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Detection of Simulated Candidemia by the BACTEC 9240 System with Plus Aerobic/F and Anaerobic/F Blood Culture Bottles

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We studied the ability of the BACTEC 9240 automated blood culture system to detect simulated candidemia, including both *Candida albicans* and non-*albicans Candida* species. Simulated blood cultures were produced using 50 *Candida* isolates and BACTEC Plus Aerobic/F and Anaerobic/F blood culture bottles. Ten milliliters of blood and a suspension of each isolate containing 1,000 CFU were introduced into each bottle and then incubated at 35°C in the BACTEC 9240 system. The system detected growth in 56 of 100 bottles. Four isolates did not have growth detected in either bottle after 21 days of incubation, resulting in four missed episodes of candidemia. If the blood culture bottles had been incubated for 5 days, an additional episode of candidemia would have remained undetected. If the bottles had been incubated for only 3 days, another episode would have been missed, resulting in up to six missed episodes of candidemia (four *Candida glabrata* isolates, one *C. albicans* isolate, and one *Candida rugosa* isolate). Terminal subculture of bottles without detected growth recovered yeast in 93% (41 of 44) of the bottles, representing 41 false negatives. In bottles where growth was detected, the time to detection was ~24 h. However, the mean time to growth detection for *C. glabrata* isolates in anaerobic medium was 22.14 ± 2.47 h, but it was 120.89 ± 35.33 h in aerobic medium ($P < 0.001$). The BACTEC 9240 system detected growth of most *Candida* isolates; however, the delayed time to detection of *C. glabrata* is clinically significant. Given the high rate of false negatives, terminal subcultures may be helpful in certain situations.

Candidemia is an increasingly common problem in hospitalized patients, with epidemiologic surveys revealing that *Candida* spp. are now the fourth most common pathogen isolated from the blood of hospitalized patients, most commonly in intensive-care unit settings (4, 10). Unfortunately, candidemia also carries the highest associated mortality (40%) of all nosocomial bloodstream infections (4). *Candida albicans* used to be the predominant cause of candidemia, accounting for >80% of all candidal isolates recovered from nosocomial yeast infections in the 1980s (1), but non-*albicans Candida* (NAC) species are now recovered with increasing frequency. *C. albicans* currently accounts for only about one-half of all nosocomial bloodstream *Candida* isolates in the United States and is even less commonly isolated in surgical and neonatal intensive-care unit settings (4, 10).

Episodes of candidemia are most commonly detected by standard blood culture media in automated blood culture systems. Multiple studies have demonstrated effective recovery of *Candida* spp. by use of the BACTEC 9240 automated blood culture systems (6, 8, 9, 11, 14). However, these earlier studies generally addressed *C. albicans* recovery; few NAC were recovered, and the performance of the system specifically for these species has not been well defined. Additionally, earlier studies were all conducted on clinical specimens, many without terminal subculture, and therefore the number of false negatives may not have been fully examined. In the course of performing a study of simulated candidemia, we noted some interesting observations in the overall detection of growth and time to detection among various *Candida* spp. We report our observations of the performance characteristics of the BACTEC 9240 system in the detection of growth, the time to detection, and the utility of terminal subcultures for a broad range of *Candida* spp., using a simulated candidemia model.

MATERIALS AND METHODS

Fifty clinical isolates of *Candida* were utilized in our study, including *C. albicans* (n = 12), *Candida tropicalis* (n = 12), *Candida glabrata* (n = 9), *Candida krusei* (n = 5), *Candida lusitaniae* (n = 3), *Candida parapsilosis* (n = 3), *Candida guilliermondii* (n = 2), *Candida kefyr* (n = 2), *Candida firmetaria* (n = 1), and *Candida rugosa* (n = 1). Frozen yeast isolates, originally isolated from clinical specimens, were subcultured twice onto solid medium to ensure the isolation of pure colonies. A suspension of each specimen was made in 5 ml of normal saline and adjusted by transmittance at 530 nm (Spectronic 20D; Milton Roy, Rochester, N.Y.) to 0.5 McFarland standard (Becton Dickinson, Sparks, Md.). The resulting suspension contained $\sim 1 \times 10^6$ to 5×10^6 yeasts/ml (7). A 1:100 dilution of each yeast suspension was then performed to produce a density of 1×10^4 to 5×10^4 yeasts/ml. A 0.1-ml aliquot of this suspension was introduced into each set of blood cultures, one aerobic and one anaerobic bottle, to produce a final inoculum of ~1,000 yeast cells. Inoculum densities were verified by colony counts of serial dilutions on solid medium.

The blood culture bottles utilized in this study were the BACTEC Plus Aerobic/F and Anaerobic/F (Becton Dickinson). All bottles were inoculated with 10 ml of fresh, whole blood from healthy volunteers. The protocol was approved by the Brooke Army Medical Center institutional review board, and informed consent was obtained from all volunteers. After the addition of 0.1 ml of yeast suspension, the inoculated bottles were immediately placed in the BACTEC 9240 automated blood culture system (Becton Dickinson) for up to 21 days. This system incubates specimens at 35°C with continuous agitation and uses a fluorescence technology to indicate the quantity and rate of CO_2 production, indicative of microbial growth.

Individual blood culture bottles were removed from the automated system when growth was detected, and the time to detection was recorded. A 0.1-ml aliquot was withdrawn from each positive bottle, plated on solid medium, incubated at 30°C, and read after 24 h.

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TABLE 1. Detection of growth in simulated candidemia by the BACTEC 9240 using Aerobic Plus/F and Anaerobic Plus/F blood culture bottles

Candida sp. (no. of isolates)	No. of isolates recovered			
	Both bottles	Aerobic bottle only	Anaerobic bottle only	Neither bottle
C. albicans (12)	0	11	0	1
C. tropicalis (12)	4	8	0	0
C. glabrata (9)	4	2	1	2
C. krusei (5)	1	4	0	0
C. lusitaniae (3)	1	2	0	0
C. parapsilosis (3)	0	3	0	0
C. guilliermondii (2)	0	2	0	0
C. kefyr (2)	0	2	0	0
C. firmetaria (1)	0	1	0	0
C. rugosa (1)	0	0	0	1
Total (50)	10	35	1	4

Blood culture bottles in which no growth was detected were removed from the automated system after 21 days of incubation. Terminal subculture was performed; a 0.1-ml aliquot was removed from each bottle and then plated on solid medium. Each plate was incubated at 30°C and read daily for up to 72 h.

Statistical analysis of data was performed using SPSS software. One-way analysis of variance was followed by Student-Newman-Keuls tests. Additional tests included Fisher's exact test, McNemar tests, and Cohen's kappa test when necessary.

RESULTS

Fifty-six of the 100 bottles in this study had growth detected by the BACTEC 9240 automated blood culture system (Table 1). All *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, *C. kefyr*, and *C. firmetaria* isolates had growth detected exclusively in the aerobic medium. Four *Candida* spp. had growth detected in both aerobic and anaerobic media: *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. lusitaniae*.

Forty-four bottles had no growth detected despite 21 days of incubation. These included 5 of 50 (10%) aerobic and 39 of 50 (78%) anaerobic blood culture bottles. Though 44 bottles had no growth detected, only 4 of 50 (8%) *Candida* isolates did not have growth detected in either bottle. The four isolates were one *C. albicans*, one *C. rugosa*, and two *C. glabrata*.

Terminal subculture was performed on all 100 bottles. A comparison of growth detection by the BACTEC 9240 to terminal subculture is presented in Table 2. Forty-five of 50 (90%) aerobic bottles had growth detected by the BACTEC 9240, while 49 of 50 (98%) had positive terminal subcultures. In contrast, only 11 of 50 (22%) anaerobic bottles had growth detected by the BACTEC 9240, while 48 of 50 (96%) had positive terminal subcultures ($P < 0.001$). All 56 bottles with growth detected by the BACTEC 9240 also had positive terminal subcultures. Of the 44 bottles with no growth detected by the BACTEC 9240, 41 of 44 (93%) had positive terminal subcultures. Four of five "negative" aerobic bottles (three *C. glabrata* and one *C. rugosa*) were subculture positive; one *C. albicans* bottle was subculture negative. Thirty-seven of 39 anaerobic bottles with no growth detected by the BACTEC 9240 were subculture positive (11 *C. albicans* isolates, 8 *C. tropicalis* isolates, 4 *C. glabrata* isolates, 4 *C. krusei* isolates, 3 C. *parapsilosis* isolates, 2 *C. lusitaniae* isolates, 2 *C. guilliermondii* isolates, 2 *C. kefyr* isolates, and 1 *C. rugosa* isolate); 1 *C. albicans* isolate and the *C. firmetaria* isolate were subculture negative. A total of three of the bottles were therefore true negatives, with no growth in the anaerobic bottle of the *C. firmetaria* isolate and both the aerobic and anaerobic bottles of one *C. albicans* isolate.

Growth detection occurred primarily within the first 3 days of incubation, with 50 bottles detected as positive by day 3. Of the six remaining bottles with growth detected, three were detected on day 5, two were detected on day 6, and one was detected on day 8. Two of these "late" cultures represented the only bottle of the blood culture set with growth detected. Both were *C. glabrata* isolates in aerobic media with growth detected on day 5 and day 8.

The mean time to growth detection for each *Candida* spp. is listed in Table 3. The mean time to growth detection for most *Candida* spp., including *C. albicans*, *C. tropicalis*, and *C. para psilosis*, was ~24 h. *C. glabrata* was the only species that required a substantially longer incubation period in aerobic medium for growth detection, with a mean time to growth detec-

TABLE 2. *Candida* growth detection in the BACTEC 9240 automated blood culture system compared to growth detection by terminal subculture

Candida sp.	No./*n* (%) positive			
	Aerobic bottles		Anaerobic bottles	
	BACTEC	Subculture	BACTEC	Subculture
C. albicans	11/12 (92)	11/12 (92)	0/12 (0)	11/12 (92)
C. tropicalis	12/12 (100)	12/12 (100)	4/12 (33)	12/12 (100)
C. glabrata	6/9 (67)	9/9 (100)	5/9 (56)	9/9 (100)
C. krusei	5/5 (100)	5/5 (100)	1/5 (20)	5/5 (100)
C. lusitaniae	3/3 (100)	3/3 (100)	1/3 (33)	3/3 (100)
C. parapsilosis	3/3 (100)	3/3 (100)	0/3 (0)	3/3 (100)
C. guilliermondii	2/2 (100)	2/2 (100)	0/2 (0)	2/2 (100)
C. kefyr	2/2 (100)	2/2 (100)	0/2 (0)	2/2 (100)
C. firmetaria	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
C. rugosa	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)
Total	45/50 (90)	49/50 (98) (NS)[a]	11/50 (22)	48/50 (96) ($P < 0.001$)

[a] NS, not significant.

TABLE 3. Incubation time to growth detection by the BACTEC 9240 using Aerobic Plus/F and Anaerobic Plus/F blood culture bottles

Species (total no. of isolates)	Mean time to growth detection (h) (avg ± SD)	
	Aerobic bottles (no. of isolates)	Anaerobic bottles (no. of isolates)
C. albicans (12)	20.63 ± 1.57 (11)	ND[a]
C. tropicalis (12)	14.28 ± 1.45 (12)	48.36 ± 80.27 (4)
C. glabrata (9)	120.89 ± 35.33 (6)[b]	22.14 ± 2.47 (5)
Other *Candida* spp. (17)	19.64 ± 5.66 (16)	20.41 ± 4.29 (2)
C. krusei (5)	16.26 ± 1.35 (5)	23.44 (1)
C. lusitaniae (3)	17.21 ± 1.35 (3)	17.37 (1)
C. parapsilosis (3)	25.01 ± 0.51 (3)	ND
C. guilliermondii (2)	21.34 ± 0.53 (2)	ND
C. kefyr (2)	14.19 (2)	ND
C. firmetaria (1)	35.19 (1)	ND
C. rugosa (1)	ND	ND

[a] ND, no growth detected by BACTEC 9240 automated system.
[b] Time to growth detection of *C. glabrata* isolates in aerobic medium was significantly longer ($P < 0.001$) than that of *C. glabrata* isolates in anaerobic medium, as well as those of all other *Candida* spp. in aerobic medium.

tion of just over 5 days and a range of 3 to 8 days (69 to 178 h). The mean time to growth detection of *C. glabrata* in aerobic bottles was significantly longer than in the corresponding anaerobic bottles (121 versus 22 h; $P < 0.001$). Additionally, the mean time to growth detection of *C. glabrata* in aerobic bottles was significantly longer than the mean time to growth detection of all other *Candida* spp. in aerobic media (121 versus 18 h; $P < 0.001$).

DISCUSSION

Our group was concerned to find that only 56 of 100 bottles had growth detected by the BACTEC 9240, which resulted in four episodes of missed simulated candidemia. Forty-four bottles had no growth detected despite the fact that each bottle was seeded with ~1,000 yeast cells and incubated for up to 21 days. The majority of bottles with no growth detected were anaerobic; more troubling was the fact that a full 10% of aerobic bottles had no growth detected despite a relatively high yeast inoculum and a prolonged incubation period. Forty-one of the 44 (93%) "negative" bottles demonstrated heavy growth of yeast when terminally subcultured, representing 41 false-negative cultures. A review of the literature revealed that other authors have described a similar phenomenon with regard to recovery of *Candida* spp. with clinical use of the BACTEC 9240 (8, 12, 14). Shigei et al. terminally subcultured Plus Aerobic/F and Anaerobic/F clinical blood culture specimens in which growth was not detected after 5 days of incubation in the BACTEC 9240. Forty-four *Candida* spp. were detected by the system, 42 in aerobic and 2 in anaerobic media. Terminal subculture detected an additional 21 *Candida* spp., 4 in aerobic and 17 in anaerobic media, all representing false negatives (12). Pohlman et al. and Ziegler et al. also performed terminal subculture on "negative" Plus Aerobic/F and Anaerobic/F bottles. Although both studies evaluated only a limited number of *Candida* spp., one-third of all false negatives recovered were *Candida* spp. (8, 14). Though terminal subculture of negative blood cultures is not routinely performed by most clinical microbiology laboratories, product labels from the manufacturer of the BACTEC 9240 and the Plus/F media do recommend terminal subculture of negative bottles for maximum yield of pathogens. Difficulties with the detection of *Candida* pathogens in automated blood culture systems do not seem to be limited to this system. Borst et al. also noted that terminal subculture of negative bottles from the BacT/ALERT (Biomérieux, Durham, N.C.) automated blood culture system may be helpful for the recovery of *Candida* spp. (2).

The reason for the high rate of false negatives continues to be poorly defined. Thirty-nine of the 44 negative bottles were in anaerobic medium, with 37 of the 39 being false negatives. It is possible that the growth rate, and hence CO_2 production, under anaerobic conditions might not exceed the threshold necessary for detection in the BACTEC 9240 automated blood culture system. However, this does not explain why growth was not detected in 5 of 50 aerobic bottles when *Candida* spp. are generally considered obligate aerobes (13). In this study, *C. albicans* does appear to be an obligate aerobe, with the BACTEC 9240 detecting no anaerobic growth of *C. albicans*. However, in contrast to *C. albicans*, some NAC clearly grow and are active enough in anaerobic media to be detected by the BACTEC 9240 system. Five of 9 (56%) *C. glabrata* and 4 of 12 (33%) *C. tropicalis* isolates in this study had growth detected in the Plus Anaerobic/F medium by the BACTEC 9240. Additionally, all "negative" bottles had growth detected on terminal subculture, except for one *C. albicans* and one *C. firmetaria* isolate, revealing that the yeasts remain viable in the anaerobic medium but are not detected by the BACTEC 9240.

Most clinical laboratories using automated blood culture systems incubate routine blood cultures for only 5 days and regard any organisms that do not grow fast enough to be detected before that time as likely contaminants. This may not be true for pathogens that possess slower growth characteristics or lower metabolic activity, such as yeasts. Debate continues over the required duration of incubation for yeasts. Prevost-Smith and Hutton evaluated the recovery of yeast from an older version of the BACTEC system in 1992 and concluded that 5 days of incubation should be sufficient for recovery of yeast pathogens (9). Sixty-one *Candida* spp. were isolated, usually within 48 h. Only two *C. glabrata* isolates were isolated, and these were recovered after an average of 96 h. Interestingly, this group continued incubation for up to 21 days and subcultured on days 7 and 21 but isolated no further *Candida* spp. Reisner and Woods looked at 88 *Candida* spp. recovered in the BACTEC 9240 Plus Aerobic/F medium (11). Most were detected within 48 h, except for two *C. glabrata* isolates on day 5 and one *C. albicans* isolate on day 6. These investigators ultimately recommended 6 days of incubation for optimal recovery of yeast, influenced somewhat by the delayed recovery of other yeasts, including *Cryptococcus neoformans* and *Histoplasma capsulatum*. More recently Bourbeau and Pohlman have suggested that only 3 days of incubation are required to isolate clinically relevant blood pathogens, including *Candida* spp. (3). Thirty-seven *Candida* spp. were isolated from BacT/ALERT FAN aerobic medium, and all were detected within 72 h, though no terminal subcultures were performed. An increasing number of clinical microbiology laboratories are now adopting this shorter incubation period.

Our microbiology laboratory currently employs a 5-day in-

cubation period for all routine blood cultures; longer durations can be requested by the physician when clinically indicated. The delay in detection of *C. glabrata* growth in the aerobic media is of some concern even with 5 days of incubation. Six of the nine isolates had detectable growth in the aerobic medium, but the average time to positive was 120.89 h, just slightly longer than the time after which these specimens would have been flagged as negative. This would have led to one additional missed episode of *C. glabrata* candidemia, or two additional missed episodes if we employed a 3-day incubation period. This delayed time to recovery for *C. glabrata* isolates in the BACTEC 9240 system using Plus Aerobic/F bottles was also noted by Fricker-Hildago et al., who demonstrated a mean time to detection of 125.92 ± 48.87 h (5). One study did report a shorter time to detection; four *C. glabrata* isolates had a mean time to detection of 21.8 h using the same medium and system (6). In our own study, *C. glabrata* growth was detected on average 4 days faster in the anaerobic medium than in the aerobic medium, a difference that is both statistically and clinically significant. Thus, even with use of both aerobic and anaerobic blood culture media and a 5-day incubation period, three of nine episodes of simulated *C. glabrata* candidemia would have been missed.

The BACTEC 9240 automated blood culture system detects the majority of *Candida* pathogens in at least one bottle of a blood culture set. We demonstrate that this system, using a simulated candidemia model, is effective for the recovery of most *C. albicans* and NAC. The BACTEC 9240 automated blood culture system alone did not detect 4 of 50 episodes of simulated candidemia. However, it would not have detected five (10%) episodes of simulated candidemia if we had followed our standard protocol of incubating aerobic and anaerobic bottles for only 5 days; six episodes of simulated candidemia would have been missed with a 3-day incubation protocol. Overall, there continues to be difficulty with the detection of *Candida* spp. in Plus Anaerobic/F medium and a delay in *C. glabrata* recovery in Plus Aerobic/F medium using the BACTEC 9240 system. With the increasing prevalence of candidemia, more study is needed to optimize the recovery of *Candida* spp. from automated blood culture systems.

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REFERENCES

1. **Beck-Sagué, C. M., W. R. Jarvis, and the National Nosocomial Infections Surveillance System.** 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980–1990. J. Infect. Dis. **167:**1247–1251.
2. **Borst, A., M. Leverstein-Van Hall, J. Verhoef, and A. Fluit.** 2000. Value of terminal subculture of automated blood cultures in patients with candidemia. Eur. J. Clin. Microbiol. Infect. Dis. **19:**803–805.
3. **Bourbeau, P. P., and J. K. Pohlman.** 2001. Three days of incubation may be sufficient for routine blood cultures with BactT/Alert FAN blood culture bottles. J. Clin. Microbiol. **39:**2079–2082.
4. **Edmond, M. B., S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, and R. P. Wenzel.** 1999. Nosocomial bloodstream infections in United States hospitals: a three year analysis. Clin. Infect. Dis. **29:**239–244.
5. **Fricker-Hidalgo, H., F. Chazot, B. Lebeau, H. Pelloux, P. Ambroise-Thomas, and R. Grillot.** 1998. Use of simulated blood cultures to compare a specific fungal medium with a standard microorganism medium for yeast detection. Eur. J. Clin. Microbiol. Infect. Dis. **17:**113–116.
6. **Jorgensen, J. H., S. Mirrett, L. C. McDonald, P. R. Murray, M. P. Weinstein, J. Fune, C. W. Trippy, M. Masterson, and L. B. Reller.** 1997. Controlled clinical laboratory comparison of BACTEC Plus aerobic/F resin medium with BacT/Alert aerobic FAN medium for detection of bacteremia and fungemia. J. Clin. Microbiol. **35:**53–58.
7. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. NCCLS document M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Pohlman, J. K., B. A. Kirkley, K. A. Easley, B. A. Basille, and J. A. Washington.** 1995. Controlled clinical evaluation of BACTEC plus aerobic/F and BacT/Alert FAN bottles for detection of bloodstream infections. J. Clin. Microbiol. **33:**2856–2858.
9. **Prevost-Smith, E., and N. Hutton.** 1992. Value of extended agitation and subculture of BACTEC NR 660 aerobic resin blood culture bottles for clinical yeast isolates. J. Clin. Microbiol. **30:**3239–3242.
10. **Rangel-Frausto, M. S., T. Wiblin, H. M. Blumberg, L. Saiman, J. Patterson, M. Rinaldi, M. Pfaller, J. E. Edwards, Jr., W. Jarvis, J. Dawson, R. P. Wenzel, and the NEMIS Study Group.** 1999. National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. Clin. Infect. Dis. **29:**253–258.
11. **Reisner, B. S., and G. L. Woods.** 1999. Times to detection of bacteria and yeasts in BACTEC 9240 blood culture bottles. Clin. Infect. Dis. **37:**2024–2026.
12. **Shigei, J. T., J. A. Shimabukuro, M. T. Pezzlo, L. M. De La Maza, and E. M. Peterson.** 1995. Value of terminal subcultures for blood cultures monitored by BACTEC 9240. J. Clin. Microbiol. **33:**1385–1388.
13. **Weinstein, M. P., S. Mirrett, L. B. Reller, L. G. Reimer, and M. L. Wilson.** 1996. Value of terminal subcultures for blood cultures monitored by BACTEC 9240. J. Clin. Microbiol. **34:**234–235.
14. **Ziegler, R., L. Johnscher, P. Martus, D. Lenhardt, and H.-M. Just.** 1998. Controlled clinical laboratory comparison of two supplemented aerobic and anaerobic media used in automated blood culture systems to detect bloodstream infections. J. Clin. Microbiol. **36:**657–661.