

Novel Rapid Antifungal Susceptibility Testing System for Yeasts Based on Fast Cell Growth

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Background

- The rise of multidrug-resistant (MDR) fungal pathogens, posing a serious global threat to human health, has increased the importance of antifungal susceptibility testing (AFST) in clinical microbiology laboratories.
- The traditional methods of AFST have turnaround times of 24 to 48 h.
- Given the urgency of adequate antifungal treatment in invasive fungal disease, rapid AFST is urgently needed in clinics to treat invasive fungal infections with the appropriate antifungal drugs and to slow the emergence of antifungal resistance.

Results

- Table 1 shows summary results for ten antifungal agents (fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, caspofungin, micafungin, anidulafungin, amphotericin B, and 5-flucytosine) in terms of essential agreement between the rapid AFST and the reference/comparator method.
- For AFST essential agreement (EA) is achieved if the MIC obtained with the test method is within 2 two-fold dilution of that obtained with the reference or comparator method according to the CLSI guideline M52. Overall, EA is more than 93% for all 10 drugs. Table 2 shows MIC values for quality controls and typical clinical strains between the rapid AFST and reference/comparator methods.

Conclusions

The performance of this reported rapid AFST system is substantially equivalent to that of the standard microdilution method and the Sensititre YeastOne method. The rapid AFST system can be an ideal alternative AFST method given that it reduces the turnaround time significantly.

Methods

➤ The rapid AFST system follows the same operation procedure of a traditional AFST as shown in Figs 1 and 2. The difference is that the rapid AFST is measuring the metabolism in fungal cells using resazurin in combination with a specifically formulated medium that promotes fast growth and metabolism of fungal cells, thus requires much shorter incubation time to obtain desired results.

➤ A total of 32 randomly selected clinical fungal strains were analyzed at the Peking Union Medical College Hospital. Species of these strains were commonly found in patients with systemic infections: *Candida albicans* (n=9), *Candida krusei* (n=5), *Candida tropicalis* (n=3), *Candida parapsilosis* (n=3), *Candida lusitanae* (n=3), *Candida glabrata* (n=7), and *Candida guilliermondii* (n=2). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality control strains.

➤ The performance of this rapid AFST method in terms of minimum inhibitory concentration (MIC) for isavuconazole was compared with the standard broth microdilution method according to CLSI M27 Ed4 guidelines. The comparator method for the remaining nine antifungal agents was the Sensititre YeastOne colorimetric panel (TREK Diagnostic Systems - West Sussex, UK). For the rapid AFST method, MICs were determined at 5-7 h of incubation, with extended incubation to 12 h for *Candida glabrata*. MICs for broth microdilution and the YeastOne panel were determined at 24 to 48 h of incubation.

Table 1 Summary of Essential agreement (EA) between reference method and the rapid AFST for *Candida* species.

Antifungal agents	Reportable Range(μg/ml)		N _{EA}	N _T	EA [N _{EA} / N _T]
	Reference method	Rapid AFST			
Fluconazole	0.12-256	0.12-64	31	32	96.88%
Itraconazole	0.016-16	0.03-16	30	32	93.75%
Voriconazole	0.008-8	0.016-8	32	32	100.00%
Posaconazole	0.008-8	0.03-16	30	32	93.75%
Isavuconazole	0.03-16	0.016-8	30	32	93.75%
Caspofungin	0.008-8	0.016-8	31	32	96.88%
Micafungin	0.008-8	0.016-8	30	32	93.75%
Anidulafungin	0.016-8	0.03-4	31	32	96.88%
5-flucytosine	0.06-64	0.12-64	32	32	100.00%
Amphotericin B	0.12-8	0.12-8	32	32	100.00%

Abbreviations: EA, essential agreement (minimal inhibitory concentration (MIC) result obtained with rapid AFST that is within two two-fold dilution of the MIC value determined by the reference method); NEA, number of tests that resulted in EA; NT, total number of clinical isolates tested.

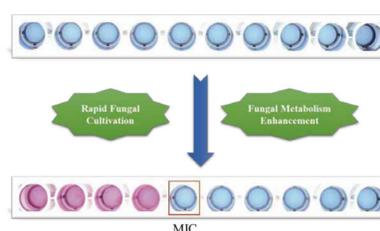


Fig 1 Detection principle of the rapid AFST.

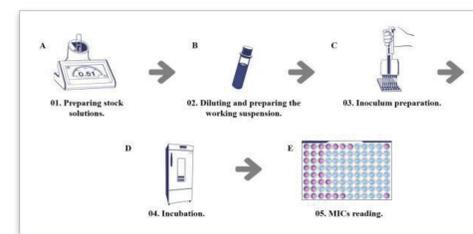


Fig 2 Operation procedure of the rapid AFST.

Table 2 MIC values obtained by reference methods and rapid AFST for quality control strains and typical strains of clinical isolates

Antifungal agents	QC strains				Clinical strains													
	<i>Candida parapsilosis</i> ATCC 22019		<i>Candida krusei</i> ATCC 6258		<i>Candida albicans</i>		<i>Candida krusei</i>		<i>Candida tropicalis</i>		<i>Candida parapsilosis</i>		<i>Candida lusitanae</i>		<i>Candida glabrata</i>		<i>Candida guilliermondii</i>	
	QC Range	Rapid AFST	QC Range	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST
Fluconazole	0.5-4	4	8-64	16	1	1	32	16	2	1	0.25	0.5	0.5	1	32	64	>256	>64
Itraconazole	0.06-0.5	0.25	0.12-1	0.25	0.06	0.12	0.25	0.25	0.25	0.25	≤0.016	0.06	0.06	0.12	0.12	0.25	>16	>16
Voriconazole	0.016-0.12	0.06	0.06-0.5	0.12	0.016	0.016	0.25	0.12	0.12	0.12	≤0.008	≤0.016	≤0.008	≤0.016	0.25	1	>8	8
Posaconazole	0.03-0.25	0.12	0.06-0.5	0.12	0.016	0.016	0.25	0.25	0.12	0.25	0.015	0.03	0.016	≤0.03	1	0.5	1	2
Isavuconazole	0.016-0.06	0.03	0.06-0.5	0.06	0.03	0.03	0.06	0.12	0.12	0.12	≤0.03	≤0.016	0.03	0.016	0.03	0.016	4	4
Caspofungin	0.25-1	0.5	0.12-1	0.12	0.03	0.12	0.5	0.12	0.06	0.03	0.5	0.25	0.12	0.06	0.06	0.06	0.12	0.06
Micafungin	0.5-2	1	0.06-0.25	0.06	≤0.008	≤0.016	0.06	0.06	0.03	0.03	1	0.5	0.06	0.03	0.016	≤0.016	0.12	0.12
Anidulafungin	0.25-2	0.5	0.03-0.12	0.03	≤0.016	≤0.03	0.03	0.03	0.25	0.12	1	0.25	0.12	0.03	0.03	0.03	0.5	0.25
5-flucytosine	0.06-0.25	0.25	4-16	4	≤0.06	≤0.12	8	4	≤0.06	≤0.12	≤0.06	≤0.12	≤0.06	≤0.12	≤0.06	≤0.12	>64	>64
Amphotericin B	0.25-2	0.5	0.5-2	0.5	0.5	0.25	0.5	0.5	1	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5