

Evaluation of Bactec Mycosis IC/F and Plus Aerobic/F Blood Culture Bottles for Detection of *Candida* in the Presence of Antifungal Agents

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Clinical practice guidelines recommend performing follow-up cultures for patients with candidemia in order to determine the time when *Candida* is cleared from the bloodstream. Since this requires culturing blood samples from patients undergoing antifungal treatment, we evaluated two blood culture bottles (the Bactec Mycosis IC/F [MICF], specifically adapted to the growth of fungi, and the Bactec Plus Aerobic/F [PAF], containing resins to inactivate anti-infective agents) for their effectiveness in detecting *Candida albicans* and *Candida glabrata* when seeded in concentrations of 1 CFU/ml and 10 CFU/ml, respectively, together with human whole blood and various antifungal agents in therapeutic peak serum concentrations (C_{max}). Significant differences between the MICF and PAF vials for the detection of *Candida* spp. were found when inoculated with caspofungin (0/12 versus 8/12) ($P < 0.001$) or amphotericin B (3/12 versus 12/12) ($P < 0.001$). Inoculation of fluconazole or voriconazole did not influence the effectiveness of detection in the MICF and PAF bottles ($P = 1.0$). Neither the MICF nor the PAF bottles detected *Candida* spp. reliably when seeded together with anidulafungin (1/12 versus 1/12) ($P = 1.0$) or micafungin (0/12 versus 1/12) ($P = 1.0$). The times to positivity of both bottles were significantly prolonged when antifungal agents were added compared to those of controls without antimycotic drugs ($P < 0.001$). Overall, the results of this *in vitro* study indicate that the PAF bottles detected *Candida* spp. more reliably than the MICF bottles when supplemented with certain antifungal agents. Consequently, clinical studies should evaluate whether this holds true when blood cultures from patients undergoing antifungal treatment are performed.

A retrospective assessment among 14,414 patients in 76 countries demonstrated that *Candida* bloodstream infections affect 6.9 of 1,000 patients in intensive care units (ICUs) and that candidemia is associated with a high mortality rate (42%) and prolonged ICU lengths of stay (1). While *Candida albicans* is still the predominant species in invasive infections, the prevalence and clinical impact of non-*C. albicans* species, which are associated with increasing resistance to antifungal agents (2), are increasing (3).

Hence, the management of patients with candidemia and the use of appropriate microbiological diagnostic tests are of utmost importance. Therefore, some studies have evaluated the commercial Bactec system for its ability to detect *Candida* bloodstream infections. With this system, Bactec Mycosis IC/F (MICF) blood culture bottles, which are specifically adapted to the growth of *Candida* spp., were demonstrated to have a significantly shorter time to positivity (TTP) and a higher *Candida* detection rate than conventional (an)aerobic media (such as the Bactec Plus Aerobic F [PAF] and Bactec anaerobic bottles) (4–8).

The Infectious Diseases Society of America (IDSA) and a panel of European microbiologists and infectious disease specialists (within the European Society of Clinical Microbiology and Infectious Diseases [ESCMID]) recommend that the duration of antifungal therapy for patients with candidemia be prolonged for 2 weeks after the clearance of *Candida* from the bloodstream (and resolution of symptoms attributable to candidemia) (9, 10). This recommendation implies that after confirmation of candidemia and the start of antimycotic therapy, daily follow-up blood cultures should be performed in order to determine the time of bloodstream clearance. However, neither the IDSA nor the ESCMID specifies the diagnostic medium which should be used in these cases, and up to now the diagnostic applicability of the *Can-*

*did*a bloodstream infection detection systems with respect to their ability to detect *Candida* during the course of antifungal therapy has not been systematically evaluated.

The aim of this study was to analyze which *Candida*-spiked blood culture bottle detects candidemia with the highest sensitivity and the shortest TTP and also in the presence of defined amounts of antifungal agents. For comparing the Bactec MICF and PAF bottles, *in vitro* experiments were performed using (i) defined quantities of *Candida albicans* and the emerging species *Candida glabrata*, (ii) antifungal agents added in concentrations corresponding to their respective therapeutic peak serum concentrations, and (iii) volumes of human blood as recommended by the manufacturer.

Overall, the results of this study can be used to guide clinical diagnostic tests for patients with proven candidemia and to guide specific recommendations by the IDSA and ESCMID regarding this issue.

MATERIALS AND METHODS

Preparation of *Candida* suspensions. *Candida albicans* (ATCC 90028) and *Candida glabrata* (ATCC 90030) were cultured on Kimmig agar plates (bioMérieux, Marcy l'Etoile, France) for 48 h at 37°C. From these cultures, colonies were suspended in 0.85% sodium chloride (3 ml *in vitro*

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diagnostic medium; bioMérieux), vortexed, and diluted to a McFarland standard of 0.5 density. From this suspension, decimal dilution series were prepared with concentrations of 1 CFU/ml and 10 CFU/ml. The final inoculum size of each suspension was checked by plating and incubating the suspension on a Kimmig agar plate for 48 h at 37°C.

Susceptibility testing. The MICs of both ATCC strains for all tested antifungal agents were determined using the gradient diffusion method (Etest; bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Briefly, several colonies from an overnight agar plate were emulsified in 0.85% saline solution and adjusted to a 0.5 McFarland standard. A plate rotator was used to distribute the inoculum evenly over an RPMI 1640 agar plate (Sigma-Aldrich, St. Louis, MO, USA). The Etest strips were positioned on agar plates and dried for approximately 15 min, and the inoculated plates were incubated at 37°C. The results were read according to the manufacturer's instructions. This procedure was performed three times for all antifungal agents and each ATCC strain. Each final MIC reported was defined as the median of the three MIC values read at 24 h.

Blood culture systems used. The blood culture bottles used in this study were Bactec Mycosis IC/F (MICF) bottles (medium comprising 1.0% brain heart infusion broth, 0.5% soybean-casein digest broth, 0.035% yeast extract, 0.6% sucrose, 0.1% dextrose, 0.05% *m*-inositol, 0.0001% ferric ammonium citrate, 0.05% sodium polyanethanol sulfonate [SPS], 0.024% saponin, 0.0037% chloramphenicol, 0.001% tobramycin, 0.01% antifoaming agent; recommended for inoculation of 8 to 10 ml blood) and Bactec Plus Aerobic/F (PAF) bottles (medium comprising 3.0% soybean-casein digest broth, 0.25% yeast extract, animal tissue digest, 0.05% amino acids, sugar, sodium citrate, 0.005% SPS, 0.025% vitamins, 0.005% antioxidants/reductants, 16.0% nonionic adsorbing resin, 1.0% cationic exchange resin; recommended for inoculation of 8 to 10 ml blood).

Preparation of blood samples. For inoculation of the blood culture bottles according to the manufacturer's recommendation, venous whole-blood samples obtained for quality control purposes from healthy human volunteer donors not receiving systemic medication were retrieved. Donors provided written informed consent. Samples were retrieved in commercial sterile bags (Leukotrap WB T&B; Pall Medical, Port Washington, NY) and inoculated into the blood culture bottles within 4 h after retrieval from the donor.

Preparation of antifungal agents. For inoculation into the blood culture bottles, we reconstituted the antifungal agents listed below according to the recommendations of the manufacturers. A stock solution containing the maximum plasma concentration (C_{max}) for adults (when using a standard dosing regimen) was prepared for amphotericin B deoxycholate (Fungizone; Bristol-Myers Squibb) (C_{max} , 2.9 µg/ml) (11), anidulafungin (Ecalta; Pfizer) (C_{max} , 8.6 µg/ml) (12), caspofungin (Cancidas; Merck Sharp & Dohme) (C_{max} , 12.1 µg/ml) (13), micafungin (Mycamine; Astellas) (C_{max} , 10.1 µg/ml) (12), fluconazole (Diflucan IV; Pfizer) (C_{max} , 4.68 µg/ml) (14), and voriconazole (Vfend; Pfizer) (C_{max} , 5.402 µg/ml) (15).

Inoculation of the blood culture bottles in the first test series. Each test series set of blood culture bottles consisted of 24 MICF and 24 PAF bottles, which were inoculated using sterile precautions with a *Candida* suspension (100 µl *C. albicans* suspension or 200 µl *C. glabrata* suspension, each at final concentrations of 1 CFU/ml and 10 CFU/ml in the bottle), 10 ml blood, and 100 µl of each of the six antifungal agents (at the concentrations mentioned above). In addition, for each CFU concentration, one culture vial with MICF medium and one with PAF medium were inoculated with blood and a *Candida* suspension without antifungal agents as controls. This inoculation protocol was repeated three times, yielding a total of 168 culture sets in this test series.

Incubation and data analysis. All blood culture bottles were incubated at 37°C in a BD Bactec 9240 system for 14 days. After 14 days, 100-µl samples from all negative bottles were streaked onto Kimmig agar plates and incubated for 48 h at 37°C. For all bottles detected as positive within 14 days, the TTPs were recorded. Statistical analyses were performed us-

ing the chi-square test, Fisher's exact test, or the Kruskal-Wallis test (SPSS statistical software, version 21).

Additional test series for echinocandins. After finishing the first test series as described above, which demonstrated significant differences between the MICF and PAF bottles, we repeated the same tests for caspofungin, anidulafungin, and micafungin using identical procedures for the preparation of the *Candida* suspensions and the human blood samples and inoculation of the bottles. The protocol for preparing the antifungal agents was changed by using stock solutions containing 50%, 25%, 12.5%, and 6.25% of the C_{max} for each antifungal agent when inoculating the blood culture bottles. All remaining procedures for inoculation and incubation were the same as those described above. For each of the four concentrations of the antifungal agents and each of the three echinocandins, the experiments were again performed three times for *C. albicans* and *C. glabrata* with inoculum suspensions of both 1 CFU/ml and 10 CFU/ml. This resulted in a second test series of 288 culture bottles.

RESULTS

A total of 168 seeded blood culture bottles were incubated in the first test series. For all controls containing *Candida* spp. without antifungal agents, growth was detected by the blood culture system. The median TTPs for the controls in MICF and PAF bottles were 21.1 h and 24.4 h ($P = 0.015$) for *C. albicans* and 22.0 h and 43.2 h ($P = 0.002$) for *C. glabrata*, respectively. For the controls without antifungal agents, the median TTPs for different inoculum sizes (1 CFU/ml and 10 CFU/ml) of *C. albicans* were 24.9 h and 21.4 h ($P = 0.07$) and for *C. glabrata* were 33.5 h and 31.3 h ($P = 0.24$), respectively.

Testing the susceptibility of the reference strains used for inoculation yielded median MICs for *C. albicans* of 0.19 mg/liter for amphotericin B, 1.0 mg/liter for fluconazole, 0.023 mg/liter for voriconazole, 0.19 mg/liter for caspofungin, 0.004 mg/liter for anidulafungin, and 0.016 mg/liter for micafungin. For *C. glabrata*, the MICs were 0.125 mg/liter for amphotericin B, 24 mg/liter for fluconazole, 0.5 mg/liter for voriconazole, 0.25 mg/liter for caspofungin, 0.006 mg/liter for anidulafungin, and 0.012 mg/liter for micafungin.

For the following calculations, the controls were excluded from the analysis, and only the culture bottles spiked with antifungal agents were included. Overall, when *Candida* spp. were inoculated in a concentration of 1 CFU/ml, the BD Bactec 9240 system yielded positive results in 16/36 (44%) cases for both *C. albicans* and *C. glabrata* ($P = 1.0$). When 10 CFU/ml inocula were used, 20/36 (56%) *C. albicans* and 22/36 (61%) *C. glabrata* cultures were detected ($P = 0.13$). Overall, growth was detected more frequently in the PAF bottles (46/72 [64%]) than in the MICF bottles (28/72 [39%]) ($P = 0.004$). The TTPs did not vary between the MICF and PAF bottles when we compared only the positive culture bottles ($P = 0.548$). The test results for the various antifungal agents are shown in Table 1. The growth of *Candida* spp. was more sensitively detected in MICF and PAF bottles in the presence of peak concentrations (C_{max}) of fluconazole and voriconazole. When *Candida* spp. were inoculated with anidulafungin and micafungin in MICF and PAF bottles, the BD Bactec 9240 system mostly did not detect growth ($P = 1.0$). For amphotericin B ($P < 0.001$) and caspofungin ($P = 0.001$), we observed that the level of detection of *Candida* spp. was significantly less in MICF than in PAF bottles. There were no significant differences regarding the growth of *C. albicans* versus that of *C. glabrata*.

The TTPs of blood culture bottles without antifungal agents (i.e., controls) and those supplemented with antifungals were sig-

TABLE 1 Comparison of Bactec Mycosis IC/F and Bactec Plus Aerobic/F blood culture bottles for detection of *Candida* spp. in the presence of therapeutic peak serum concentrations of different antifungal agents

Antifungal agent	<i>Candida</i> spp. detected separately (no. of culture sets with growth detected/no. of culture sets tested [%] and <i>P</i> value) by ^a :						Total <i>Candida</i> spp. detected (no. of culture sets with growth detected/no. of culture sets tested [%] and <i>P</i> value) by:		
	Bactec Mycosis IC/F bottles			Bactec Plus Aerobic/F bottles			Bactec Mycosis IC/F bottles	Bactec Plus Aerobic/F bottles	<i>P</i>
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>P</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>P</i>			
Amphotericin B	3/6 (50)	0/6 (0)	0.18	6/6 (100)	6/6 (100)	1.0	3/12 (25)	12/12 (100)	<0.001
Fluconazole	6/6 (100)	6/6 (100)	1.0	6/6 (100)	6/6 (100)	1.0	12/12 (100)	12/12 (100)	1.0
Voriconazole	6/6 (100)	6/6 (100)	1.0	6/6 (100)	6/6 (100)	1.0	12/12 (100)	12/12 (100)	1.0
Anidulafungin	0/6 (0)	1/6 (17)	1.0	0/6 (0)	1/6 (17) ^a	1.0	1/12 (8)	1/12 (8) [#]	1.0
Caspofungin	0/6 (0)	0/6 (0)	1.0	4/6 (67)	4/6 (67)	1.0	0/12 (0)	8/12 (67)	0.001
Micafungin	0/6 (0)	0/6 (0)	1.0	1/6 (17)	0/6 (0)	1.0	0/12 (0)	1/12 (8)	1.0

^a In one case, growth was undetected by the automated blood culture system, but *C. glabrata* grew on solid medium when samples from negative blood culture bottles were plated after 14 days of incubation.

nificantly different ($P < 0.001$) (Fig. 1). The median TTPs were 336 h (i.e., the maximum incubation time) for all three echinocandins tested, 67 h for amphotericin B, 36 h for voriconazole, 32 h for fluconazole, and 23 h for controls without antifungal agents (Fig. 1).

Compared to detection with inoculation of the C_{max} of caspofungin in MICF bottles (as shown in Table 1), *C. glabrata* was first detected at $0.125 \times$ the C_{max} (Table 2); in contrast, in PAF bottles, the growth of *C. albicans* and *C. glabrata* was more sensitively detected at all concentrations of caspofungin. For micafungin, *Candida* spp. were detected better in PAF bottles at $0.5 \times$ the C_{max} , while neither *C. albicans* nor *C. glabrata* was detected in the MICF bottles with concentrations higher than $0.125 \times$ the C_{max} . For anidulafungin, *C. glabrata* was detected more reliably from $0.5 \times$ the C_{max} and below in PAF bottles but not before $0.25 \times$ the C_{max}

in MICF bottles. *C. albicans* was detected in the presence of anidulafungin from $\geq 0.125 \times$ the C_{max} in PAF bottles and $0.0625 \times$ the C_{max} in MICF bottles.

DISCUSSION

The aim of this study was to investigate to what extent *Candida* spp. in quantities typically found in the blood of patients with candidemia (16) are detected in two different Bactec blood culture bottles when inoculated together with therapeutic concentrations of various antifungal agents. The study question arose from the IDSA's and ESCMID's recommendations to perform follow-up cultures for patients undergoing antifungal treatment to guide the length of therapy (9, 10). The evidence for this recommendation is based on several prospective clinical studies, where this procedure was associated with therapeutic success (17–19). However, none

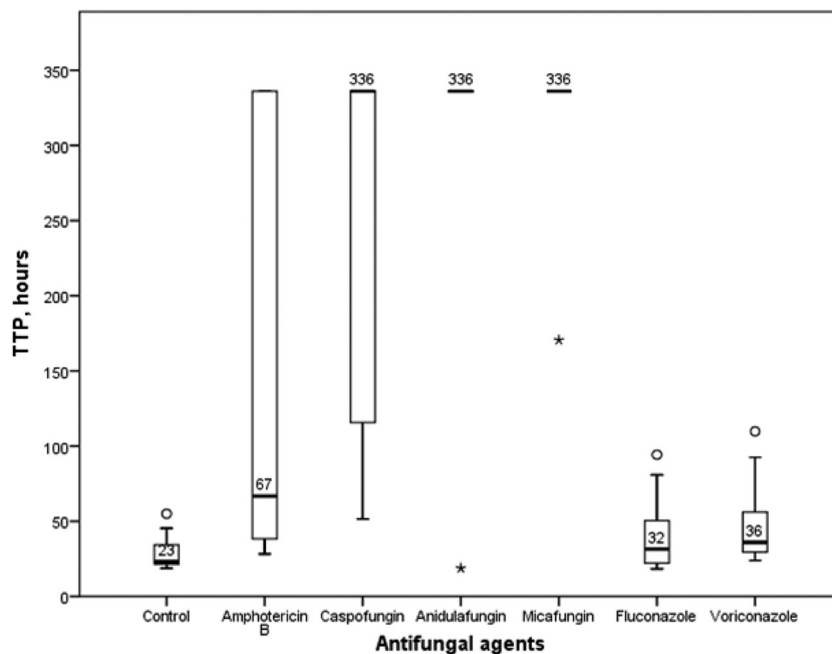


FIG 1 Times to positivity (TTPs) in hours for blood culture bottles without antifungal agents (controls) and supplemented with different types of antifungal agents (maximal incubation period, 336 h). Box plots indicate the medians (thick black bars) and 25th and 75th percentiles. Whiskers indicate the smallest and the highest values not classified as outliers. Outliers are indicated by circles and extreme outliers by asterisks.

TABLE 2 Comparison of Bactec Mycosis IC/F and Bactec Plus Aerobic/F blood culture bottles for detection of *Candida* spp. in the presence of different concentrations of echinocandins

C_{\max} of antifungal agents used	<i>Candida</i> spp. detected separately (no. of culture sets with growth detected/no. of culture sets tested [%] and <i>P</i> value) by ^a :						Total <i>Candida</i> spp. detected (no. of culture sets with growth detected/no. of culture sets tested [%] and <i>P</i> value) by:		
	Bactec Mycosis IC/F bottles			Bactec Plus Aerobic/F bottles			Bactec Mycosis IC/F bottles	Bactec Plus Aerobic/F bottles	<i>P</i>
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>P</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>P</i>			
$0.5 \times C_{\max}$									
Anidulafungin	0/6 (0)	0/6 (0)	1.0	0/6 (0)	6/6 (100)	0.002	0/12 (0)	6/12 (50)	0.014
Caspofungin	0/6 (0)	0/6 (0)	1.0	5/6 (83)	4/6 (67)	1.0	0/12 (0)	9/12 (75)	<0.001
Micafungin	0/6 (0)	0/6 (0)	1.0	3/6 (50)	2/6 (33)	1.0	0/12 (0)	5/12 (42)	0.037
$0.25 \times C_{\max}$									
Anidulafungin	0/6 (0)	6/6 (100)	0.002	0/6 (0)	6/6 (100)	0.002	6/12 (50)	6/12 (50)	1.0
Caspofungin	0/6 (0)	0/6 (0)	1.0	5/6 (83)	6/6 (100)	1.0	0/12 (0)	11/12 (92)	<0.001
Micafungin	0/6 (0)	0/6 (0)	1.0	6/6 (100)	5/6 (83)	1.0	0/12 (0)	11/12 (92)	<0.001
$0.125 \times C_{\max}$									
Anidulafungin	0/6 (0)	4/6 (67)	0.061	5/6 (83)	4/6 (67)	1.0	4/12 (33)	9/12 (75)	0.10
Caspofungin	0/6 (0)	0/6 (0)	1.0	6/6 (100)	5/6 (83)	1.0	0/12 (0)	11/12 (92)	<0.001
Micafungin	6/6 (100)	6/6 (100)	1.0	6/6 (100)	5/6 (83)	1.0	12/12 (100)	11/12 (92)	1.0
$0.0625 \times C_{\max}$									
Anidulafungin	3/6 (50)	6/6 (100)	0.182	6/6 (100)	6/6 (100)	1.0	9/12 (75)	12/12 (100)	0.217
Caspofungin	0/6 (0)	2/6 (33)	0.455	5/6 (83)	6/6 (100)	1.0	2/12 (17)	11/12 (92)	<0.001
Micafungin	6/6 (100)	6/6 (100)	1.0	6/6 (100)	6/6 (100)	1.0	12/12 (100)	12/12 (100)	1.0

of these studies described the method of microbiological determination, which may limit the quality of evidence supporting the recommendation.

The rationale for choosing Bactec MICF bottles for these experiments was that in experiments evaluating the general applicability of different commercial blood culture systems for detecting *Candida* spp., Bactec MICF bottles, which include supplements specifically adapted for the growth of fungi, were shown to be more sensitive and to detect *Candida* spp. faster than most other conventional blood culture media (4–8). This finding was confirmed by the results of our study, since for controls inoculated without antifungal agents, we found reduced median TTPs in MICF bottles compared to those in PAF bottles (21.1 h versus 24.4 h for *C. albicans* and 22.0 h versus 43.2 h for *C. glabrata*, respectively). Bactec PAF bottles were chosen for comparison with MICF bottles, because they contain a standard medium for the detection of bacterial and fungal pathogens not specifically adapted to fungi and include resins, which can bind and inactivate antifungal agents and other drugs. Several investigations demonstrated that Bactec PAF bottles yielded better results for detecting bacteremia in patients undergoing pharmacotherapy, indicating that the resins have an effect on the inactivation of potential inhibitors of microbiological pathogen growth (20, 21).

In this study, we have shown that the presence of fluconazole and voriconazole had no effects on *Candida* species detection in MICF and PAF bottles, i.e., *Candida* spp. grew reliably in both bottles. For fluconazole, this finding might be due to the fact that the *C. albicans* and *C. glabrata* strains used in this study had high MICs (1.0 mg/liter and 24 mg/liter, respectively) and low C_{\max} /MIC quotients for this agent compared with those for the other antifungals tested. Another possible explanation for why the two azoles tested did not have major effects on the detection of *Can-*

*did*a spp. in the spiked bottles is that compared with echinocandins, which are fungicidal against *Candida*, azoles are fungistatic (22, 23).

However, for most of the other drugs tested, we found that their presence in therapeutic serum peak concentrations had a significant impact on the detection of *Candida* spp. For the inoculation of amphotericin B and the three echinocandins, we found that more sensitive detection in MICF than in PAF bottles (as demonstrated for the controls without antifungal agents in this study) did not hold true. Theoretically, one might assume that in these cases, the ability of the resins included in the Bactec PAF medium to bind and inactivate antimycotics led to significantly better detection in the PAF bottles. However, we have shown that when the PAF and MICF bottles were spiked with anidulafungin and micafungin, *Candida* spp. mostly remained undetected in both bottles. Since echinocandins are considered the therapy of choice for candidemia in the majority of cases (9), for these antifungal agents, we evaluated in more detail how the quantity of the drug inoculated affected the growth of *Candida* spp. Therefore, we supplemented the MICF and PAF bottles with concentrations of 6.25% to 50% of the C_{\max} values for these drugs. Overall, we found that the recovery rate was improved (Table 2), but *Candida* spp. (especially *C. glabrata*) were mostly not detected in the MICF bottles before the concentrations were reduced to 6.25% to 12.5% of the C_{\max} . Since the minimum serum concentrations with the standard echinocandin therapy are usually within a range of 1 to 6 mg/liter (13, 24, 25), i.e., about 10 to 50% of the C_{\max} of the agent, detection is not reliable even when the cultures are collected shortly before serum trough levels of the echinocandins are expected.

A major limitation of this study is that it was performed completely *in vitro* using *Candida* ATCC reference strains (rather than

clinical *Candida* isolates) and whole-blood samples from volunteers not taking any systemic medication. This does not reflect the actual situation and underlines the need for performing studies in a clinical setting to confirm our findings.

In summary, in this *in vitro* study, we have shown significant differences between the abilities of two blood culture bottles (Bactec Aerobic/F and Mycosis IC/F) used in a commercial blood culture detection system to detect putative *C. albicans* and *C. glabrata* bloodstream infections when the bottles were spiked with therapeutic concentrations of antifungal agents. These results clearly indicate that it is not sufficient to use MICF bottles when cultures from patients undergoing antifungal therapy are performed in order to determine the time of *Candida* clearance from the bloodstream. However, clinical studies should be performed to confirm these findings. Moreover, our observations prompt the necessity for performing clinical studies to document more accurately the preferred microbiological candidemia diagnostic tests to increase the evidence for recommendations suggesting that blood cultures be performed while the patient is being treated with antifungals.

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