

Rationale Document for EUCAST clinical breakpoints

Agent	Flucytosine (5-fluorocytosine)	
Current version	1.0	22th July 2025
Previous versions		

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

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1. Introduction

Flucytosine (5-fluorocytosine, 5-FC) is a fluorinated pyrimidine analogue with *in vitro* activity against a variety of fungal pathogens, including *Candida* spp., *Cryptococcus* spp., and several dematiaceous fungi such as *Cladophialophora bantiana*. Its antifungal activity is primarily mediated by intracellular conversion to 5-fluorouracil, which inhibits fungal RNA and DNA synthesis.

The drug is used for:

- (i) Treatment of cryptococcal meningitis, in combination with amphotericin B or fluconazole.
- (ii) Treatment of severe invasive *Candida* infections, including endocarditis, septic arthritis, eye, urinary tract, intra-abdominal infections and meningitis, as part of combination therapy.
- (iii) Treatment of chromoblastomycosis, typically as part of combination therapy.

The *in vitro* activity of 5-FC is generally excellent against *Candida* spp. (including *C. auris*) and *Cryptococcus* spp., but resistance can emerge rapidly during monotherapy, particularly in *Candida* infections, due to mutations in the enzymes required for its uptake and activation (1). This highlights the importance of combination therapy to mitigate resistance.

A notable rise in acquired resistance in *C. tropicalis* has been reported in several European countries over the past two decades, with rates up to 50%, despite low use in clinical practice (2-4). Elevated MICs has also been found in other *Candida* species, which may exhibit variable susceptibility. Finally, several *Candida* species have intrinsically higher MICs compared to *C. albicans*. These include species such as *C. krusei* and *C. norvegensis*. Correct identification and susceptibility testing are crucial to guide effective therapy.

Given its narrow therapeutic index, monitoring 5-FC serum concentrations is essential to minimise toxicity, particularly bone marrow suppression and hepatotoxicity. These considerations underscore the need for close clinical and laboratory drug monitoring when using 5-FC in antifungal therapy.

The European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined ECOFFs for 5-FC against *Candida* spp. Currently, there is not enough evidence for setting breakpoints. ECOFFs and breakpoints for other fungal species remain undefined.

2. Dosage			
		IV (mg/kg/day)	Oral (mg/day unless otherwise stated)
Adults	Loading dose	NR	NR
	Maintenance dose	4 x 25-37.5 mg/kg	4 x 25-37.5 mg/kg
Children (2 to <12 years) and young adolescents (12 to 14 years) with low body weight (<50 kg)*	Loading dose	NR	NR
	Maintenance dose	4 x 25-37.5 mg/kg	4 x 25-37.5 mg/kg
Maximum dose schedule		4 x 50 mg/kg	
Available formulations	5-FC is available as oral capsules (250 mg and 500 mg) and intravenous formulations. However, availability varies significantly between countries. Clinicians should verify local availability when considering treatment options.		
Comments on dosing	<p>The oral route is preferred due to high bioavailability (approximately 90%), with intravenous administration reserved for patients unable to take oral medication.</p> <p>The dose must be adjusted in patients with renal impairment due to the risk of accumulation, which may lead to toxicity. A creatinine clearance below 50 mL/min typically necessitates a reduced dosing frequency or dose.</p> <p>Close monitoring of plasma drug levels is essential to maintain therapeutic concentrations and minimize toxicity, particularly myelosuppression.</p> <p>Combination therapy with amphotericin B (or alternative antifungal agents) is strongly recommended to reduce the risk of resistance development and improve clinical efficacy in cryptococcal meningitis.</p>		
TDM	<p>Routine TDM is recommended, with plasma concentrations ideally measured 3–5 days after treatment initiation or dose adjustment.</p> <p>Peak levels >100 mg/L are associated with increased toxicity, while trough levels <25 mg/L are associated with treatment failure (5).</p>		

NR = Not recommended. * licence for paediatric patients varies across countries.

3a. MIC distributions (numbers) 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	(T)ECOFF*
<i>Candida albicans</i>	500 ^a					11	155	252	34	33	5	1	1			6					0.5
<i>Candida auris</i>	150						11	66	55	18											0.5
<i>Candida glabrata</i>	500				25	154	295	20				2	2			2					ID
<i>Candida krusei</i>	539									2	43	227	224	40	2				1		(8)
<i>Candida parapsilosis</i>	300 ^a					40	122	124	10	3											(0.5)
<i>Candida tropicalis</i>	210				4	27	64	44	10	2				1	7	46	5				(0.5)
<i>Saccharomyces cerevisiae</i>	143				11	25	49	37	19	1								1			ID
<i>Cryptococcus neoformans</i>	559							3	5	7	23	66	202	180	51	13	5	4			(16)
<i>Cryptococcus gattii</i>	52								3	3	14	14	6	5	4	1	2				ID

The table includes EUCAST 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 fewer data sets that 3 there is insufficient data (ID) set to set (T)ECOFF or ECOFF. When there otherwise is insufficient evidence, no ECOFFs have been determined (ND).

^aData sets were aggregated from 1423 MICs from 5 centres (*C. albicans*) and 636 MICs from 3 centres (*C. parapsilosis*) after each of the distributions were normalized to 100 isolates, in order to avoid that data sets with larger sample sized dominating the aggregated distribution. Due to rounding of decimal numbers, the sums are close to, but not exactly, 500 and 300, respectively.

*(T)ECOFF, Tentative ECOFF values were determined for MIC distributions with only 3 data sets. TECOFFs are indicated in parentheses.

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	(T)ECOFF*
<i>Candida albicans</i>	500 ^a					2	31	50	7	7	1	<1	<1			1					0.5
<i>Candida auris</i>	150						7	44	37	12											0.5
<i>Candida glabrata</i>	500				5	31	59	4				<1	<1			<1					ND
<i>Candida krusei</i>	539									<1	8	42	42	7	<1			<1			(8)
<i>Candida parapsilosis</i>	300 ^a					13	41	41	3	1											(0.5)
<i>Candida tropicalis</i>	210				2	13	30	21	5	1				<1	3	22	2				(0.5)
<i>Saccharomyces cerevisiae</i>	143				8	17	34	26	13	1								1			ND
<i>Cryptococcus neoformans</i>	559							1	1	1	4	12	36	32	9	2	1	1			(16)
<i>Cryptococcus gattii</i>	52								6	6	27	27	12	10	8	2	4				ND

*This table contains a percentage presentation of the MIC data from table 3a. Footnotes for Table 3a apply to this table as well. Percentages of isolates in the range between 0 and 0.5 are indicated as <1.

^a Data sets were aggregated from 1423 MICs from 5 centres (*C. albicans*) and 636 MICs from 3 centres (*C. parapsilosis*) after each of the distributions were normalized to 100 isolates, in order to avoid that data sets with larger sample sized dominating the aggregated distribution. Due to rounding of decimal numbers, the sums are close to, but not exactly, 500 and 300, respectively.

*(T)ECOFF, Tentative ECOFF values were determined for MIC distributions with only 3 data sets. TECOFFs are indicated in parentheses.

4. Pharmacokinetics					
	Intravenous formulation		Oral formulation		
	16 HIV+ (6)	64 HIV+ patients with cryptococcal meningoencephalitis (7)	Normal subjects (8)	35 healthy volunteers (9)	15 HIV+ (6)
Dosage	100 mg/kg daily	25 mg/kg q6h	2000 mg oral dose	1500 mg b.i.d. IR tablets	
Bioavailability			78% - 89%		
Tmax (h), median (range)			1-2h	7.5 (1.5–9.0)	
Cmax ± SD (mg/L) Mean (CV), [IQR Range]	63 [41-82]		30-40	40.9±8.30	30 [23–35]
Cmin ± SD (mg/L) Mean (CV), [Range]	37 [17–57]				20 [13–28]
Cav (mg/L) Mean (CV)					
Total body clearance/F± SD (L/h); Mean (CV) [IQR Range]	6.7 [4.8–8.3]	5.88±3.35			3.8 [2.7–4.3]
T ½ ± SD (h), Mean (range)			3-4h normal renal function 85h renal impairment	6.3±1.17	
Plasma AUC_{24h} ± SD (mg.h/L) total drug; Mean (CV), [Range]	1,289 [721–1,637]	890.38 (603.81-1213.70)		478.0±72.34	576 [455–847]
CSF:plasma AUC₂₄ [IQR range]		0.69 (0.58-0.82)			
Fraction unbound (IQR)	96-98%				
Volume of distribution/F *; Mean (CV), [Range]	1.41 [0.85–2.23] L/kg	17.50± 9.99 L	0.6-0.9 L/kg		0.94 [0.60–1.18] L/kg
Comments	Flucytosine was administered in combination with other drugs Cells are left empty when data are not readily available				

5. Pharmacodynamics				
Murine model of invasive candidiasis	<i>Candida albicans</i> (N=4) (10)			
T>MIC for 0.5 log CFU reduction in kidney burden compared to untreated controls	15%			
T>MIC for 1 log CFU reduction in kidney burden compared to untreated controls	22%			
T>MIC for 2 log CFU reduction in kidney burden compared to untreated controls	35%			
T>MIC for stasis compared to the kidney burden at the start of therapy	52%			
Comments				

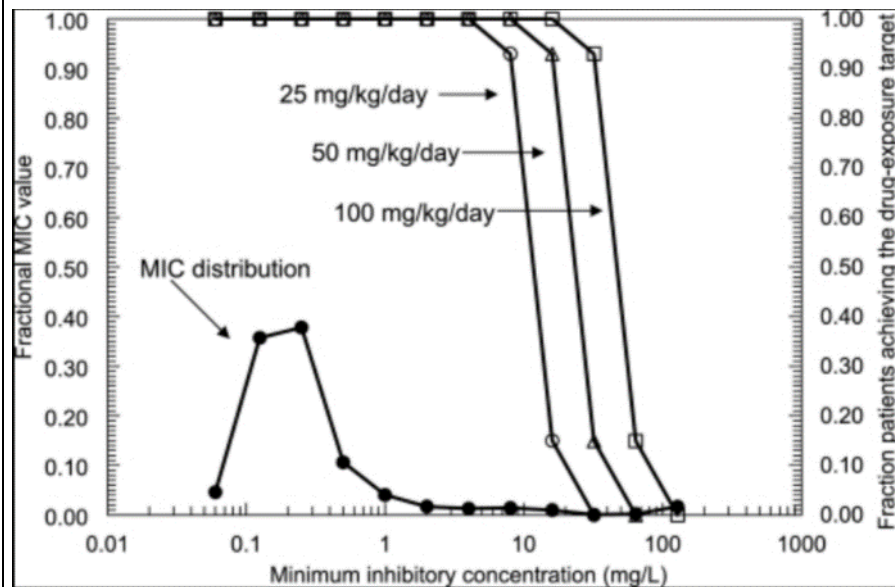
6. Monte Carlo simulations and Pk/Pd breakpoints

Fraction of the 9,999 simulated patients which achieved the drug exposure target (i.e. serum 5-FC concentrations greater than the MIC for at least 45% of the dosing interval) following the administration of 5-FC at 25, 50 and 100 mg/kg/day, depicted by open circles, triangles, and squares, respectively.

A serum level above the MIC for at least 45% of the dosing interval optimally separated the population into groups with high and low probabilities of a 2-log fall within the kidney of a *C. albicans* strain with MIC, 0.125 mg/L.

The distribution of the MICs for 1,941 *C. albicans* isolates, obtained using the EUCAST method, is shown by the solid circles. The MIC₉₀ was 1 mg/L. The simulations suggested that the *in vivo* drug exposure target (i.e., T>MIC of >45%) was attained in at least 95% of simulated patients who were infected with an isolate (of *C. albicans*) with an MIC of 16, 8 or 4 mg/L or less and who received at least 100, 50 or 25 mg/kg/day in four divided doses, respectively (10).

Data for other species are not available, however, this observation is likely transferrable to *C. auris*, *C. tropicalis*, and *C. parapsilosis* which share the same ECOFF as *C. albicans* and have similar or lower virulence.



7. Clinical data

Below is a brief summary of clinical use of 5-FC against fungal infections. For more information please refer to clinical guidelines.

5-FC is an antifungal agent first licensed in the 1970's. It is primarily used in combination therapies for cryptococcal meningitis and severe *Candida* infections, particularly those involving the central nervous system (CNS), endocardium, or urinary tract. It is usually combined with amphotericin B or other antifungals with the intention of avoiding the emergence of antifungal resistance (11). Due to the risk of resistance and potential toxicity, its use requires careful patient selection, dosing and monitoring.

Candida

Candida endophthalmitis;

5-FC concentrates in the eye and reaches approximately 100% of serum concentrations of 5-FC in vitreous humour of human eyes (12).

Candida meningitis and CNS candidiasis;

5-FC crosses the blood brain barrier well. In a study of treatment outcomes of HIV-infected patients with *Candida* meningitis, patients receiving combination therapy with 5-FC and amphotericin B exhibited a 22% improvement in survival. Mortality decreased from 33% in patients treated with amphotericin B monotherapy to 11% among those who received the combined regimen (11). LAmB, usually combined with 5-FC, is strongly recommended for the treatment of CNS candidiasis, with fluconazole, alone or in combination with 5-FC, strongly recommended as oral consolidation therapy (13).

Candida endocarditis;

The global *Candida* guideline strongly recommends initial therapy either with LAmB with or without 5-FC, or with an echinocandin (13).

Candida fungal urinary tract infection;

5-FC is excreted in the urine and can be a useful treatment option/addition in refractory *Candida* UTI and due to azole resistant *Candida* species such as *C. glabrata* and *C. auris* (14, 15). While 5-FC monotherapy has been used for urinary tract infections caused by *Candida* species, its effectiveness is limited by an associated development of resistance in 22% patients and failure on therapy.

Cryptococcus

Amphotericin B plus 5-FC combination clear cryptococci more rapidly than either amphotericin B alone or amphotericin B plus fluconazole (16, 17). Addition of 5-FC to fluconazole is a cost-efficient alternative to amphotericin B based regimens particularly attractive in low-income countries (18).

8. Clinical breakpoints					
	Organism group	MIC breakpoints (mg/L)			Notes
		S ≤	R >	ATU	
Species-related breakpoints	<i>C. albicans</i>	IE	IE		
	<i>C. parapsilosis</i>	IE	IE		
	<i>C. tropicalis</i>	IE	IE		
	<i>C. krusei</i>	IE	IE		
	<i>C. auris</i>	IE	IE		
	<i>C. neoformans</i>	IE	IE		
Species without breakpoints	Animal data suggest that isolates with MICs up to 16 mg/L can be treated, but there is no clinical evidence to support this observation. Hence, EUCAST has abstained from setting clinical breakpoints. Careful monitoring of patients with non-WT isolates is recommended.				
Clinical qualifications	CNS infections, endocarditis, endophthalmitis, urinary tract infections, intra-abdominal infections as part of combination regimens.				
Dosage	4 x 25-37.5 mg/kg				
Additional comment	Because of the rapid emergence of resistance during monotherapy, flucytosine is combined with other antifungal drugs, most frequently with amphotericin B.				

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