

Routine Incubation of BacT/ALERT FA and FN Blood Culture Bottles for More than 3 Days May Not Be Necessary

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We reviewed time to detection for 35,500 blood cultures collected in BacT/ALERT FA and FN bottles. In the first 3 days of incubation, 97.5% of the 2,609 clinically significant isolates were detected, suggesting that routine incubation for more than 3 days may not be necessary for FA and FN bottles.

It has become established over time that no more than 5 days of incubation is required for continuous-monitoring automated blood culture instruments, and indeed, regulatory agencies generally now require 5-day minimum incubation periods (6, 9). More recently, there have been reports that 5 days of incubation may not be required for some of the automated blood culture systems (2–5, 8).

In a previously published study, we reported that 3 days of incubation was generally sufficient for BacT/ALERT FAN aerobic and FAN anaerobic blood culture bottles (1). New non-vented bottles, designated FA and FN, subsequently replaced the FAN aerobic and FAN anaerobic blood culture bottles. The purpose of this study was to assess the time to positivity of the BacT/ALERT FA and FN blood culture bottles.

We retrospectively examined the results of approximately 35,500 blood cultures collected from adult patients at Geisinger Medical Center in a 30-month period. Except for occasional cultures that are collected with only a single FA aerobic bottle, sets include a FA aerobic bottle and a FN anaerobic bottle. Phlebotomists are instructed to collect 10 ml of blood for each bottle, but volumes were not verified for the blood culture sets included in this review. The BacT/ALERT 3D system was in use for all of these cultures.

Blood culture results were obtained using the BacT/ALERT instrument and software. The time to positivity of the first bottle to be flagged as positive in a set was used to determine the time to positivity of the set. Days were calculated as full 24-h periods. For example, isolates detected by the instrument between 96.1 and 120 h were classified as detected on day 5. For the purposes of this study, isolates of *Staphylococcus* spp. not *Staphylococcus aureus*, viridans streptococci, *Bacillus* spp., *Propionibacterium* spp., and aerobic diphtheroids from only one set of bottles were considered to be not clinically significant.

If a patient had a significant blood culture isolate that was first detected on day 4 or day 5 of incubation and the patient did not have another concurrent blood culture that was detected within the first 3 days of incubation, a chart review was conducted by one of us (M.F.), an infectious diseases specialist.

The purpose of the chart review was to determine if the patient's antimicrobial therapy was changed based upon the results of the positive blood culture result.

From a total of approximately 35,500 blood culture sets collected over a 30-month period, 3,706 organisms were recovered with 2,609 judged to be clinically significant (Table 1). In the first 3 days of incubation, 97.5% of the clinically significant isolates were detected. Of the 67 isolates that were detected on day 4 or 5 of incubation, 34 were recovered in concurrent cultures within the first 3 days of incubation. Chart reviews were conducted for the 23 patients with the remaining 33 isolates. For five isolates from five patients, changes were made in antimicrobial therapy based upon the positive culture on day 4 or 5. These five organisms (day of detection) were *Shewanella putrefaciens* (day 4), anaerobic gram-positive cocci (day 5), *S. aureus* (day 5), *S. aureus* (day 5), and *Candida glabrata* (day 5). None of these five patients died during this septic episode.

The continuous-monitoring automated blood culture instruments generally employed 6- or 7-day incubation periods when they were introduced. Over time, it became established that no more than 5 days of incubation was required for the BacT/ALERT blood culture system when standard BacT/ALERT blood culture bottles were used (6, 9).

When the BacT/ALERT blood culture system was introduced, only bottles with the designations of standard aerobic and standard anaerobic were available. At a later time, additional bottles with the designations FAN aerobic and FAN anaerobic were introduced. The FAN bottles were designed to enhance the recovery of fastidious bacteria, bacteria from patients receiving antimicrobial therapy, and yeasts in comparison to the standard BacT/ALERT blood culture bottles. Several studies have suggested that 5 days of incubation is not necessary for the FAN blood culture bottles (1–3).

Cornish et al. examined time to detection for FAN bottles in two studies. In the first study, they demonstrated that a BacT/ALERT FAN aerobic bottle combined with a BacT/ALERT standard anaerobic bottle detected 95% of all clinically significant isolates within 3 days of incubation (2). In a second study, they demonstrated that all clinically significant episodes of bloodstream infections were detected within 4 days of incubation when paired FAN aerobic and FAN anaerobic bottles were utilized (3).

We reported that 97% of all clinically significant bacterial and fungal isolates were detected within the first 3 days of

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TABLE 1. Time to recovery of microorganisms from blood cultures with BacT/ALERT FA and FN bottles

Organism ^a	No. (%) of organisms recovered on day:					Total no. of organisms recovered
	1	2	3	4	5	
Significant isolates						
Gram-positive cocci						
<i>Staphylococcus aureus</i>	587	161	23	7	8	786
<i>Staphylococcus lugdunensis</i>	4	3	0	0	0	7
<i>Staphylococcus</i> sp. not <i>S. aureus</i>	256	165	10	3	0	434
<i>Streptococcus pneumoniae</i>	74	1	0	0	0	75
Beta-hemolytic streptococci	72	8	1	0	0	81
Enterococci	132	18	1	0	1	152
Other streptococci	79	11	2	1	0	93
Aerobic gram-positive bacilli						
Enterobacteriaceae						
<i>Citrobacter</i> sp.	16	0	1	0	0	17
<i>Enterobacter</i> sp.	33	5	0	0	1	39
<i>Escherichia coli</i>	297	22	4	4	0	327
<i>Escherichia hermannii</i>	1	0	0	0	0	1
<i>Klebsiella oxytoca</i>	22	1	0	0	0	23
<i>Klebsiella pneumoniae</i>	120	4	1	1	0	126
<i>Kluyvera</i> sp.	1	0	0	0	0	1
<i>Hafnia alvei</i>	2	0	0	0	0	2
<i>Morganella morganii</i>	7	2	0	0	0	9
<i>Proteus mirabilis</i>	37	3	0	1	0	41
<i>Proteus vulgaris</i>	4	0	0	0	0	4
<i>Providencia</i> sp.	6	0	0	0	0	6
<i>Salmonella</i> sp.	7	0	0	0	0	7
<i>Serratia marcescens</i>	19	7	1	1	0	28
Other GNB						
<i>Acinetobacter anitratus</i>	10	3	0	0	0	13
<i>Aeromonas</i> sp.	1	0	0	0	0	1
<i>Alcaligenes xylosoxidans</i>	2	3	1	1	0	7
<i>Burkholderia cepacia</i>	1	0	0	0	0	1
<i>Comamonas acidovorans</i>	1	0	0	0	0	1
<i>Eikenella corrodens</i>	0	1	1	0	0	2
<i>Flavimonas oryzihabitans</i>	1	0	0	0	0	1
<i>Haemophilus influenzae</i>	3	1	1	0	0	5
<i>Moraxella osloensis</i>	2	0	0	0	0	2
<i>Neisseria meningitidis</i>	2	0	0	0	0	2
<i>Pasteurella multocida</i>	4	0	0	0	0	4
<i>Pseudomonas aeruginosa</i>	53	9	2	0	0	64
<i>Pseudomonas stutzeri</i>	0	3	0	0	0	3
<i>Pseudomonas</i> sp.	2	0	0	0	0	2
<i>Shewanella putrefaciens</i>	0	0	0	1	0	1
<i>Stenotrophomonas maltophilia</i>	5	6	1	0	0	12
Other gram-negative bacilli	6	4	1	1	0	12
Yeast						
<i>Candida albicans</i>	3	19	11	3	1	37
<i>Candida glabrata</i>	0	2	13	7	2	24
<i>Candida lusitanae</i>	3	1	0	0	0	4
<i>Candida parapsilosis</i>	3	12	5	0	1	21
<i>Candida tropicalis</i>	11	2	0	0	0	13
<i>Cryptococcus neoformans</i>	0	0	0	3	0	3
<i>Malassezia pachydermatis</i>	0	1	1	4	5	11
Yeast, no I.D.	0	2	3	0	0	5
Anaerobes						
<i>Bacteroides fragilis</i> group	8	15	1	1	1	26
<i>Bacteroides uniformis</i>	0	0	0	2	0	2
<i>Bacteroides urealyticus</i>	0	0	0	2	0	2
<i>Bifidobacterium</i> sp.	0	0	0	1	0	1
<i>Clostridium perfringens</i>	6	0	0	0	0	6
<i>Clostridium septicum</i>	1	1	0	0	0	2
<i>Clostridium</i> sp.	4	2	0	0	0	6
<i>Eubacterium lentum</i>	0	0	2	0	1	3
<i>Fusobacterium</i> sp.	0	3	3	0	0	6

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TABLE 1—Continued

Organism ^a	No. (%) of organisms recovered on day:					Total no. of organisms recovered
	1	2	3	4	5	
Anaerobic gram-positive bacilli	2	3	0	0	0	5
Anaerobic gram-positive cocci	1	2	2	1	2	8
Anaerobic gram-negative bacilli	2	1	0	1	0	4
Total	1,935 (74)	513 (20)	94 (4)	44 (2)	23 (1)	2,609
Probable contaminants						
<i>Staphylococcus</i> sp. not <i>S. aureus</i>	352	460	56	8	4	880
Viridans streptococci	68	22	2	1	1	94
<i>Propionibacterium</i> sp.	0	2	0	1	3	6
<i>Neisseria</i> sp.	2	2	1	0	0	5
<i>Aerococcus</i> sp.	0	1	0	0	0	1
<i>Lactobacillus</i> sp.	0	1	1	0	1	3
<i>Bacillus</i> sp.	12	10	3	2	2	29
Aerobic non-spore-forming GPR	13	38	12	10	5	78
Unidentified gram-positive cocci	1	1	1	0	0	3
Total	448	537	76	22	16	1,099

^a GNB, gram-negative bacilli; I.D., identification; GPR, gram-positive rods.

incubation using FAN aerobic and FAN anaerobic blood culture bottles (1). In our study, from a total of 1,242 clinically significant isolates, there were a total of only 14 clinically significant isolates from 13 patients that were recovered on day 4 or 5 of incubation and were not detected by either a paired bottle from the same set or from a concurrent blood culture within the first 3 days of incubation. For only 1 of these 14 isolates was there any change in antimicrobial therapy based on this positive result (1). We concluded that 3 days of incubation was probably sufficient for BacT/ALERT FAN aerobic and FAN anaerobic blood culture bottles, which are no longer marketed.

New nonvented BacT/ALERT bottles, designated FA and FN, have since replaced the FAN anaerobic and FAN anaerobic blood culture bottles. In addition to a change in medium formulation, the FA/FN bottles have a different type of sensor than the FAN bottles. The FA bottles contain a different concentration of charcoal, a smaller volume of broth media, and more headspace gas than the FAN aerobic bottles and are unvented, while the FAN aerobic bottles required transient venting. In an evaluation that compared FA bottles to FAN aerobic bottles, Mirrett and colleagues reported that significantly more isolates of *Burkholderia cepacia*, *Candida albicans*, *Cryptococcus neoformans*, and total microorganisms were recovered from the FA bottles (7).

In this study, we reviewed the time to detection of positive results from approximately 35,500 blood cultures collected in FA and FN bottles. We determined that in our hospital, a change in therapy was made on average only once every 6 months based upon a positive blood culture detected on day 4 or 5 of incubation. We acknowledge that other changes may have been made in patient management based upon a positive blood culture on day 4 or 5; however, changes in patient management are not always measurable even by chart review.

When consideration is given to shortening the time for incubation of routine blood cultures to 3 days, it is reasonable to

ask whether certain fastidious species of bacteria may routinely require longer periods of incubation and, therefore, be missed by the 3-day incubation period (1). While it is difficult to definitively address this question, the fact remains that this study included approximately 35,500 blood cultures collected during a 2-year period in a tertiary care medical center, and no species of bacteria was recovered that routinely required more than 3 days of incubation. Importantly, we are unaware of any published studies of blood culture time to positivity that have included as many total sets as this study. Nonetheless, there are certain microorganisms, such as *Brucella* sp., that would not be detected by a 3-day or even a 5-day incubation period. Requests for culture of *Brucella* sp. or other known fastidious organisms should prompt either a longer incubation period or the use of an alternative blood culture method.

Perhaps more problematic is the question of whether 3 days of incubation is adequate for detection of yeast routinely isolated in blood culture bottles. In an earlier evaluation of time to positivity for BacT/ALERT FAN aerobic and FAN anaerobic blood culture bottles, we detected 40 isolates of yeast from 1,242 clinically significant isolates (3.2% of significant isolates) (1). In this study, we detected 118 isolates of yeast from 2,609 clinically significant isolates (4.5% of significant isolates). We acknowledge that a valid statistical comparison cannot be performed between the results of separate studies. Nonetheless, these results are consistent with the results of Mirrett et al., who reported significantly more isolates of *Candida albicans*, *Cryptococcus neoformans*, and total yeast from FA bottles than from FAN aerobic blood culture bottles (7).

A closer examination of the time to detection of yeast isolated in this study (Table 1) indicates that 26 yeast isolates (22% of all yeasts detected) were detected on day 4 or day 5 of incubation. In contrast, overall in this study, only 2.5% of total significant isolates were detected on day 4 or 5 of incubation. Nine of the 26 yeast detected on day 4 or 5 of incubation were isolates of *Malassezia pachydermatis* from one patient who also

had other isolates detected on day 1 or day 2 of incubation. The three isolates of *Cryptococcus neoformans* (two patients) were detected by cryptococcal antigen test. Of the remaining 14 yeast isolates, antimicrobial therapy was changed only for one patient with *C. glabrata*. Thus, in summary, from a total of 118 yeast isolates, antimicrobial therapy was altered for only one yeast isolate from one patient based upon a result that was detected on day 4 or 5 of incubation. We acknowledge that similar conclusions may not be applicable to institutions with a higher incidence of fungal infections. Moreover, in our institution, the diagnosis of cryptococcosis is often made by the use of the cryptococcal antigen test, usually prior to detection by culture. As previously noted, both patients in this review with *Cryptococcus neoformans* in blood cultures were positive with the cryptococcal antigen test.

Similar studies have been performed with other automated blood culture systems to determine whether a 5-day incubation period is required for these systems as well (4, 5, 8). Care should be taken when trying to compare the performances of different blood culture systems due to modifications in media, software, and bottles that occur on a routine basis. In essence, any study result represents a snapshot in time for a particular blood culture system.

In conclusion, we have demonstrated that 3 days of incubation may be sufficient for the detection of routine bacteria and yeast when utilizing BacT/ALERT FA and FN blood culture bottles at Geisinger Medical Center. We emphasize that this is a full 72 h and not some fraction of a third day. Our results are not necessarily valid for other types of BacT/Alert blood culture bottles or for other commercial blood culture systems. We note minimal therapeutic benefit by extending the incubation period for FA/FN bottles beyond 3 days. We caution others to recognize that our results may be influenced by the geographic area in which we are located, the patient population that we serve, and the antimicrobial ordering patterns of our clinicians.

Moreover, delayed entry was not a factor for any of these cultures, since all were collected at our institution. The fact that we utilize two blood cultures collected simultaneously (total recommended volume for adults, 40 ml) may also influence our results. We believe that additional studies should be performed at other institutions to determine how widely applicable our results may be in different geographic areas as well as different patient populations. In our own laboratory, we continue to employ a 5-day incubation for routine blood cultures, due in part to current regulatory requirements but also due to current instrument capacity.

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