Caspofungin MICs for Candida spp. Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? Antimicrob Agents Chemother 2013;57:5836–42. https://doi.org/10.1128/AAC.01519-13.

[5] Arendrup MC, Perlin DS. Echinocandin resistance: an emerging clinical problem? Curr Opin Infect Dis 2014;27:484–92. https://doi.org/10.1097/QCO.0000000000000111.

[6] Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. EUCAST technical note on Candida and micafungin, anidulafungin and fluconazole. Mycoses 2014;57:377–9. https://doi.org/10.1111/myc.12170.

[7] Reboli AC, Rotstein C, Pappas PG, Chapman SW, Kett DH, Kumar D, et al. Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med 2007;356:2472–82. https://doi.org/10.1056/NEJMoa066906.

[8] Mora-Duarte J, Betts R, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med 2002;347:2020–9. https://doi.org/10.1056/NEJMoa021585.

[9] Sipsas N V, Lewis RE, Tarrand J, Hachem R, Rolston K V, Raad II, et al. Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001-2007): stable incidence but changing epidemiology of a still frequently lethal infection. Cancer 2009;115:4745–52. https://doi.org/10.1002/cncr.24507.

[10] Forrest GN, Weekes E, Johnson JK. Increasing incidence of Candida parapsilosis candidemia with caspofungin usage. J Infect 2008;56:126–9. https://doi.org/10.1016/j.jinf.2007.10.014.

[11] Clinical and Laboratory Standards Institute (CLSI). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. CLSI document M27-S4. 2012.

[12] Chiotos K, Vendetti N, Zaoutis TE, Baddley J, Ostrosky-Zeichner L, Pappas P, et al. Comparative effectiveness of echinocandins versus fluconazole therapy for the treatment of adult candidaemia due to Candida parapsilosis : a retrospective observational cohort study of the Mycoses Study Group (MSG-12): Table 1. J Antimicrob Chemother 2016;71:3536–9. https://doi.org/10.1093/jac/dkw305.

[13] Mesini A, Mikulska M, Giacobbe DR, Del Puente F, Gandolfo N, Codda G, et al. Changing epidemiology of candidemia: increase in fluconazole-resistant Candida parapsilosis. Mycoses 2020. https://doi.org/10.1111/myc.13050.

[14] Souza ACR, Fuchs BB, Pinhati HMS, Siqueira RA, Hagen F, Meis JF, et al. Candida parapsilosis Resistance to Fluconazole: Molecular Mechanisms and *In Vivo* Impact in Infected Galleria mellonella Larvae. Antimicrob Agents Chemother 2015;59:6581–7. https://doi.org/10.1128/AAC.01177-15.

[15] Pinhati HMS, Casulari LA, Souza ACR, Siqueira RA, Damasceno CMG, Colombo AL. Outbreak of

candidemia caused by fluconazole resistant Candida parapsilosis strains in an intensive care unit. BMC Infect Dis 2016;16:433. https://doi.org/10.1186/s12879-016-1767-9.

[16] Grossman NT, Pham CD, Cleveland A a., Lockhart SR. Molecular Mechanisms of Fluconazole Resistance in Candida parapsilosis Isolates from a U.S. Surveillance System. Antimicrob Agents Chemother 2015;59:1030–7. https://doi.org/10.1128/AAC.04613-14.

[17] Siopi M, Tarpatzi A, Kalogeropoulou E, Damianidou S, Vasilakopoulou A, Vourli S, et al. Epidemiological Trends of Fungemia in Greece with a Focus on Candidemia during the Recent Financial Crisis: a 10-Year Survey in a Tertiary Care Academic Hospital and Review of Literature. Antimicrob Agents Chemother 2020;64:1–17. https://doi.org/10.1128/AAC.01516-19.

[18] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis 2016;62:1–50. https://doi.org/10.1093/cid/civ933.

[19] Ullmann a J, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli S, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect 2012;18 Suppl 7:53–67. https://doi.org/10.1111/1469-0691.12041.

[20] Hope WW, Castagnola E, Groll AH, Roilides E, Akova M, Arendrup MC, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: prevention and management of invasive infections in neonates and children caused by Candida spp. Clin Microbiol Infect 2012;18 Suppl 7:38–52. https://doi.org/10.1111/1469-0691.12040.

[21] Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 2012;18 Suppl 7:19–37. https://doi.org/10.1111/1469-0691.12039.

[22] Helleberg M, Jørgensen KM, Hare RK, Datcu R, Chowdhary A, Arendrup MC. Rezafungin In Vitro Activity against Contemporary Nordic Clinical Candida Isolates and Candida auris Determined by the EUCAST Reference Method. Antimicrob Agents Chemother 2020;64:1–10. https://doi.org/10.1128/AAC.02438-19.

[23] Pfaller MA, Castanheira M, Messer SA, Rhomberg PR, Jones RN. Comparison of EUCAST and CLSI broth microdilution methods for the susceptibility testing of 10 Systemically active antifungal agents when tested against Candida spp. Diagn Microbiol Infect Dis 2014;79:198–204. https://doi.org/10.1016/j.diagmicrobio.2014.03.004.

[24] Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W. Differential in vivo activities of anidulafungin, caspofungin, and micafungin against Candida glabrata isolates with and without FKS resistance mutations. Antimicrob Agents Chemother 2012;56:2435–42. https://doi.org/10.1128/AAC.06369-11.

[25] Lackner M, Tscherner M, Schaller M, Kuchler K, Mair C, Sartori B, et al. Positions and numbers of FKS mutations in Candida albicans selectively influence in vitro and in vivo susceptibilities to echinocandin treatment. Antimicrob Agents Chemother 2014;58:3626–35. https://doi.org/10.1128/AAC.00123-14.

[26] Masih A, Singh PK, Kathuria S, Agarwal K, Meis JF, Chowdhary A. Identification by Molecular Methods and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry and Antifungal Susceptibility Profiles of Clinically Significant Rare Aspergillus Species in a Referral Chest Hospital in Delhi, India. J Clin Microbiol 2016;54:2354–64. https://doi.org/10.1128/JCM.00962-16.

[27] Resendiz-Sharpe A, Mercier T, Lestrade PPA, van der Beek MT, von dem Borne PA, Cornelissen JJ, et al. Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients. J Antimicrob Chemother 2019;74:2759–66. https://doi.org/10.1093/jac/dkz258.

[28] Stensvold CR, Jørgensen LN, Arendrup MC. Azole-Resistant Invasive Aspergillosis: Relationship to Agriculture. Curr Fungal Infect Rep 2012;6:178–91. https://doi.org/10.1007/s12281-012-0097-7.

[29] Jørgensen KM, Astvad KMT, Hare RK, Arendrup MC. EUCAST Susceptibility Testing of Isavuconazole: MIC Data for Contemporary Clinical Mold and Yeast Isolates. Antimicrob Agents Chemother 2019;63:1–11. https://doi.org/10.1128/AAC.00073-19.

[30] Gregson L, Goodwin J, Johnson A, McEntee L, Moore CB, Richardson M, et al. In vitro susceptibility of Aspergillus fumigatus to isavuconazole: Correlation with itraconazole, voriconazole, and posaconazole. Antimicrob Agents Chemother 2013;57:5778–80. https://doi.org/10.1128/AAC.01141-13.

[31] Arendrup MC, Meletiadis J, Mouton JW, Guinea J, Cuenca-Estrella M, Lagrou K, et al. EUCAST technical note on isavuconazole breakpoints for Aspergillus, itraconazole breakpoints for Candida and updates for the antifungal susceptibility testing method documents. Clin Microbiol Infect 2016;22:571.e1-4. https://doi.org/10.1016/j.cmi.2016.01.017.

[32] Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. Breakpoints for antifungal agents: an update from EUCAST focussing on echinocandins against Candida spp. and triazoles against

Aspergillus spp. Drug Resist Updat 2013;16:81–95. https://doi.org/10.1016/j.drup.2014.01.001.

[33] Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann J-W, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002;347:408–15. https://doi.org/10.1097/00019048-200205000-00033.

[34] Lortholary O, Gangneux J-P, Sitbon K, Lebeau B, de Monbrison F, Le Strat Y, et al. Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005-2007). Clin Microbiol Infect 2011;17:1882–9. https://doi.org/10.1111/j.1469-0691.2011.03548.x.

[35] Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R, et al. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. Intensive Care Med 2012;38:1761–8. https://doi.org/10.1007/s00134-012-2673-2.

[36] Denning DWD, Cadranel J, Beigelman-Aubry C, Ader F, Chakrabarti A, Blot S, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Respir J 2016;47:45–68. https://doi.org/10.1183/13993003.00583-2015.

[37] Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW, European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST), Arendrup MC, et al. EUCAST technical note on Aspergillus and amphotericin B, itraconazole, and posaconazole. Clin Microbiol Infect 2012;18:E248-50. https://doi.org/10.1111/j.1469-0691.2012.03890.x.

Table 1. EUCAST breakpoints for *Candida* species valid from 04-02-2020. New or revised breakpoints are underscored. ATU, Area of Technical Uncertainty, is a single MIC value, the interpretation of which can be performed via the regular breakpoints but which often needs further attention as explained in footnotes.

Antifungal agent	*Candida albicans*			*Candida dubliniensis*		*Candida glabrata*		*Candida krusei*		*Candida parapsilosis*		*Candida tropicalis*		Non-species related breakpoints[1]	
	S ≤	R >	ATU	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B[2]	1	1		1	1	1	1	1	1	1	1	1	1	IE	IE
Anidulafungin[2,3]	0.03	0.03				0.06	0.06	0.06	0.06	4	4	0.06	0.06	IE	IE
Fluconazole[4]	2	4		2	4	0.001[5]	16	-	-	2	4	2	4	2	4
Itraconazole[2]	0.06	0.06		0.06	0.06	IE[6]	IE[6]	IE[6]	IE[6]	0.125	0.125	0.125	0.125	IE	IE
Micafungin[2,3]	0.016	0.016	0.03[7]			0.03	0.03	IE[8]	IE[8]	2	2	IE[8]	IE[5]	IE	IE
Posaconazole[2]	0.06	0.06		0.06	0.06	IE[6]	IE[6]	IE[6]	IE[6]	0.06	0.06	0.06	0.06	IE	IE
Voriconazole[9]	0.06[10]	0.25[10]		0.06[10]	0.25[10]	IE	IE	IE	IE	0.125[10]	0.25[10]	0.125[10]	0.25[10]	IE	IE

- No breakpoints. Susceptibility testing is not recommended.

IE Insufficient evidence that the organism or group is a good target for therapy with the agent.

[1] Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific *Candida* species. They are for use only for organisms that do not have specific breakpoints.

[2] No data to support an I category for amphotericin B according to the new definition of I

[3] Isolates that are susceptible to anidulafungin as well as micafungin should be considered susceptible to caspofungin, until caspofungin breakpoints have been established. EUCAST breakpoints have not yet been established for caspofungin, due to significant inter-laboratory variation in MIC ranges for caspofungin.

[4] High dose for fluconazole is required isolates in the I-category

[5] The entire *C. glabrata* is in the I category. MICs against *C. glabrata* should be interpreted as resistant when above 16 mg/L. Susceptible category (≤0.001 mg/L) is simply to avoid missclassification of "I" strains as "S" strains.

[6] The ECOFFs for these species are in general higher than for *C. albicans*.

[7] If S to anidulafungin, report as S and add the following comment: "Isolates susceptible to anidulafungin with micafungin MIC of 0.03 mg/L do not harbour an *fks* hot spot mutation conferring resistance to the echinocandins".
If not S to anidulafungin, report as R and refer to reference laboratory for *fks* sequencing and confirmation of MICs.

[8] Micafungin MICs for *C. tropicalis* are 1-2 two-fold dilution steps higher than for *C. albicans* and *C. glabrata*. In the clinical study successful outcome was numerically slightly lower for *C. tropicalis* than for *C. albicans* at both dosages (100 and 150 mg daily). However, the difference was not significant and whether it translates into a relevant clinical difference is unknown. MICs for *C. krusei* are approximately three two-fold dilution steps higher than those for *C. albicans* and, similarly, those for *C. guilliermondii* are approximately eight two-fold dilutions higher. In addition, there were only a small number of cases involved these species in the clinical trials. This means there is insufficient evidence (IE) to indicate whether the wild-type population of these pathogens can be considered susceptible to micafungin.

[9] For *Candida* the I category is introduced to acknowledge that the increased exposure obtained by iv dosing is sufficient (potentially confirmed by TDM). There is not enough information available for the response to voriconazole of infections caused by *Candida* isolates with higher MICs.

[10] Strains with MIC values above the S/I breakpoint are rare or not yet reported. The identification and antifungal susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint they should be reported resistant. A clinical response of 76% was achieved in infections caused by the species listed below when MICs were lower than or equal to the epidemiological cut-offs. Therefore, wild type populations of *C. albicans, C. dubliniensis, C. parapsilosis* and *C. tropicalis* are considered susceptible.

Table 2. EUCAST breakpoints for *Aspergillus* species valid from 04-02-2020. New or revised breakpoints are highlighted in underscored font. ATU, Area of Technical Uncertainty, is a single MIC value, the interpretation of which can be performed via the regular breakpoints but which often needs further attention as explained in footnotes.

Antifungal agent	***A. flavus***			***A. fumigatus***			***A. nidulans***			***A. niger***		***A. terreus***		
	S ≤	**R >**	**ATU**	**S ≤**	**R >**	**ATU**	**S ≤**	**R >**	**ATU**	**S ≤**	**R >**	**S ≤**	**R >**	**ATU**
Amphotericin B[1]	-	-		1	1		-	-		1	1	-	-	
Isavuconazole[2,3]	1	2	2[4]	1	2	2[4]	0.25	0.25		IE[5]	IE[5]	1	1	
Itraconazole[1,3,6]	1	1	2[7]	1	1	2[7]	1	1	2[7]	IE[5]	IE[5]	1	1	2[7]
Posaconazole[3,6,8]	IE[5]	IE[5]		0.125	0.25	0.25[9]	IE[5]	IE[5]		IE[5]	IE[5]	0.125	0.25	0.25[9]
Voriconazole [1,3,6]	IE[5]	IE[5]		1	1	2[10]	1	1	2[10]	IE[5]	IE[5]	IE[5]	IE[5]	

- No breakpoints. Susceptibility testing is not recommended.

IE Insufficient evidence that the organism or group is a good target for therapy with the agent.

[1] No data to support an "I" category according to the new definition of "I"

[2] Isavuconazole MIC = 2 mg/L should not be interpreted as I but only as ATU

[3] Itraconazole and posaconazole R isolates but S to voriconazole and isavuconazole are not uncommon in azole-treated patients. Refer the isolate to a reference laboratory for CYP51A sequencing and confirmation of MICs.

[4] If voriconazole wild-type: (*A. flavus*: voriconazole MIC ≤2 mg/L; *A. fumigatus*: voriconazole MIC ≤1 mg/L) report as isavuconazole S and add the following comment: The MIC of 2 mg/L is one dilution above the S breakpoint but within the wild-type isavuconazole MIC range due to a stringent breakpoint susceptibility breakpoint. See rationale documents for more information.
If voriconazole non wild-type: report as isavuconazole R and refer to reference laboratory for *CYP51A* sequencing and confirmation of MICs."

[5] The ECOFFs for these species are in general one two-fold dilution higher than for *A. fumigatus*

[6] Monitoring of azole trough concentrations in patients treated for fungal infection is recommended.

[7] Report as R with the following comment: "In some clinical situations (non-invasive infections forms) itraconazole can be used provided sufficient exposure is ensured".

[8] Normally, values between the S and R categories should be classified as “I”, but in the case of Posaconazole and *A. fumigatus* MIC = 0.25 mg/L should not be interpreted as I but only as ATU. How to act on this is described in footnote [9].

[9] If S to itraconazole: report as S and add the following comment: "The MIC is 0.25 mg/L and thus one dilution above the S breakpoint due to overlapping wt and non-wt populations".
If not S to itraconazole: report as R and refer to reference laboratory for *CYP51A* sequencing and confirmation of MICs.

[10] Report as R with the following comment: "In some clinical situations (non-invasive infections forms) voriconazole can be used provided sufficient exposure is ensured".

Table 3. "EUCAST breakpoints are based on the adult dosages indicated below. Alternative dosing regimens which result in equivalent exposure are acceptable. The table should not be considered an exhaustive guidance for dosing in clinical practice. The table neither replaces specific local, national, or regional dosing guidelines, nor does it replace manufacturer's licensed dosage recommendations according to SPCs.

Azoles	**Standard dose**[1]	**Increased Exposure Dose**	**Special situations**
Fluconazole	A single initial dose of 800 mg followed by 400 mg once daily (or 6 mg/kg) iv/oral	800 mg (or 12 mg/kg) once-daily iv/oral	Indicated doses are those appropriate for invasive candidiasis Mucosal infections (Mendling et al; Mycoses. 2012;55 Suppl 3:1-13): Standard doses is 100-200 mg once daily and increased dose 800 mg once daily (for *C. glabrata*)
Itraconazole	200 mg twice daily the first day followed by 100*-400** mg daily iv/po Target trough level***: >0.5 mg/L for prophylaxis, >1 mg/L for therapy		*Superficial infections only **Daily doses up to 200 mg twice daily may be given depending on the infection. Capsules have 30% lower bioavailability than the oral solution ***HPLC assay method and Parent compound only.
Isavuconazole	200 mg three times daily for 2 days followed by 200 mg once daily		
Posaconazole	Tablets/iv: 300 mg twice daily the first day followed by 300 mg once daily Oral suspension: 200 mg four times daily or 400 mg twice daily Target trough level: >0.7 mg/L for prophylaxis / >1.25 mg/L for therapy		
Voriconazole	6 mg/kg twice daily the first day followed 4 mg/kg twice daily iv 400 mg twice daily followed by 200 mg twice daily po Target trough level: >0.5 for prophylaxis, 2-5.5 mg/L for therapy	*Candida*: The I-category only applies for the iv dosage (not the standard oral dose)	Increased exposure can be achieved by elevated dosage (note non-linear kinetics in adults) or with a proton pump inhibitor, in patients with low blood levels.
Amphotericin B formulations	**Standard dose**	**Increased Exposure Dose**	**Special situations**
Liposomal amphotericin B	3 mg/kg once daily		Increased doses up to 7 mg/kg (or even 10 mg/kg e.g. *Mucorales* CNS infections) can be used in specific

			situations.
Amphotericin B deoxycholate	1 mg/kg once daily		
ABLC	5 mg/kg once daily		
Echinocandins	**Standard dose**	**Increased Exposure Dose**	**Special situations**
Anidulafungin	A single initial dose of 200 mg followed by 100 mg once daily		
Caspofungin	A single initial dose of 70 mg followed by 50* mg once daily (weight ≤ 80 kg) or 70 mg once daily (weight > 80 kg)		*Continue with 70 mg once daily after loading dose if weight >80 kg
Micafungin	100 mg once daily (weight >40 kg) 2 mg/kg once daily in patients weighing <40 kg	200 mg once daily (weight >40 kg) 4 mg/kg once daily in patients weighing <40 kg	Increased dose indicated in patients not responding to standard dose Standard dose for chronic aspergillosis is Micafungin 150 mg once daily (Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Resp J 2016)

[1] Duration of treatment only indicated for loading doses, because the total duration of therapy is not only dependent on the type and site of infection but also on the underlying disease of the patient. Please consult clinical management guidelines for recommendations on total duration."

Table 4. Summary table of current EUCAST ECOFFs (WT ≤; mg/L, in blue) and susceptibility breakpoints (S ≤; mg/L, in black) for *Candida* species, *Saccharomyces* (*S.*) *cerevisiae* and *Cryptococcus* (*C.*) *neoformans* and *Cryptococcus gattii*. Tentative ECOFFs are indicated in brackets[a]. ND (not done). – (dash) EUCAST recommends not to test as the species is intrinsically resistant to the agent in question.

Drug	Species											
	Candida									*Saccharomyces*	*Cryptococcus*	
	albicans	*dubliniensis*	*glabrata*	*krusei*	*parapsilosis*	*tropicalis*	*guilliermondii*	*lusitaniae*	*kefyr*	*cerevisiae*	*neoformans*	*gattii*
Amphotericin B												
WT ≤	1	0.25	1	1	1	1	[0.5]	[0.5]	[1]	[0.5]	[1]	[0.5]
S ≤	1	1	1	1	1	1	ND	ND	ND	ND	1	ND
Anidulafungin												
WT ≤	0.03		0.06	0.06	4	0.06						
S ≤	0.03		0.06	0.06	4	0.06					-	-
Fluconazole												
WT ≤	0.5	[0.5]	16	128	2	1	[16]		[1]			
S ≤	2	2	0.001	-	2	2	ND		ND			
Itraconazole												
WT ≤	0.06	0.06	2	1	0.125	0.125	2	0.125				
S ≤	0.06	0.06	ND	ND	0.125	0.125	ND	ND				
Micafungin												
WT ≤	0.016		0.03	0.25	2	0.06						
S ≤	0.016		0.03	ND	2	ND					-	-
Posaconazole												
WT ≤	0.06	0.06	1	0.5	0.06	0.06	0.25				0.5	1
S ≤	0.06	0.06	ND	ND	0.06	0.06	ND				ND	ND
Voriconazole												
WT ≤	0.03	0.03	1	1	0.06	0.125					0.5	
S ≤	0.06	0.06	ND	ND	0.125	0.125					ND	

[a] Tentative ECOFFs are set on dataset that do not full fill the criteria described in EUCAST SOP 10.1 available at the www.eucast.org website (e.g. fewer than 5 distributions, fewer than 100 isolates per species etc.) Tentative ECOFFs therefore may change when more data emerge.

Table 5. Summary table of current EUCAST ECOFFs (WT ≤; mg/L, in blue) and susceptibility breakpoints (S ≤; mg/L, in black) for *Aspergillus* species, and *Fusarium* species. Tentative ECOFFs are indicated in brackets. ND (not done). – (dash) EUCAST recommends not to test as the species is intrinsically resistant to the agent in question.

Drug	**Species**						
	A. flavus	*A. fumigatus*	*A. nidulans*	*A. niger*	*A. terreus*	*Fusarium* (*Gibberella*) *fujikuroi* SC	*Fusarium solani* SC
Amphotericin B							
WT ≤	4	1	[4]	[0.5]	8	[8]	[8]
S ≤	-	1	-	1	-	ND	ND
Isavuconazole							
WT ≤	2	2	0.25	4	1		
S ≤	1	1	0.25	ND	1		
Itraconazole							
WT ≤	1	1	1	4	0.5		
S ≤	1	1	1	ND	1		
Posaconazole							
WT ≤	0.5	0.25	0.5	0.5	0.25		
S ≤	ND	0.125	ND	ND	0.125		
Voriconazole							
WT ≤	2	1	1	2	2		
S ≤	ND	1	1	ND	ND		