EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

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Rationale for EUCAST clinical breakpoints

Agent	**Itraconazole and *Aspergillus***

Current version	**2.0**	**4th of February, 2020**
Previous versions	1.1	6th of June, 2012
	1.0	11st of January, 2012

Foreword

EUCAST
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Centre for Disease Prevention and Control (ECDC), and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002. The current remit of EUCAST is to harmonise clinical breakpoints for existing drugs in Europe, to determine clinical breakpoints for new drugs, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

Information on EUCAST and EUCAST breakpoints is available on the EUCAST website at http://www.EUCAST.org.

EUCAST rationale documents
EUCAST rationale documents summarise the information on which the EUCAST clinical breakpoints are based.

Availability of EUCAST document
All EUCAST documents are freely available from the EUCAST website at http://www.EUCAST.org.

Citation of EUCAST documents
This rationale document should be cited as: “European Committee on Antimicrobial Susceptibility Testing. Itraconazole and *Aspergillus* spp: Rationale for the clinical breakpoints, version 2.0, 2020. http://www.eucast.org.

1. Introduction

Itraconazole is a triazole antifungal agent active in vitro against *Aspergillus* spp., *Candida* spp., dermatophytes, *Histoplasma* spp., *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Fonsecaea* spp., *Cladosporium* spp., *Blastomyces dermatitidis*, and various other yeasts and fungi. The agent is approved for many indications, but licensed indications vary between European countries.

The mould species most frequently causing human infections include *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus* and *Aspergillus niger*. The in vitro activity of itraconazole against these species of *Aspergillus* is fairly uniform, although differences do occur even between the recently described and rarer "sub" species belonging to the species complexes (e.g. *Aspergillus lentulus* belongs to the *A. fumigatus* complex and is multidrug resistant). Acquired resistance is reported with increasing frequency even among isolates obtained from azole-naive patients. Thus, correct species identification and susceptibility testing is of utmost importance.

The European Committee on Antimicrobial susceptibility Testing - Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined breakpoints for itraconazole against *Aspergillus* spp. These breakpoints are tentative and will be revised after two years.

In version 2.0 of this rationale document, the clinical breakpoints have been revised due to the revised definition of the "I" category. The resistance breakpoint has been lowered and an ATU (Area of Technical Uncertainty) category has been introduced as insufficient evidence exist to support an "I" category (Susceptible, Increased exposure) category with high exposure for *Aspergillus* isolates with MIC= 2 mg/L.

2. Dosage

	Austria	Belgium	Czech Republic	Denmark	Estonia	Germany	Greece	France	The Netherlands	Norway	Russia	Spain	Sweden	Turkey	UK
Minimum daily dose (mg/day)	200	200	NA	200	NA	NA	200	200	200	200	400	200	400	200	200
Most common daily dose (mg/day)	200	400	200 iv maintenance or 400 oral	400 plus TDM	NA	200 iv maintenance. Oral dosing 5 mg/kg plus TDM	200	400	400		5 mg/kg	200	200 x 2	200 iv	200
Loading dose (mg/day)	200 x 2 day 1 & 2	600 for 4 days	400 for 2 days	No	NA	200 x 2 iv day 1 & 2 None for oral dosing	200 x 2 iv day 1 & 2 (same dose for solution)	200 x 3 day 1-3	200 x 3 day 1-4	400	No	200 x 2 day 1 & 2	200 x 3-4 for 4 days for capsules	400 iv for 2 days	200 x 2 day 1 & 2
Maintenance dose (mg/day)	200	400	200	400	NA		200	400		200		400	400		200 (400 if severe)
Most common number of daily doses for which the indicated doses are divided	1	2	2 when 400, 1 if 200	2 (but 1 in minimal dose)	NA	1 (iv maintenance dosing) 2 (oral dosing)	2	1 (but 2 if 400)	2 (but 1 in minimum dose)		1	1	2	2 (oral)	1 (but 2 if 400)
Maximum daily dose (mg/day)	400	400	NA	400	NA	NA	400	400			7 mg/kg	400	800	400	400
Available formulations	oral solution; capsules	capsules (oral solution indicated only for candidiasis)	iv and oral solutions; capsules	capsules	PO capsules and oral solution	iv; oral solution; (capsules available)	iv; oral suspension and capsules available	oral solution; capsules	iv; oral solution; capsules	iv; oral liquid and capsules.	oral solution; capsules available but not used for *Aspergillus*	iv; oral solution	oral solution; capsules	iv ; oral solution; capsules	iv; oral solution; capsules

TDM = therapeutic drug monitoring
NA = not available

3a. MIC distributions (numbers) and epidemiological cut-off (ECOFF) values (mg/L)

	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	ECOFF
Aspergillus flavus	0	0	2	2	0	11	59	162	116	25	11	0	0	4	0	0	0	0	0	1
Aspergillus fumigatus	0	0	1	2	8	16	155	895	832	202	54	30	14	58	121	1	0	0	0	1
Aspergillus nidulans	0	0	0	0	2	7	33	39	19	4	5	1	2	4	0	0	0	0	0	1
Aspergillus niger	0	0	0	0	0	1	6	18	104	117	21	7	5	25	3	0	0	0	0	4
Aspergillus terreus	0	0	0	1	5	24	200	341	54	5	3	0	2	1	0	0	0	0	0	0.5
Aspergillus versicolor	0	0	0	0	0	1	2	4	11	10	1	0	1	1	0	0	0	0	0	ND
Aspergillus sydowii	0	0	0	0	0	1	8	8	18	10	1	2	1	7	0	0	0	0	0	ND

The table includes MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. When there is insufficient evidence no epidemiological cut-off has been determined (ND).

3b. MIC distributions (%) and epidemiological cut-off (ECOFF) values (mg/L)

	N	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	ECOFF
Aspergillus flavus	392	0	0	1	1	0	3	15	41	30	6	3	0	0	1	0	0	0	0	0	1
Aspergillus fumigatus	2389	0	0	0	0	0	1	6	37	35	8	2	1	1	2	5	0	0	0	0	1
Aspergillus nidulans	116	0	0	0	0	2	6	28	34	16	3	4	1	2	3	0	0	0	0	0	1
Aspergillus niger	307	0	0	0	0	0	0	2	6	34	38	7	2	2	8	1	0	0	0	0	4
Aspergillus terreus	636	0	0	0	0	1	4	31	54	8	1	0	0	0	0	0	0	0	0	0	0.5
Aspergillus versicolor	31	0	0	0	0	0	3	6	13	35	32	3	0	3	3	0	0	0	0	0	ND
Aspergillus sydowii	56	0	0	0	0	0	2	14	14	32	18	2	4	2	13	0	0	0	0	0	ND

The table includes MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. When there is insufficient evidence no epidemiological cut-off has been determined (ND).

4. Breakpoints prior to harmonisation (mg/L) S≤ / R>

	Europe	CLSI
General breakpoints:		
	NA	NA
Species specific breakpoints:		
	NA	NA

NA = Not available

5. Pharmacokinetics

Dosage (mg)	capsules 200 single dose	oral solution 200 single dose	iv formulation 200 x 2 day 1 & 2 followed by 200 mg daily until day 4	
Cmax (mg/L)	0.3-0.5	0.3-0.55	3-6	
Cmin (mg/L)			0.5-1	
Cav (mg/L)	0.5-2			
Total body clearance/F (L/h)	39.6		16.7	
T ½ (h); Mean (CV %)	22-36	22-36	32.5	
AUC96h (mg.h/L) total drug	4-10	5-15		
Fraction unbound (%)	0.2	0.2	0.2	
Volume of distribution/F (L/kg)	11	11	>700 L	
Comments	• Itraconazole bioavailability is around 55% but displays considerable variation on oral administration. Absorption is affected by formulation (optimised by cyclodextrin in the oral solution), gastric pH, prandial state and the timing of dose administration relative to the time of a meal. • Strategies to maximize itraconazole exposure after administration of capsules include administration with or immediately after a meal, with an acid beverage (cola or juice), or in divided doses. The administration of any acid inhibitors should be avoided if possible. • Strategies to maximize itraconazole exposure after administration of oral solution include administration during fasting (30% greater exposure), or in divided doses. The administration of any acid inhibitors should be avoided if possible. • Itraconazole is metabolised to hydroxyl-itraconazole which also has antifungal activity. In HPLC determinations these two compounds are measured individually whereas in a bioassay the combined antifungal activity is measured and expressed in itraconazole equivalents. • Itraconazole displays non-linear pharmacokinetics. • Itraconazole has multiple drug-drug interactions.			
References	• Itraconazole product information : http://www.produktresume.dk/docushare/dsweb/GetRendition/Document-11131/html and http://www.medicines.org.uk/EMC/medicine/7395/SPC/Sporanox+Capsules/ • Poirier JM and Cheymol G. Clin Pharmacokinet. 1998; 35: 461-73. • Willems L, van der Geest R and de Beule K.. J Clin Pharm Therapeut 2001; 26: 159-169. • de Beule K and Van Gestel J.. Drugs 2001; 61 Suppl 1: 27-37. • Barone JA et al. Antimicrob Agents Chemother 1998; 42: 1862-65.			

6. Pharmacodynamics				
*f*AUC/MIC for stasis				
*f*AUC/MIC for 2 log reduction				
*f*AUC/MIC from clinical data				

Comments	• No pharmacodynamic data are available for EUCAST MICs and *Aspergillus* spp.
	• The AUC/MIC is general regarded as the significant parameter for *Candida* infections and the same is likely to be so for *Aspergillus*, but no data are available for *Aspergillus*.
	• Outcome in animal models and patients with invasive pulmonary aspergillosis is associated with exposure (with better outcome for patients with higher trough levels), but no international agreement has been reached regarding the exact target range. Examples are listed below:
	  ○ In a rabbit model of invasive aspergillosis near-peak concentrations of itraconazole + hydroxy-itraconazole (by bioassay) below 6 mg/L were associated with failure (Berenguer)
	  ○ In a clinical study of invasive aspergillosis (Denning) levels of itraconazole + hydroxy-itraconazole (by bioassay) greater than 5 mg/L were associated with better outcome.
	  ○ Target trough concentrations of itraconazole $\geq$0.5 mg/L (by HPLC) in the setting of prophylaxis has been associated with fewer breakthrough infections and survival in a haematological population (Glasmacher, Boogaerts). Levels above 17 mg/L have been associated with toxicity (Lestner)
	• In established infections higher levels may be required, but remain undefined.			

References	• Berenguer, J et al., Antimicrob Agents Chemother 1994; 38: 1303-1308. • Denning DW et al., Am J Med. 1994; 97: 135-144. • Denning DW et al. Am J Med 1989; 86: 791-800. • Walsh TJ, et al. Clin Inf Dis 2008; 46: 327–60. • Glasmacher A et al, Mycoses 1999; 42:443-51. • Boogaerts MA et al. Mycoses 1989; 32 Suppl 1:103-8. • Lestner JM et al. Clin Inf Dis 2009; 49: 928–30.			

7. Monte Carlo simulations and Pk/Pd breakpoints

Not available for EUCAST data because there is no clear Pk/Pd target defined.

8. Clinical data

Aspergillosis

Orally administered itraconazole has been used to treat patients with invasive aspergillosis [Stevens; Denning]. In the largest open multicentre trial of 76 evaluable patients (39 with no prior antifungal treatment, 16% failing amphotericin B therapy and 45% with prior iv amphotericin B), 30 patients (39%) had a complete or partial response, with success rates varying widely according to site of disease and underlying disease group. Four percent had stable disease. The overall failure rate at the end of the study was 56% including 26% classified as itraconazole therapy failures and 30% failing for other reasons. Of note, a substantial number of patients who failed therapy had undetectable levels of itraconazole in blood whereas none of the responders had undetectable levels [Denning]. More recent studies of the parenteral formulation of β-hydroxy-propyl-cyclodextrin itraconazole in the treatment of invasive pulmonary aspergillosis that was refractory to various forms of amphotericin B have been reported, with overall response rates of 32-52% [Caillot 2001 and 2003]. Salvage therapy with itraconazole for treatment of invasive pulmonary aspergillosis that is refractory to primary therapy despite appropriate levels of voriconazole is not recommended because most isolates resistant to voriconazole are also resistant to itraconazole. Therapy with itraconazole for treatment of invasive pulmonary aspergillosis that has failed posaconazole prophylaxis despite appropriate levels of posaconazole in the haematology setting is not recommended because isolates resistant to posaconazole in mostly are also resistant to itraconazole.

Walsh TJ et al., Clin Infect Dis 2008; 46: 327–60.
Denning DW et al. NIAID Am J Med 1994; 97: 135–44.
Caillot D et al. Clin Infect Dis 2001; 33: 83–90.
Stevens DA, Lee JY. Arch Intern Med 1997; 157: 1857–62.
Caillot D. Acta Haematol 2003; 109: 111–8.

Acquired Resistance

The first cases of itraconazole resistant *A. fumigatus* were from the late 1980s, yet the vast majority has been detected since the turn of the millennium. The frequency is largely undefined, as many centres do not routinely test the susceptibility of their *Aspergillus* isolates. Resistance has currently been reported in Belgium, Canada, China, Denmark, France, Norway, Spain, Sweden, The Netherlands, UK and the USA. Most commonly the resistance is linked to point mutations in the target gene *cyp51A*. However, at some centres a significant proportion of the isolates with elevated itraconazole MICs lack such mutations, suggesting the other mechanisms like efflux pumps or up-regulation of target production may also play a role. Importantly, *A. fumigatus* isolates with acquired resistance mechanisms have been increasingly found in the environment, probably due to agricultural azole fungicide use, and have also been found in azole-naïve patients failing therapy. The EUCAST MICs for such isolates vary with the underlying mechanism but are >4 mg/L for the most commonly-identified mutants (alterations at G54, G138, M220, and the environmental phenotype TR-L98H). Correlation of in vitro itraconazole MIC data with clinical outcome has not been done as such data sets are not available for EUCAST MIC method.

Denning DW et al. *Antimicrob Agents Chemother* 1997; 41: 1364-8.
Snelders E et al. *PLoS Med* 2008; 5: e219.
Snelders E et al. *Appl Environ Microbiol* 2009; 75: 4053-7.
Mortensen KL *et al*. Antimicrob Ag Chemother 2010; 54: 4545-9.
Mortensen KL *et al.* J Clin Microbiol 2011; 49: 2243-51.
Dannaoui E et al. J Med Microbiol 2006; 55: 1457-9.
Howard SJ et al. *Emerg Infect Dis* 2009; 15: 1068-76.
Arendrup MC et al. Development *PLoS One* 2010; 5: e10080.
van der Linden JWM et al *Clin Inf Dis* 2009; 48: 1111-3.
Howard S, Arendrup MC. Med Mycol. 2011; 49 Suppl 1: S90-5.
Chryssanthou. Scan J Infect Dis. 1997; 29: 509-12.

9. *Aspergillus* clinical breakpoints

Non-species-related breakpoints

There is insufficient evidence to set non-species-related breakpoints.

Species-related breakpoints

Organism group	MIC breakpoints (mg/L)			Notes
	S ≤	R >	ATU	
Aspergillus flavus	1	1	2	Report as R with the comment that in some clinical situations (non-invasive infections) itraconazole can be used provided sufficient exposure is ensured.
Aspergillus fumigatus	1	1	2	
Aspergillus nidulans	1	1	2	
Aspergillus terreus	1	1	2	

Breakpoints were based on microbiological data and clinical experience.

Clinical information shows that the wild type population of *A. fumigatus* is susceptible to itraconazole. No clinical studies have so far presented outcome data for a significant number of cases involving the other species. Although there is inadequate clinical information on outcome for wild type populations of *A. flavus, A. nidulans,* and *A. terreus*, the MIC distributions are similar to (or a single dilution lower than) that obtained for *A. fumigatus.* Therefore, EUCAST AFST considers wild type populations of these species as susceptible to itraconazole. There is insufficient evidence that isolates with an MIC of 2 mg/L is susceptible at standard dosing, but also which high dose target might be efficacious therapy for such isolates. However, with very few oral options for outpatient with non-acute infections itraconazole may be considered provided sufficient exposure is ensured.

Species without breakpoints

The MIC values for isolates of *A. niger* and *A. versicolor* are in general higher than those for *A. fumigatus*. Whether this translates into a poorer clinical response is unknown. There is insufficient evidence (IE) to set breakpoints for these species.

Clinical qualifications	In addition to the licensed indications, the EUCAST AFST considers itraconazole appropriate therapy for chronic and allergic syndromes caused by wild type isolates of *Aspergillus* species.
Dosage	The EUCAST breakpoints apply to licensed dosing. Dosage adjustment may be acquired according to serum concentrations.
Additional comment	Isolates susceptible to itraconazole but resistant to voriconazole or posaconazole are so far extremely rare (but reported for a minority of the environmental TR_{46}/Y121F/T289A isolates), whereas itraconazole resistant isolates may or may not be resistant to voriconazole and posaconazole, depending on the underlying mechanism. Isolates with elevated itraconazole MIC should be checked for cross resistance to other azole agents active against *Aspergillus* spp. Itraconazole absorption is affected by gastric pH, prandial state and the timing of dose administration relative of the time of a meal, and a correlation between itraconazole plasma concentration and outcome has been found (see 7. Clinical data). Monitoring of itraconazole trough concentrations in patients treated for fungal infection is recommended. Breakpoints for itraconazole are mainly based upon ECOFFs and clinical experience with wild type and resistant mutants. Breakpoints will be reviewed when more data are available for *Aspergillus* species which were not assigned breakpoints during the present review, when more PK/PD data are available or when there are further data related to optimal drug exposures for itraconazole.

10. Exceptions noted for individual national committees
None