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Value of Extended Agitation and Subculture of BACTEC NR 660 Aerobic Resin Blood Culture Bottles for Clinical Yeast Isolates

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From 10,351 blood cultures, we prospectively studied 1,000 BACTEC NR 660 aerobic resin blood culture bottles (26+ and Peds Plus) for patients suspected of having yeast septicemia to determine whether extended agitation and subculturing would increase the recovery of yeasts. Aerobic bottles were agitated continuously for 144 h. On day 7, 1,000 culture-negative aerobic bottles which had fungal blood culture requests were agitated for an additional 14 days. During this time they were subcultured twice and read twice by BACTEC NR 660. On days 1 to 7, 81 bottles were culture positive for yeasts from 36 patients, which included 44 isolates of *Candida albicans*, averaging 1.4 days to detection, and 12 isolates of *Cryptococcus neoformans*, averaging 3.8 days to detection. The average detection time for all yeasts was 2.2 days. On days 7 to 21, no yeasts were detected by BACTEC or recovered from the subcultures. We conclude that when continuously agitated for at least 5 full days (120 h), the BACTEC NR 660 aerobic resin bottles reliably isolate yeasts, and it is unnecessary to subculture or hold these bottles beyond 5 days. It also eliminates the need for an additional blood culture system for yeast detection, thus saving (i) confusion in the collection process, (ii) patients' blood and money, and (iii) laboratory technologists' time.

A positive blood culture for yeasts is often a medical emergency for the increasing number of immunocompromised patients in hospitals and medical centers today. It is essential that a blood culture system be able to recover yeasts as rapidly as possible so that important therapeutic management choices may be made by physicians for these patients. To provide better patient care and decrease expense and confusion, it would be advantageous for hospital laboratories to utilize a single blood culture system which quickly and reliably recovers both bacteria and yeasts (10, 13). If a routine bacterial blood culture system is inadequate for the detection of yeasts and necessitates the use of a separate fungal blood culture system with restrictive criteria for use, the delay in recovering yeasts could be a cause of serious morbidity and perhaps mortality.

The BACTEC NR 660 system utilizes infrared spectrophotometry for the detection of positivity by measuring CO_2 in the headspace of the blood culture bottles. The amount of measured CO_2 in each bottle is printed out as a growth value index (GVI). In the routine BACTEC numbering system, day 1 bottles (day of collection) are not 1 day old (24 h) until day 2. Our two 660 instruments agitated the bottles on days 1 to 4. For days 5 to 7, we installed a glass-front 37C incubator (model 3918; Forma Scientific Instruments, Marietta, Ohio), which would hold orbital agitators (Becton-Dickinson Diagnostic Instruments, Sparks, Md.) that fit the BACTEC NR 660 trays.

We have 15 years of experience utilizing the BACTEC system for both bacteria and yeasts and feel it reliably isolates both when the aerobic bottles are continuously agitated for the full incubation period, instead of only 24 to 48 h as recommended by the manufacturer. In order to establish whether 21 days of incubation with agitation and two subcultures yield significant yeast isolates in the BACTEC NR 660, we prospectively studied 1,000 blood cultures from patients suspected of having yeast septicemia.

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MATERIALS AND METHODS

Blood was drawn in the usual manner by placing 8 to 10 ml in each of the high-volume resin bottles (26+ and 27+), and 3 to 5 ml of blood was placed in the low-volume resin bottles (Peds Plus and 17A). Routine bacterial aerobic blood culture bottles were continuously agitated for 144 h at 37°C, while the anaerobic bottles remained stationary at the same temperature.

The BACTEC NR 660 aerobic bottles were read twice on the day of collection (day 1), three times on the second day, and once daily on days 3 to 7. All instrument-positive aerobic bottles were Gram stained and subcultured to 5% sheep blood–Trypticase soy agar, MacConkey agar, and chocolate agar plates. Yeast isolates were referred to the mycology laboratory for identification to the species level. Species identification was accomplished by cornmeal-Tween 80 morphology and the API 20C system. Yeasts giving unusual or unidentifiable codes were submitted to a reference laboratory.

One thousand aerobic blood culture bottles that were negative for growth on day 7 and accompanied by a fungal blood culture request were submitted to the mycology laboratory, where they underwent an additional 14 days of agitation at 37°C. On days 7 and 21, a 0.5-ml sample from each bottle was subcultured to a commercial blood-Trypticase soy agar plate (TSA II-5% sheep blood, BBL-prepared media; Becton-Dickinson) and incubated for 5 days at 37°C. On days 10 and 21, each bottle was read by the BACTEC NR

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TABLE 1. Yeasts isolated from 10,351 BACTEC aerobic 26+ and Peds Plus blood culture bottles which were incubated and agitated for 7 days[a]

Isolate	No. of positive cultures[b]	Avg detection time (days)	Range (days)
Candida albicans	44	1.4	0–4
Cryptococcus neoformans	12	3.8	3–5
Candida parapsilosis	10	1.7	1–3
Torulopsis candida	6	1.0	1
Candida tropicalis	3	1.0	1
Torulopsis glabrata	2	4.0	3–5
Pichia ohmeri	3	1.7	1–2
Candida lusitaniae	1	3.0	3
Total (%)	81 (0.8)	2.2	

[a] A total of 1,000 blood cultures were read and subcultured on days 7 to 21, with no yeasts isolated.
[b] Results are given for 36 patients.

660. Final reports were sent after 21 days, pending results of the terminal subculture.

RESULTS

On days 1 to 7, 81 aerobic bottles taken from 36 patients were positive for yeasts (Table 1). The predominant isolate was *Candida albicans*, with 44 isolations; *Cryptococcus neoformans* was second with 12 isolations. The average time to detection for *C. albicans* was 1.4 days, while for *C. neoformans* it was 3.8 days. There was one isolate of *Torulopsis glabrata* and two isolates of *C. neoformans* which required 5 days of incubation. Overall detection time for all yeasts was 2.2 days.

Figure 1A is a graph of GVI readings for 12 *C. neoformans* isolates in our study. Although BACTEC detected four of our isolates after 72 h of incubation on day 4 (isolates D, E, F, and L), the GVI jumped substantially in six instances at 96 h of incubation on day 5 (isolates B, C, H, I, J, and K) and in two instances at 120 h of incubation on day 6 (isolates A and G). There were two instances when the bottle's GVI reading was interpreted as positive by the technologist because of a change in the delta growth value from the previous day's reading (isolates B and K); BACTEC detected both on the following day (at 120 h). No additional yeasts were recovered after 120 h.

No yeasts were recovered from the 1,000 aerobic bottles read twice by BACTEC and subcultured twice during the 14 additional days of agitation (days 7 to 21). Two bottles grew contaminating bacteria and one grew a mold contaminant, for a contamination rate of 0.3%. Regarding the type of medium used, there was not a notable difference in the recovery rates with 26+ and Peds Plus. There were four instances in which four patients had positive yeast cultures both in a 26+ bottle and a Peds Plus bottle for samples drawn at the same time; they were detected on the same day. Sixteen blood cultures from six patients drawn within 1 to 3 days in both 26+ and Peds Plus bottles did not show a notable difference in the detection times for the two bottle types, even though there is as much as a twofold difference in the volume of blood collected. The time of detection (in days) for 14 of the bottles was the same, and for two bottles there was one day's difference; one was detected one day earlier in the 26+ and the other was detected one day earlier in the Peds Plus.

DISCUSSION

Agitation (shaking) and venting provide most yeasts with enhanced growth because they increase oxygenation. Huahua et al. (4) have shown that when (an average of) 17.6 CFU of *C. albicans* per ml was introduced and incubated in blood culture bottles, more colonies were produced from the agitated bottles than from the stationary bottles. They also reported that the growth of *C. albicans* and *C. neoformans* was enhanced by oxygenation, while *T. glabrata* growth was not enhanced (but not inhibited). We, too, found that *T. glabrata* was the single exception which did not benefit from continuous agitation in the 26+ or Peds Plus bottles. *T. glabrata* is known to grow better on chocolate agar than on whole blood agar. Moreover, improved recovery and enhanced growth of *T. glabrata* were observed in a BACTEC blood culture medium formulated to recover fungi which contained a lytic agent (7a). One might speculate that continuous agitation of this lytic fungal medium would result in optimum isolation of *T. glabrata*.

In this study, all yeasts, including *C. neoformans*, were recovered by 120 h of agitation. There are several reports which question the ability of the BACTEC NR 660 to detect *C. neoformans* (7, 11, 13). One report states that the BACTEC system is unsatisfactory for isolating *C. neoformans*

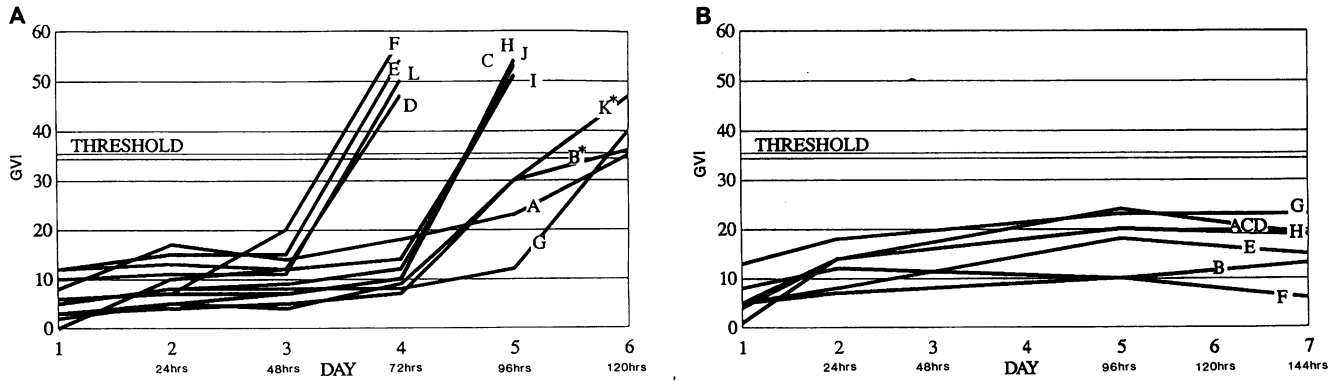

FIG. 1. BACTEC GVI readings for *C. neoformans* by NR 660 shaken for 144 h (A) and the radiometric 460 system shaken only for the first 24 h (B). The results shown in panel A represent 12 isolates (A to L) from the current study; the asterisks indicate detection because of a change in the GVI (delta growth value) from the previous day's reading. The results in panel B are those of Robinson et al. (11) for eight isolates.

TABLE 2. Comparison of detection times (in days) of common yeast isolates in BACTEC and lysis-centrifugation systems

Isolate	Detection time (days) by:						
	BACTEC				Lysis-centrifugation[a] results from reference:		
	Shaken 144 h[b]		Shaken 24 h[c]				
	NR 660	460	NR 660	460	2	13	9
C. albicans	1.4	1.6	4.08	3.7	2.4	2.5	3.0
C. tropicalis	1.0	1.9	3.85	2.4	1.7	1.9	2.5
C. parapsilosis	1.7	1.5	4.67	1.3	3.4	2.4	2.6
C. neoformans	3.8	5.0	None	7.0	3.5	3.4	3.6
T. glabrata	4.0	5.4	3.0	7.0	4.3	3.7	4.1

[a] A Dupont isolator, now manufactured by Wampole Laboratories, Cranbury, N.J., was used.
[b] Aerobic bottles were continuously shaken for 144 h. Results for the NR 660 were from the present study; those for the 460 are from reference 10.
[c] Aerobic bottles were shaken for the first 24 h and remained stationary for the remaining incubation period, as recommended by the manufacturer. Results for the NR 660 are from reference 8; those for the 460 are from reference 2.

(13). Our 12 isolates of *C. neoformans* were detected in an average of 3.8 days. In two other bottles of samples collected on the same day from two patients with blood cultures positive for *C. neoformans*, there was no growth from the subcultures. This may indicate that there are so few of these yeasts in the bloodstream of patients with cryptococcemia that collecting multiple aerobic blood cultures is beneficial in order to yield a higher percentage of positive cultures. Figure 1A shows a sharp rise in the GVI, clearly documenting that *C. neoformans* can and does produce enough CO_2 to be detected by the BACTEC NR 660 when the bottles are continuously agitated for at least 120 h. For comparison, Fig. 1B is a chart of GVI readings from eight isolates of *C. neoformans* from bottles agitated for 24 h in a study by Robinson et al. (11), which suggested that this organism does not produce enough CO_2 to be detected by the BACTEC radiometric 460. The radiometric 460 and NR 660 are equivalent with regard to mean time for detection of yeasts (5).

In the past 15 years of monitoring blood cultures for both bacteria and fungi in our medical center, the BACTEC 460 or 660 system has detected, within 7 days, some unusual filamentous or yeast-like fungi from true infections, including *Wangiella dermatitidis*, *Fusarium solani*, *Aspergillus flavus*, *Aspergillus fumigatus*, and the actinomycete *Nocardia asteroides*. However, laboratories located in areas of endemicity for dimorphic fungi or those whose patient populations include patients with systemic infections should establish their own protocol for isolating dimorphic, filamentous or slowly growing fungi from blood cultures.

Table 2 shows a comparison of detection times (in days) for the BACTEC NR 660 and the BACTEC radiometric 460 systems with agitation for 144 h versus 24 h and also for the lysis-centrifugation system (Dupont isolator) (2, 8–10, 13). In both of our studies utilizing 144 h of agitation, our detection times for the recovery of yeasts are, for most yeasts, less than those reported for the same systems with agitation for only 24 h. We conclude that extended agitation is a valuable addition to the BACTEC NR 660 protocol, and the simple installation of orbital agitation equipment in incubators provides the system with an effective enhancement for yeasts.

Our data do not support other reports (1–3, 9, 12, 13) which suggest that optimum recovery of both bacteria and yeasts is accomplished only by the use of two separate blood culture systems, since our detection times for yeasts in the BACTEC NR 660 are shorter than or comparable to those reported for the lysis-centrifugation system (Table 2). Further, the contamination rate of 0.3% in our study is more acceptable than that of 4.8% (3) and as much as 12.8% (6) reported for the lysis-centrifugation system. When two separate blood culture systems are utilized in a hospital, physicians often feel obliged to use both systems because many of their patients are compromised, and they worry that they will miss yeast septicemia if the fungal blood culture method is not used in addition to the bacterial blood culture system. In our previous study (10), we found that the early isolation of yeasts in the BACTEC radiometric 460 bottles was a serendipitous finding, viz, the physician drew blood cultures for bacteria because the patient had symptoms of sepsis. When yeasts instead of bacteria were recovered from the BACTEC bottles, the physician was able to quickly change management plans, such as replacing or removing contaminated catheters or switching from antibacterial to antifungal therapy early in the infection. On the other hand, when we used the two separate systems (10), many of our patients had an enormous amount of blood collected and there were additional laboratory charges and technologists' time, but without additional benefit. If the system is equally reliable for both bacteria and yeasts, the use of a single blood culture system in hospitals (i) alleviates confusion in the blood collection process because it does not require special permission, fungal blood culture request, or different bottles or tubes; (ii) saves technologists' time, laboratory time, and hospital expense; (iii) saves the patient both blood and money; and, most importantly, (iv) enables the physician to have a reliable culture for both bacteria and yeasts with the same blood culture system and the same order whether he or she has considered yeast septicemia as a possibility, consequently increasing the frequency of diagnosing yeast infections earlier.

We have shown that when continuously agitated for at least 5 full days (120 h), the BACTEC NR 660 aerobic resin blood culture bottles, 26+ and Peds Plus, reliably isolate yeasts, including *C. neoformans*. When the bottles have the extended agitation time, it is not necessary to incubate beyond 5 days or subculture in order to isolate common clinical yeast isolates.

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