

## Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms

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**BACKGROUND:** Approximately 1 in 2000 platelet components are bacterially contaminated. The time to detection of 15 seeded organisms in platelets recovered from an automated culture system was studied.

**STUDY DESIGN AND METHODS:** Isolates of *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*, *Clostridium perfringens*, *Corynebacterium* species, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Streptococcus pyogenes*, and *Streptococcus viridans* were inoculated into Day 2 apheresis platelet components to obtain a final concentration of approximately 10 and 100 CFU per mL (2 units/organism). Each bag was sampled 10 times (20 mL/sample). Four mL of each sample was inoculated into standard aerobic and anaerobic bottles and into aerobic and anaerobic bottles containing charcoal; 2 mL was inoculated into pediatric aerobic bottles (so as to maintain a 1:10 ratio of sample to media) and 1 mL into thioglycollate broth.

**RESULTS:** With the exception of *P. acnes*, all organisms were detected in a mean of 9.2 to 25.6 hours. A range of 10 serial dilutions in inoculating concentrations was associated with an overall 10.1-percent difference in detection time. A mean of 74.4 and 86.2 hours (100 and 10 CFU/mL inocula, respectively) was required for the detection of *P. acnes* in anaerobic bottles.

**CONCLUSION:** Bacteria thought to be clinically significant platelet contaminants can be detected in 9.2 to 25.6 hours when the starting concentration is approximately 10 to 100 CFU per mL. *P. acnes* required considerably longer incubation times for detection (in either aerobic or anaerobic bottles). However, *P. acnes* is of questionable clinical significance. Such a detection system could be used in either a blood collection center or a transfusion service to screen platelet concentrates for bacterial contamination. Such testing (with sterile sampling performed so as to maintain a closed-bag system) would be expected to save lives and might allow an extension of platelet storage.

It is known that 1 in 1000 to 1 in 2000 platelet units are bacterially contaminated, and it is estimated that severe morbidity or death due to bacterially contaminated platelets occurs in as many as 100 to 150 patients every year in the United States alone.<sup>1-3</sup> It has been suggested that bacteremia is the most common transfusion-related infection today and that the risk of receiving bacterially contaminated platelets may be 50 to 250 times higher than the combined risk per unit of transfusion-related infection with HIV-1/2, HCV, HBV, and HTLV-I/II.<sup>4,5</sup> Various techniques have been suggested to screen platelets for bacterial contamination, but none have received general acceptance, because of a lack of sensitivity, their nonspecificity, the personnel time required, the expense, and the lack of an approved test.<sup>6</sup>

In the early 1980s, platelet storage for 7 days was approved in the United States. This 7-day storage was based on acceptable in vitro function, in vivo recovery, and survival data.<sup>7-11</sup> However, because of the increasing risk of the proliferation of bacteria over time, the outdate was reduced to the current 5 days. Recently, reports from Europe have advocated the use of bacteria culturing of platelets on Days 2 or 3 to extend the shelf life of platelets to 7 days, thereby reducing the outdating of platelets and preserving a limited medical resource.<sup>12,13</sup> Such a strategy is considered cost-effective.<sup>14</sup>

We studied the use of an automated liquid media culture system (BacT/ALERT 3D, Organon Teknika, Durham,

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**ABBREVIATIONS:** ATCC = American type culture collection; PC(s) = platelet concentrate(s).

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NC), currently available, as a possible means of rapid and reliable identification of the contamination of platelets with 14 bacterial and 1 fungal organisms, all of which are known to contaminate platelet units.

## MATERIALS AND METHODS

American type culture collection (ATCC) or clinical isolates of 15 microorganisms (Table 1) were inoculated into Day 2 apheresis platelets at both 10 and 100 CFU per mL (2 platelet units/organism). In the case of *Enterobacteriaceae* (*Klebsiella oxytoca*, *Escherichia coli*, *Serratia marcescens*, and *Enterobacter cloacae*), multiple isolates were screened in advance to identify plasma-resistant strains.

The inoculum dose of 10 CFU per mL was chosen for two reasons: 1) from the few studies that have attempted to quantitate the degree of bacterial contamination on the day that the contamination was suspected, it can be deduced that most contaminated platelet components had bacterial contamination of no more than 10 CFU per mL<sup>15-19</sup>; and 2) blood inoculation experiments with final inoculation concentrations of less than 5 CFU per mL are often complicated by inconsistent growth.<sup>20-23</sup>

All components were inoculated with bacterial suspensions obtained from cultures placed overnight on 5-percent sheep blood agar plates. The turbidity of the suspensions was adjusted to match a 0.5 McFarland standard. Serial dilutions of these suspensions yielded concentrations from which small aliquots (0.5-5 mL) were inoculated. The actual inoculum was confirmed from quantitative culture of the McFarland dilutions and with aliquots of the postinoculation platelets. To ensure a final bag concentration of 10 CFU per mL, bags with both 10 and 100 CFU per mL were pre-

pared (to allow for the vagaries of concentration that can be seen with nephelometry and McFarland standards).

Each bag was sampled 10 times (20 mL/sample, with a syringe). Four mL of each sample was inoculated into standard aerobic, standard anaerobic, activated charcoal aerobic, and anaerobic bottles (FAN; BacT/Alert 3D, Organon Teknika), and 2 mL of each sample was inoculated into the pediatric aerobic bottles (Pedi-BacT, Organon Teknika) so as to maintain a 1-in-10 dilution. FAN bottles contain activated charcoal and are designed to minimize the effect of any antibiotics in a blood sample. An additional 1 mL of each sample was inoculated into thioglycollate broth. Before this inoculation, two 20-mL samples from each bag were similarly inoculated into culture bottles and thioglycollate broth to ensure baseline sterility of the original apheresis bags. Thus, in total, 1800 BacT/ALERT 3D bottles were inoculated (15 organisms × 2 apheresis units × 12 samples/apheresis unit × 5 bottles/sample) in these experiments.

All sampling and inoculum injections of the apheresis bags were performed aseptically through a sampling-site coupler. Quantitative cultures of the McFarland suspensions were performed by serial 1-in-10 dilutions of 0.1 mL of platelets on sheep blood agar, with incubation at 35°C for 24 to 48 hours. Colonies were then counted from plates with 25 to 300 colonies, and the CFU per mL were calculated. Postinoculation quantitative cultures were similarly performed by placing 0.1 to 0.5 mL of the platelet suspension on sheep blood agar and incubating at 35°C for 24 to 48 hours. The limit of culture sensitivity was 2 to 10 CFU per mL (depending on the volume plated). All cultures were performed in duplicate, and the counts were averaged.

Bottles were incubated in the BacT/ALERT 3D system until the sensor indicated that they were positive for 2 weeks. All positive bottles were Gram's-stained to confirm expected Gram's-staining characteristics and morphology.

Ten bottles of each type were cultured for each organism at postinoculation concentrations of 10 or 100 CFU per mL. A bottle type was classified as supporting growth (by bottle type) if 8 or more bottles were positive, as not supporting growth if 2 or fewer bottles were positive, or as inconclusive if 3 to 7 bottles were positive.

## RESULTS

The numbers of CFU from the final inoculating concentrations were determined by quantitative culture of McFarland dilutions and postinoculum aliquots. These are summarized in Table

**TABLE 1. The number of CFU grown from final inoculating concentrations determined by quantitative culture of McFarland dilutions and postinoculum aliquots**

Organism	Source	10 CFU/mL		100 CFU/mL	
		Inoculated	Recovered	Inoculated	Recovered
<i>B. cereus</i>	ATCC 11778	4.2	<3	42	55
<i>E. coli</i>	ATCC 25922	10.5	10	105	135
<i>S. aureus</i>	ATCC 27217	13.1	10	131	175
<i>S. marcescens</i>	ATCC 43862	11.5	7	115	110
<i>B. subtilis</i>	ATCC 6633	12.1	6	121	155
<i>C. perfringens</i>	ATCC 13124	1.3	3	13	30
<i>K. oxytoca</i>	Clinical	12.4	7	124	210
<i>P. aeruginosa</i>	ATCC 27853	17.8	7	178	135
<i>S. epidermidis</i>	ATCC 49134	2.0	<3	20	25
<i>S. pyogenes</i>	ATCC 19615	0.9	<3	9	20
<i>E. cloacae</i>	Clinical	8.6	11	86	195
<i>P. acnes</i>	Clinical	17.4	<3	174	110
<i>S. viridans</i>	Clinical	2.1	<10	21	25
<i>C. albicans</i>	ATCC 10231	36.8	15	368	560
<i>Corynebacterium</i> sp.	Clinical	12.3	11	123	165
Mean*		10.9	7.3	108.7	140.3
SD		9.1	3.8	91.4	133.2

\* For the calculation of the mean and SD, values below detection were taken at the limit of detection: for example, <3 was taken as 3 CFU per mL.

1. Times to detection (mean hours) of each organism by the BacT/ALERT 3D microbe-detection system are presented in Figs. 1 and 2 and Tables 2 and 3. The average inoculum for a final concentration of 10 CFU per mL and 100 CFU per mL was 10.9 CFU per mL and 108.7 CFU per mL, respectively. The mean time to detection in 10 CFU per mL for all organisms except *P. acnes* ranged from 10.2 to 25.6 hours (all bottles). The mean detection time for *P. acnes* was 86.2 hours. Mean detection times in 100 CFU per mL for all organisms except *P. acnes* ranged from 9.2 to 20.8 hours (all bottles). The mean detection time for *P. acnes* was 74.4 hours. As expected, the growth of *Bacillus subtilis*, *Corynebacterium* species, *Candida albicans*, and *Pseudomonas*

*aeruginosa* was not supported in either type of anaerobic bottles; the growth of *Clostridium perfringens* was not supported in the FAN aerobic bottles; and the growth of *P. acnes* was not supported in either the FAN aerobic or FAN anaerobic bottles. One each of the pediatric aerobic (10 CFU/mL) and standard aerobic (10 CFU/mL) bottles (both inoculated from the same syringe sample), and one FAN aerobic bottle (100 CFU/mL) were not reactive in the *C. albicans* experiments. Two each of the pediatric and standard aerobic bottles (100 CFU/mL) (all of these bottles inoculated from the same two syringe samples), and one FAN aerobic bottle (100 CFU/mL) were not reactive in the *Corynebacterium* sp. experiments. All bottle types could be categorized as either supporting or not supporting the growth

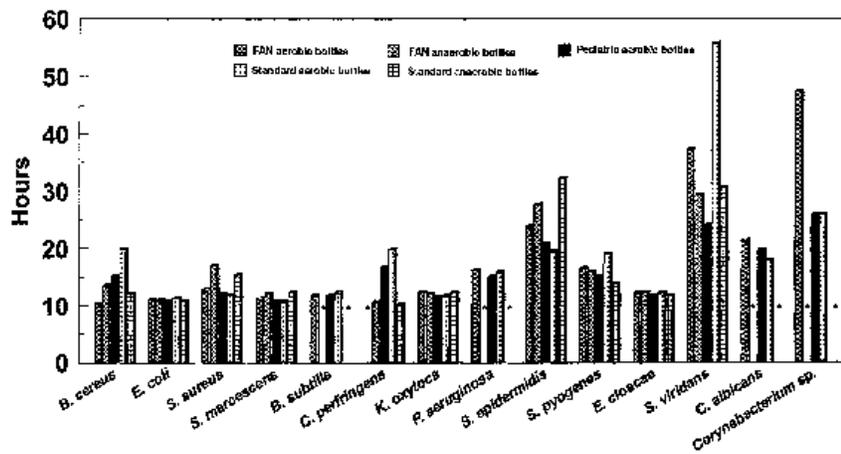


Fig. 1. Mean time to detection by BacT/ALERT 3D of 14 microbial organisms (excluding *P. acnes*) inoculated at 10 CFU per mL, shown by bottle type. Mean time to detection of all organisms except *P. acnes* ranged from 10.2 to 25.6 hours (all bottles). The mean detection time for *P. acnes* was 86.2 hours. Actual mean  $\pm$  SD = 10.9  $\pm$  9.1 CFU per mL. \* = no growth.

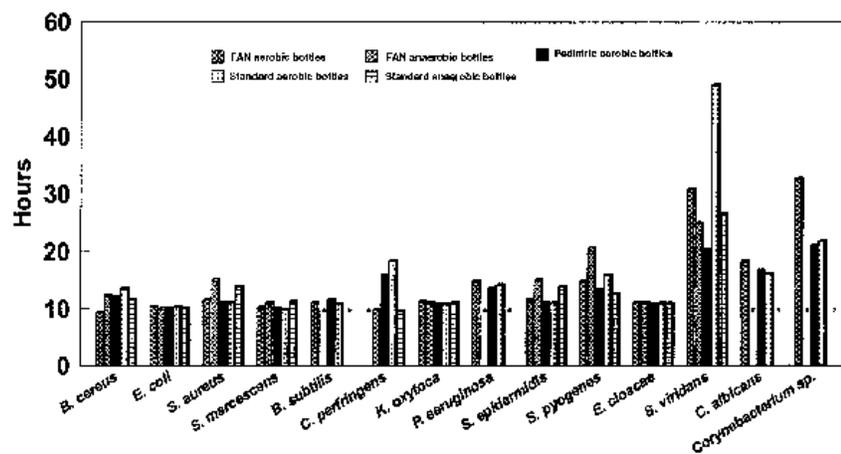


Fig. 2. Mean time to detection by BacT/ALERT 3D of the 14 microbial organisms (excluding *P. acnes*) inoculated at 100 CFU per mL, shown by bottle type. Mean time to detection of all organisms except *P. acnes* ranged from 9.2 to 20.8 hours (all bottles). The mean detection time for *P. acnes* was 74.4 hours. Actual mean  $\pm$  SD = 108.7  $\pm$  91.4 CFU per mL. \* = no growth.

supporting or not supporting the growth of each organism. Similarly, 2 of the postinoculation (10 CFU/mL) thioglycollate broths did not grow in the *Corynebacterium* sp. experiments, and one of the *S. epidermidis* postinoculation (10 CFU/mL) experiments did not grow. It is presumed that a lack of homogenous distribution of the organisms and "sampling error" were the cause of these few observed exceptions to the expected growth. Five (0.28%) of the 1800 bottles were lost to mishandling (breakage and improper insertion or removal).

Table 2 summarizes the shortest mean detection time per organism: on average, the higher inoculum was 10.1 percent (range, 6.9-18.8%) faster than the lower inoculum (a concentration range of 10 serial dilutions). Detection times for all organisms were (in appropriate medium) comparable to or faster than those in thioglycollate broth. Detection times (mean hours and the quickest bottle type) for the 10 and 100 CFU per mL inoculation experiments are summarized in Table 2.

## DISCUSSION

Unlike viral contamination of blood components that is detected from a sample obtained at the time of donation, bacterial contamination of blood components frequently requires time for the organisms to proliferate before they become detectable. For example, Blajchman et al.<sup>15</sup> with the Canadian Red Cross, using a similar, automated detection system (Bactec, Becton Dickinson, Cockeysville, MD) cultured random-donor platelet concentrates (PCs) on Days 1 (N = 16,290

**TABLE 2. Times to detection and the quickest type of bottle for the 10 and 100 CFU per mL inoculation experiments**

Organism	Time to detection*	Quickest bottle type
<i>B. cereus</i>		
10 CFU/mL	10.3 ± 0.2	FAN aerobic
100 CFU/mL	9.2 ± 0.1	FAN aerobic
<i>B. subtilis</i>		
10 CFU/mL	11.6 ± 0.2	FAN aerobic
100 CFU/mL	10.8 ± 0.2	Standard aerobic
<i>C. albicans</i>		
10 CFU/mL	17.9 ± 0.9	Standard aerobic
100 CFU/mL	15.9 ± 0.2	Standard aerobic
<i>C. perfringens</i>		
10 CFU/mL	10.2 ± 0.3	Standard anaerobic
100 CFU/mL	9.4 ± 0.2	Standard anaerobic
<i>Corynebacterium</i> species		
10 CFU/mL	25.6 ± 0.5	Pediatric standard aerobic
100 CFU/mL	20.8 ± 0.6	Pediatric standard aerobic
<i>E. cloacae</i>		
10 CFU/mL	11.7 ± 0.1	Standard anaerobic
100 CFU/mL	10.7 ± 0.2	Pediatric standard aerobic
<i>E. coli</i>		
10 CFU/mL	10.7 ± 0.1	Standard anaerobic
100 CFU/mL	9.8 ± 0.1	FAN anaerobic
<i>K. oxytoca</i>		
10 CFU/mL	11.6 ± 0.1	Pediatric standard aerobic
100 CFU/mL	10.6 ± 0.1	Pediatric standard aerobic
<i>P. acnes</i>		
10 CFU/mL	86.2 ± 1.8	Standard anaerobic
100 CFU/mL	74.4 ± 1.1	Standard anaerobic
<i>P. aeruginosa</i>		
10 CFU/mL	15.0 ± 0.2	Pediatric standard aerobic
100 CFU/mL	13.5 ± 0.1	Pediatric standard aerobic
<i>S. aureus</i>		
10 CFU/mL	11.8 ± 0.1	Standard aerobic
100 CFU/mL	10.9 ± 1.2	Standard aerobic
<i>S. epidermidis</i>		
10 CFU/mL	19.4 ± 0.4	Standard aerobic
100 CFU/mL	16.6 ± 0.1	Standard aerobic
<i>S. marcescens</i>		
10 CFU/mL	10.7 ± 0.1	Pediatric standard aerobic
100 CFU/mL	9.7 ± 0.1	Standard aerobic
<i>S. pyogenes</i>		
10 CFU/mL	13.8 ± 0.5	Standard anaerobic
100 CFU/mL	12.5 ± 0.3	Standard anaerobic
<i>S. viridans</i>		
10 CFU/mL	23.9 ± 2.3	Pediatric standard aerobic
100 CFU/mL	20.2 ± 0.4	Pediatric standard aerobic

\* Times to detection are given as mean ± SD hours. Times to detection for the 10 CFU per mL inoculation experiments in the pediatric aerobic bottles (the smallest inoculum size) are summarized in Table 3.

PCs) and 3 (N = 10,065 PCs) after preparation.<sup>15</sup> Of the 16,290 PCs cultured on the day of collection, 4 were found to be culture-positive, but an additional 3 that were culture-negative on the day of collection were culture-positive after 2 further days of storage. On the basis of these results, Blajchman et al. concluded that cultures from the day of collection may be inadequate for detecting all contaminated PC units.

Recently, a study of the growth characteristics of *Bacillus cereus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *S. marcescens*, *Staphylococcus aureus*, and *S. epidermidis* in 165 inoculated units of platelets found that, by Day 3, 100 percent of all units contaminated with *B. cereus*, *P.*

*aeruginosa*, *K. pneumoniae*, *S. marcescens*, and *S. aureus* and 91.7 percent of units contaminated with *S. epidermidis* had a concentration of 10 CFU per mL or greater.<sup>24</sup> The data in that report suggested that the vast majority of bacterially contaminated platelets could be detected with culture after 3 days of storage.

The present study suggests that bacteria thought to be clinically significant platelet contaminants can be detected in 9.2 to 25.6 hours when the starting concentration is approximately 10 to 100 CFU per mL. The detection of *P. acnes*, which can occasionally be isolated from platelets, grows poorly in the aerobic environment of platelet storage, and is of questionable clinical significance, takes considerably longer to detect (in either aerobic or anaerobic bottles). These findings are comparable to those in the experiments conducted by Wagner and Robinette,<sup>25</sup> in which 36 PCs were inoculated with *S. epidermidis* and *E. coli* (12 each inoculated to 0.1, 1.0, or 10 CFU/mL). This study found that, with *S. epidermidis*, all (36/36) of the PCs had a concentration of  $\geq 0.5$  CFU per mL by 48 hours (but not at 24 hours) (sampling volumes were 0.5, 1.0, or 2.0 mL, which corresponded to minimal detectable concentrations of 2, 1, or 0.5 CFU/mL, respectively). With *E. coli*, by 24 hours, all (36/36) of the PCs had a concentration of  $\geq 0.5$  CFU per mL (sampling volumes were 0.5, 1.0, or 2.0 mL, which corresponded to minimal detectable concentration of 2, 1, or 0.5 CFU/mL, respectively). Growth times, while similar, were somewhat slower than those seen in the present study (12.1-12.9

hours for 10 CFU/mL *E. coli*, compared to 10.7 hours in the present study, and 46.7-51.6 hours for 10 CFU/mL *S. epidermidis*, compared to 19.4 hours in the present study). However, these greater times were likely due to the smaller inoculation volumes employed (0.5-2 mL vs. 2-4 mL in the present study) or possibly to strain differences. The growth times observed were also similar to those in recent spiking experiments reported by Liu et al.<sup>26</sup> with aerobic bottles inoculated with only 1 CFU per mL of *E. coli* (mean, 12.55 hours), *B. cereus* (mean, 16.32 hours), and *S. aureus* (mean, 31.28 hours).

Taken together, the bacteria growth studies and the automated culture studies suggest that the use of an auto-

**TABLE 3. Times to detection for the 10 CFU per mL inoculation experiments with the small-inoculation pediatric aerobic bottle**

Organism	Time to detection*
<i>B. cereus</i>	15.1 ± 2.7
<i>B. subtilis</i>	11.8 ± 0.4
<i>C. albicans</i>	19.7 ± 2.0
<i>C. perfringens</i>	16.7 ± 0.4
<i>Corynebacterium</i> species	25.6 ± 0.5
<i>E. cloacae</i>	11.7 ± 0.1
<i>E. coli</i>	10.8 ± 0.2
<i>K. oxytoca</i>	11.6 ± 0.1
<i>P. acnes</i>	285 ± 34.2
<i>P. aeruginosa</i>	15.0 ± 0.2
<i>S. aureus</i>	11.9 ± 0.2
<i>S. epidermidis</i>	20.8 ± 1.7
<i>S. marcescens</i>	10.7 ± 0.1
<i>S. pyogenes</i>	15.2 ± 0.5
<i>S. viridans</i>	23.9 ± 2.4

\* Values given as mean ± SD hours.

mated culturing system on Day 3 of platelet storage would detect the vast majority of clinically significant bacteria and provide reliable culture results before the 5-day outdate of the platelets. However, it is possible that bacterially contaminated platelets might still be transfused while the culture is growing. Detection of many, but not all, of these would be expected if a culture was also performed early in storage. Alternatively, simultaneous performance of a quicker, but less sensitive assay (such as an RNA detection or a DNA amplification assay) could also minimize the transfusion of bacterially contaminated components while the culture was growing.<sup>23,27,28</sup>

Although a panel of 5 bottles was used in these studies, the data suggest that, for the clinically significant organisms (excluding *P. acnes*, which is of questionable clinical significance) studied, all organisms could be detected in a reasonable time with the use of aerobic bottles alone (Table 3). The infrequent failure of detection in 1 to 2 bottles out of 10 suggests that 2 bottles per culture should be examined to minimize “sampling error” at low bacteria concentrations. In this setting, there was no advantage to the use of the FAN bottles.

We conclude that automated microbe-detection systems could be utilized in either a blood collection center or a hospital transfusion service to rapidly screen PCs for bacterial contamination. However, to reach a concentration of 10 CFU per mL (the concentration validated in this study), it would be necessary for some storage time to elapse before testing. Bacterial testing can be expected to save lives and might allow an extension of platelet storage.

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