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Time to Detection of Positive BacT/Alert Blood Cultures and Lack of Need for Routine Subculture of 5- to 7-Day Negative Cultures

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Consecutive BacT/Alert blood cultures which were instrument negative following a 7-day incubation were subcultured. Eighteen (0.2%) of 11,476 bottles had growth on subculture. Eleven of these eighteen isolates were considered contaminants on the basis of the identity of the organism and lack of other positive blood cultures from the same patient. In addition, analysis of time to instrument detection for approximately 2,900 positive blood cultures indicates that 5 or 6 days of incubation is sufficient for the routine detection of clinically significant organisms from BacT/Alert blood cultures. These data indicate that subculture of 5- to 7-day instrument-negative BacT/Alert blood culture bottles is not necessary.

It has been previously shown that routine subculture of 5- to 7-day negative conventional or BACTEC (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) radiometric blood cultures provides little information of clinical relevance (1–7, 9). Indeed, the routine practice of terminal subculture of 7-day negative specimens has largely been discontinued.

With the advent of new technology for culture of blood, including different methods of microbial detection, differences in medium formulations, and different methods of culture agitation, it seems appropriate to determine whether that which was learned from conventional and radiometric detection systems is applicable to newer systems. BacT/Alert (Organon Teknika Corporation, Durham, N.C.) is a new automated blood culture system which utilizes a supplemented tryptic soy broth and provides continuous incubation and agitation of all culture bottles. The system monitors culture bottles six times per hour for color change in a sensor which detects H^+ generated from the reaction of water with CO_2 produced by growing microorganisms (10). In a limited clinical trial with a prototype research instrument, 1 of 16 cultures which was negative by BacT/Alert but positive by BACTEC 460 was observed to have growth upon terminal subculture (10). In a large-scale comparative clinical study which also examined the false-negative rate in a subset of the total paired cultures obtained, 2 of 63 cultures which were negative by BacT/Alert but positive by the BACTEC nonradiometric system had growth upon terminal subculture (12).

To determine the false-negative rate associated with the routine clinical use of BacT/Alert, terminal subcultures were performed on consecutive 7-day negative blood cultures; a total of 6,070 consecutive negative cultures which included 11,476 bottles were blind subcultured from 12 November 1990 to 28 February 1991.

During the periods of study, blood cultures were obtained by phlebotomy, house staff, and nursing departments. Skin antisepsis prior to venipuncture was accomplished by cleansing with alcohol and betadine according to standard procedures. The volume of blood recommended for BacT/Alert aerobic and anaerobic bottles was 5 ml. BacT/Alert cultures were incubated with continuous rocking (50 to 60 times per minute) and monitored at 10-min intervals according to the manufacturer's preset specifications for the entire 7-day incubation; the threshold for positive cultures was determined by the manufacturer's preset algorithm. At the end of 7 days of incubation, approximately 0.1 ml of the blood mixture was aseptically removed from negative-culture bottles and plated onto chocolate agar (Remel Laboratories, Lenexa, Kans.). Subcultures from aerobic bottles were incubated for 2 days at 36°C with 5% CO_2. Subcultures from anaerobic bottles were incubated for 2 days at 36°C in an anaerobic chamber with 5% CO_2, 10% H_2, and 85% N_2.

Eighteen (0.2%) of the 11,476 terminal subcultures from BacT/Alert bottles had growth. These false-negative cultures included 7 aerobic and 11 anaerobic bottles and were from a total of 16 different patients. Eleven of the isolates were considered contaminants on the basis of the identity of the organism and lack of other positive blood cultures from the same patient (11). Organisms in this group were isolated from anaerobic cultures unless otherwise noted and included four coagulase-negative staphylococci (one aerobic culture), one *Micrococcus* species (aerobic culture), one *Corynebacterium* species (aerobic culture), one *Peptostreptococcus* species, and four *Propionibacterium* species (one aerobic culture). An additional coagulase-negative staphylococcus was recovered from an anaerobic culture of blood from a patient who had one instrument-positive culture out of three other cultures collected within a 12-h period. False-negative isolates of possible clinical significance included one *Pseudomonas aeruginosa* isolate in an anaerobic culture from a patient with lymphoma, urosepsis, and a rapidly deteriorating medical condition who had no other culture from any site which was positive with the same organism; three *Staphylococcus aureus* isolates (two aerobic cultures) from one patient who had a catheter tip culture and a subsequent instrument-positive blood culture with the same organism; and one *Torulopsis glabrata* in an anaerobic culture from a patient who had multiple other negative cultures. In another patient, *T. glabrata* was isolated from terminal subculture of an anaerobic bottle but was also recognized by the BacT/

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TABLE 1. Time to instrument detection of positive blood cultures

Organism (no. of patients)	No. of positive cultures	Cumulative % of cultures positive at time (h)						
		24	48	72	96	120	144	168
Staphylococcus aureus (176)	350	86	96	99	99	99	100	
Coagulase-negative staphylococci (659)	898	66	91	96	97	99	100	
Micrococcus spp. (28)	28	34	82	86	97	97	97	100
Streptococcus pneumoniae (98)	134	99	100					
Beta-hemolytic streptococci (46)	73[a]	99	100					
Other streptococci (144)	180[b]	85	97	98	99	99	100	
Enterococcus spp. (65)	104	96	99	99	99	99	99	100
Members of the *Enterobacteriaceae* (285)	494[c]	85	93	96	98	99	100	
Pseudomonas aeruginosa (38)	76	76	99	100				
Other nonfermenters (8)	16[d]	69	75	81	88	94	100	
Aeromonas hydrophila (3)	6	83	83	83	83	83	100	
Campylobacter jejuni (1)	1	0	0	0	0	100		
Haemophilus influenzae (10)	11	73	100					
Gardnerella vaginalis (2)	3	67	67	67	67	67	100	
Kingella kingae (1)	1	100						
Cardiobacterium hominis (1)	4	25	100					
Moraxella spp. (6)	6[e]	67	100					
Neisseria spp. (11)	12[f]	83	100					
Acinetobacter spp. (12)	17	94	100					
Bacillus spp. (19)	28	59	86	90	93	96	97	100
Corynebacterium jeikeium (3)	11	27	91	91	91	100		
Other corynebacteria (61)	71	14	63	82	87	93	96	100
Lactobacillus spp. (4)	6	0	17	33	100			
Listeria monocytogenes (3)	3	67	100					
Bacteroides spp. (26)	36[g]	8	68	81	92	95	100	
Clostridium spp. (13)	19[h]	63	84	89	95	95	95	100
Anaerobic gram-positive cocci (13)	16	25	44	63	88	94	94	100
Propionibacterium spp. (195)	198	0	0	1	6	23	63	100
Candida spp. (29)	67[i]	40	87	99	99	100		
Torulopsis glabrata (9)	16	13	38	69	75	81	100	
Cryptococcus neoformans (7)	18	0	0	33	89	94	100	
Tricosporon beigelii (1)	2	50	100					

[a] Includes *S. pyogenes* (17), *S. agalactiae* (37), group C (5), group G (14).
[b] Includes *S. bovis* (9), *S. constellatus* (2), *S. intermedius* (7), *S. mitis* (15), *S. morbillorum* (3), *S. salivarius* (10), *S. sanguis* (15), *S. anginosus* (1), other alpha-hemolytic streptococci (83), other gamma-hemolytic streptococci not group D (18), nonenterococcal group D (5), nutritionally variant streptococci (12).
[c] Includes *Escherichia coli* (276), *Citrobacter* spp. (8), *Enterobacter* spp. (32), *Klebsiella* spp. (127), *Morganella morganii* (9), *Proteus mirabilis* (20), *Providencia* spp. (6), *Salmonella* spp. (6), *Serratia* spp. (10).
[d] Includes *Pseudomonas fluorescens* (1), *Xanthomonas maltophila* (9), *Achromobacter xylosoxidans* (3), other *Alcaligenes* spp. (1), *Flavobacterium* spp. (1), CDC group VEI (1).
[e] Includes *M. catarrhalis* (2), *M. osloensis* (2), *M. nonliquifaciens* (1), *M. atlantae* (1).
[f] Includes *N. gonorrhoeae* (1), *N. meningitidis* (4), other *Neisseria* spp. (7).
[g] Includes *B. fragilis* (20), *B. thetaiotaomicron* (3), *B. distasonis* (1), *B. ovatus* (4), *B. vulgatus* (1), *B. ureolyticus* (1), other *Bacteroides* spp. (6).
[h] Includes *C. perfringens* (8), *C. tertium* (5), other *Clostridium* spp. (6).
[i] Includes *C. albicans* (50), *C. parapsilosis* (6), *C. lusitaniae* (9), *C. tropicalis* (2).

Alert system in multiple other bottles. Since subcultures from anaerobic bottles were incubated only anaerobically, it is possible that our results underestimate the total number of obligate aerobes present but undetected in anaerobic culture bottles.

The above data were collected from terminal subcultures of 7-day instrument-negative cultures. To assess the potential impact of shorter incubation periods on the value of terminal subcultures for the detection of clinically significant organisms from instrument-negative cultures, we determined the time to instrument detection for all positive cultures in our laboratory from 12 November 1990 to 31 March 1992. Results of this analysis are presented in Table 1 and can be used to determine the proportion of false-negative cultures which would have resulted from shorter incubation periods. In general, these data suggest no significant difference in the proportion of clinically significant aerobic bacteria, facultative anaerobic bacteria, and *Candida* spp. that would have been undetected with 5- or 6-day incubations and those undetected in a 7-day incubation. The data indicate that ≥99% of cultures positive for staphylococci, streptococci, enterococci, members of the family *Enterobacteriaceae*, *P. aeruginosa*, *Haemophilus influenzae*, *Neisseria* spp., *Acinetobacter* spp., *Listeria monocytogenes*, and *Candida* spp. were positive within 5 days; in fact, ≥96% of these cultures were positive within 2 to 3 days. Although a few cultures of enterococci, *Bacteroides* spp., *Clostridium* spp., and *Cryptococcus* spp. required >5 days for detection, analysis of data for earliest positive cultures from patients (data not shown) indicates that 100% of patients with these organisms were identified in <5 days. Although the number of cultures with *T. glabrata* is small, approximately 20% of positive culture and patient results for this organism would have been undetected with a 5-day incubation, whereas none would have been undetected with a 6-day incubation. With the notable exception of anaerobic diphtheroids, 5- or 6-day incubations would not have significantly reduced the detection of organisms generally shown to be clinically insignificant. Five- or six-day incubations, however, would have significantly reduced the proportion of

Propionibacterium spp. detected by 77 and 37%, respectively; since none of these isolates was considered clinically significant, decreased detection of this organism would reduce our laboratory work load and expense and eliminate unnecessary concern for patients and users of laboratory information.

The organisms isolated from our 7-day instrument-negative blood cultures were primarily contaminants or provided no new clinical information. Although a few isolates were significant or possibly significant, the low false-negative rate confirms earlier reports (10, 12) and does not warrant the routine subculture of 7-day instrument-negative BacT/Alert blood cultures. Furthermore, the analysis of time to instrument detection for approximately 2,900 positive blood cultures indicates that 5 or 6 days of incubation is sufficient for the routine detection of bacteria and yeasts from blood of our patient population and that subculture after 5 or 6 days of incubation would also be an extremely low-yield effort. Our data are in agreement with other reports describing the failure of blind subcultures to improve detection of clinically significant organisms in 5- to 7-day negative cultures (1–7, 9). These previous reports have also shown that clinically significant organisms are generally detected within 3 days of incubation and that organisms considered contaminants, e.g., aerobic and anaerobic diphtheroids, coagulase-negative staphylococci, and micrococci, are the organisms most frequently isolated from terminal subcultures. These reports have also shown that the isolation of clinically significant organisms, e.g., *S. aureus*, members of the *Enterobacteriaceae*, and fungi, from terminal subcultures was generally from patients who had other cultures which were positive. On the basis of these data, the expensive and labor-intensive practice of routine subculture of 5- to 7-day negative blood cultures is not recommended (8).

In summary, the lack of utility in subcultures of 5- to 7-day negative conventional or radiometric cultures also applies to BacT/Alert cultures. In selected cases, however, such as suspected endocarditis with fastidious slowly growing organisms or symptoms of persistent or recurrent infection in the absence of positive cultures, extended incubation and terminal subculture may be worthwhile (3, 8). On the basis of our data, we have concluded that it is reasonable to decrease the routine incubation time from 7 days to 5 or 6 days. To allow for the detection of yeasts, anaerobes, and other slowly growing organisms and to decrease the detection of *Propionibacterium*, a routine incubation of 6 days will be implemented in our laboratory. Each laboratory should review its own data in the context of the patient population it serves before making similar decisions.

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