EVALUATION OF THE NEW FUNGIFAST® AFG YEAST ANTIFUNGAL SUSCEPTIBILITY TEST: COMPARISON WITH CLSI/NCCLS M27-A2- OR E-TEST METHOD

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Introduction

Antifungal susceptibility testing of yeasts are increasingly requested due to the emergence of resistant isolates and the larger number of antifungal agents available. Methods should be reliable, reproducible and easy to perform. FUNGIFAST® AFG (INTERNATIONAL MICROBIO) is a new commercially developed tray containing 5 antifungals: amphotericin B (AMB), flucytosine (5FC), fluconazole (FCA), itraconazole (ITZ) and voriconazole (VOR). This tray is designed to give MIC results in 24h or 48h. We evaluated the value of this system in comparison with the CLSI (formerly NCCLS) M27-A2 method or the E-Test method (AB Biodisk), on 92 yeast clinical isolates representing 12 species.

Material and Methods

Ninety-two isolates were evaluated: 81 clinical isolates, 11 collection strains (rare species or from deep sites). Two Quality Control strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) were included weekly as controls. Species distribution was: *C. albicans* 31.5%, *C. glabrata* 23.9%, *C. tropicalis* 10.9%, *C. parapsilosis* 7.6% and other *Candida* and non *Candida* species 26% (8 species). Each isolate was subcultured for 24h in CANDICHROM® II medium. The strains were tested in parallel with E-Test method (RPMI agar) for AMB, or CLSI M27-A2 broth micro dilution method for other antifungal agents. For CLSI M27-A2, the endpoints were obtained by reading visually and spectrophotometrically (MIC 70% in comparison with growth control) after 48h. For FUNGIFAST® AFG, 10µL of inoculated suspension medium at 2McF were added to the MES FUNGI medium, and 100µL were inoculated in each well. Colorimetric reading was performed at 24h (or 48h) with a change in the growth indicator from blue to pink.

Results and conclusion

FUNGIFAST® AFG method demonstrated no lack of growth for the clinical isolates tested, and 85% of the MIC values were obtained after 24h of incubation. QC strains MIC values were in good conformity within the limits reported. Very good categorical agreement was obtained with AMB, 5FC and VOR (100%, 91.3%, and 88% respectively), and good for ITZ (75% with 23 minor discrepancies) and FCA (71.7% with 25 minor and 1 major discrepancies). Regarding these two last azoles, MICs tended to be slightly higher mainly with *C. albicans* and *C. glabrata* strains. Three very major discrepancies (3 *C. albicans*) were observed with VOR and because 2 were found susceptible when evaluated by E-Test method, these isolates certainly presented significant trailing growth particularly with the CLSI M27-A2 method. Thus, the results for isolates with significant trailing growth should be carefully interpreted, and such strains should be tested repeatedly. We plan to evaluate secondarily these strains by E-Test method, and new reading criteria for FUNGIFAST® AFG interpretation. The FUNGIFAST® AFG test is rapid, reproducible, easy to perform and could be a suitable alternative for routine antifungal susceptibility testing of clinical isolates of yeasts.

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Evaluation of the new FUNGIFAST® AFG yeast antifungal susceptibility test: comparison with CLSI M27-A2 or the E-Test method

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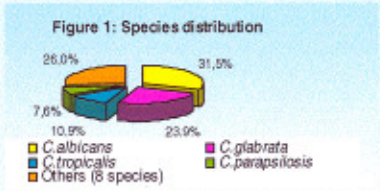
Objective

- Emergence of resistant isolates + larger number of antifungal agents → requirement for a reliable antifungal susceptibility testing of yeasts (1).
- FUNGIFAST® AFG (INTERNATIONAL MICROBIO) is a new commercial tray based on a microdilution method allows the testing of 5 antifungals: amphotericin B (AMB), flucytosine (5 FC), fluconazole (FCZ), itraconazole (ITZ) and voriconazole (VOR).
- This tray is evaluated in comparison with CLSI (formerly NCCLS) M27-A2 method or E-Test method (AB Biodisk) on 92 yeast clinical isolates (12 species).

Methods

81 clinical isolates + 11 collection strains (rare species or yeasts from deep sites) were evaluated

Figure 1: Species distribution



2 Quality Control strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) were included as controls

Each strain was subcultured for 24h in CANDICHROM® II medium and tested in parallel with E-Test (RPMI agar) for amphotericin B, or CLSI M27-A2 broth micro dilution method for other antifungals.
For CLSI M27-A2, MICs 70% were read spectrophotometrically after 48h.
For FUNGIFAST® AFG, 10µL of inoculated suspension medium (2McF) was added to the MES FUNGI medium, and 100µL was inoculated in each well.
Colorimetric reading was performed at 24h (or 48h) with a change in the growth indicator from blue to pink or white.

Results

FUNGIFAST® AFG did not demonstrate a lack of growth, and 85% of the MIC values were obtained after 24h. The MIC values of the QC strains showed good agreement and were within the reported limits.

Table 1: Agreement (%) between FUNGIFAST ® AFG vs. reference methods

Antifungal agents	Fungifast (%)
AMB	100
5 FC	91,3
VOR	88
ITZ	75
FCZ	71,7

Figures 2 and 3: Fungifast® AFG tray

We observed :
- minor discrepancies mostly with FCZ (25) and ITZ (23). MICs tended to be slightly higher with FUNGIFAST ® AFG (mainly with *C.albicans* and *C.glabrata*).
- 1 major discrepancy with FCZ and VOR for the same isolate of *C.albicans*.
- 3 very major discrepancies with VOR for 3 *C.albicans* (2 were susceptible by E-Test method).

Table 2: Isolates both resistant (%) with the 2 methods for the most representative *Candida* species

MIC µg/mL / *Candida* species	AMB MIC >2	5 FC MIC ≥ 32	VOR MIC ≥ 4	ITZ MIC ≥1	FCZ MIC ≥ 64
C.albicans (n=29)	0	0	44,8	44,8	34,5
C.glabrata (n=22)	0	0	4,5	63,6	18,1
C.tropicalis (n=10)	0	20	0	10	0
C.parapsilosis (n=7)	0	0	0	0	0

For 1/3 of *C.albicans*, the resistance is observed amongst the three azoles.

A high percentage of *C.albicans* and *C.glabrata* appeared to be resistant to ITZ especially (trailing growth phenomenon (2) ?).

Discussion-Conclusion

- FUNGIFAST® AFG test is simple to perform and the colorimetric reading is less subjective than that of the reference method.
- The panel includes voriconazole - not always available in other commercial testing systems.
- On the basis of *in vitro* evaluation, this new kit can be recommended as a simple, reproducible and rapid method for the routine antifungal susceptibity testing of yeast clinical isolates.
- To avoid difficulties in the interpretation of trailing growth, new reading criteria are being considered.

References

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(2) PFALLER M.A. et al. Activities of fluconazole and voriconazole against 1 586 recent clinical isolates of *Candida* species determined by broth microdilution, disk diffussion, and Etest methods: report from the ARTEMIS global antifungal susceptibility programm 2001. J. Clin. Microbiol.2003, **41**:1440-1446.