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Evaluation of the BacT/Alert automated blood culture system for detecting bacteria and measuring their growth kinetics in leucodepleted and non-leucodepleted platelet concentrates

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Vox Sanguinis

Background and Objective To evaluate the BacT/Alert automated blood culture system for the detection of bacteria in platelet concentrates, and to determine bacterial growth kinetics in leucodepleted and non-leucodepleted units.

Materials and Methods Apheresis (Cobe Leucocyte Reduction System™ [LRS]) and pooled buffy coat-derived (Optipress™) platelet concentrates (PCs) were tested. Six organisms were used for spiking the PCs: *Clostridium perfringens*, *Bacillus cereus*, Group B Streptococcus, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli*. Units were inoculated to give a final concentration of ≈ 1 and 50 colony-forming units (CFU)/ml. On days 0, 2 and 5, BacT/Alert standard aerobic and anaerobic bottles were inoculated with a 5-ml fill volume and bacteria were enumerated.

Results The BacT/Alert Automated blood culture system gave rapid determination times of spiked units, with all positives detected within 48 h and 98·1% detected within 24 h. In general, as the inoculum concentration increased, the detection time decreased. Rapid growth was obtained with all organisms tested except for *B. cereus*, which failed to grow on four occasions. Bacterial numbers on day 2 ranged from 10^5 to 10^{11} CFU/ml and on day 5 ranged from 10^4 to 10^{12} CFU/ml. Growth was not significantly greater in leucodepleted units.

Conclusions The study confirmed that PCs are an excellent growth medium for bacteria. Rapid and substantial growth was obtained with all organisms under test. Leucodepletion does not appear to enhance bacterial proliferation. The BacT/Alert automated blood culture system could rapidly detect contamination of units. Bacterial screening using an automated blood culture system is therefore a potential option.

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Introduction

Bacterial transmission remains the major component of morbidity and mortality associated with transfusion-transmitted infection.

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In the United States during the time-period 1986–91, bacterial contamination accounted for 15·9% of all transfusion-related fatalities [1]. From 1994 to March 1998, the French Blood Agency Haemovigilance surveillance system attributed 18 deaths (four occurring in 1997) to blood components contaminated with bacteria [2,3]. Bacterial transfusion transmission was reported to the Haemovigilance surveillance system as the most frequently identifiable cause of death [3]. In the UK, the Serious Hazards of Transfusion (SHOT) surveillance system reported that of 26 transfusion-transmitted infections in 1995–2000, 15 were caused by bacteria [4].

Of all blood components used for transfusion, platelet concentrates (PCs) are the most common cause of bacterial

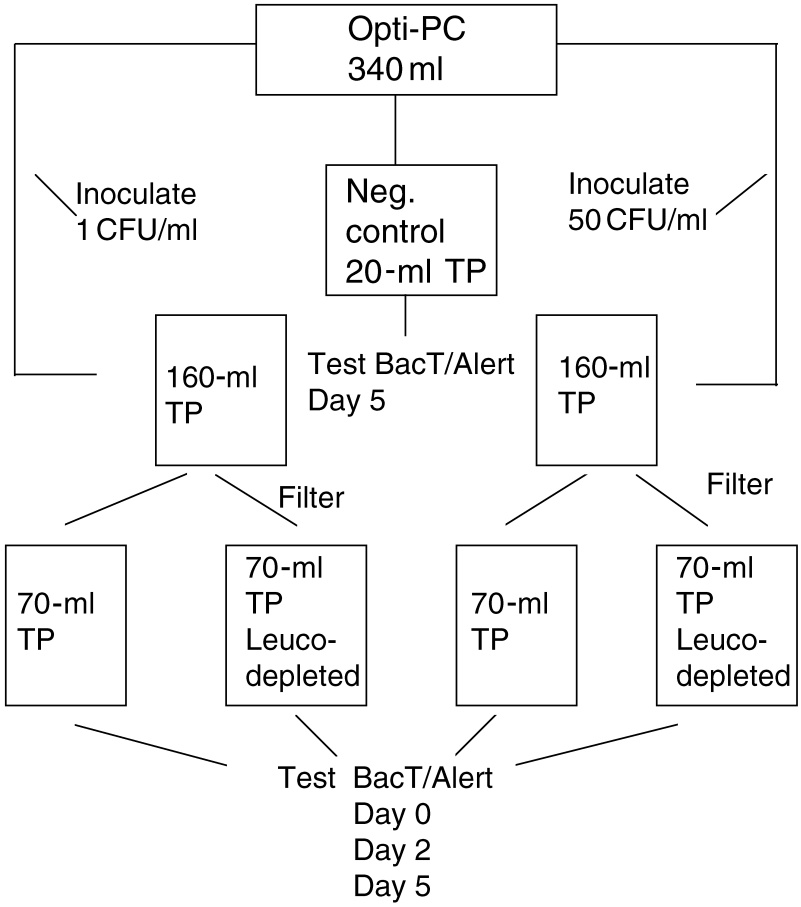

Fig. 1 Flow diagram of Opti-Press™ platelet preparation for the BacT/Alert study. All transfer packs (TPs) were incubated at 22 °C in a platelet shaker. CFU, colony-forming units; Neg., negative.

transmission [5]. The current storage temperature (22 °C), whilst optimal for platelet viability and function, also facilitates bacterial multiplication. In a retrospective study performed at the National Blood Service, North London Centre, the confirmed bacterial contamination rate was found to be 0·7% (one in 137) in pooled platelets and 0·4% (one in 251) in apheresis platelets [6].

Strategies to reduce bacterial transmission include improved disinfection of the venepuncture site, diversion of the initial 10–20 ml of blood from the collection bag and screening of donations using an automated blood culture system.

In order to evaluate the performance of the BacT/Alert automated blood culture system (Organon Teknika, Cambridge, Cambs., UK) in the detection of bacteria in PCs, units were spiked with known concentrations of bacteria. Apheresis and pooled buffy coat-derived units (Optipress™; Baxter Health Care, Newbury, Berks., UK) were tested. Leucodepleted and non-leucodepleted Optipress™ pooled platelets were used. All apheresis platelets were leucodepleted by the Cobe Leucocyte Reduction System™ (LRS) (Cobe Laboratories, Quedgeley, Glos., UK).

Growth kinetics of the bacteria in these units were studied to determine whether bacteria proliferate more rapidly in leucodepleted units than in non-leucodepleted units. The possibility has been raised that bacteria will grow more rapidly in leucodepleted units because of a reduction in bactericidal activity owing to the removal of phagocytic polymorphonuclear and mononuclear cells. This is of particular significance to the National Blood Service in England as all PCs are now leucodepleted as a measure to reduce any potential for transmission of variant Creutzfeldt-Jakob Disease (vCJD).

Materials and methods

Optipress™ pooled and Cobe LRS™ apheresis PCs were inoculated with six different types of bacteria to give a final concentration of ≈ 1 colony-forming unit (CFU)/ml (low inoculum) in one series and 50 CFU/ml (high inoculum) in another. All experiments were performed in duplicate. Serial dilutions and inoculum were prepared in one-quarter strength Ringers containing 0·1% Triton (for dispersion). The organisms chosen were:

(1) *Clostridium perfringens* (Fatal transmission; North London Centre 1998 [7]).

(2) *Bacillus cereus* (Transmission; Leeds Centre 1996 [8]).

(3) Group B Streptococcus (Transmission; North London Centre 1996, unpublished).

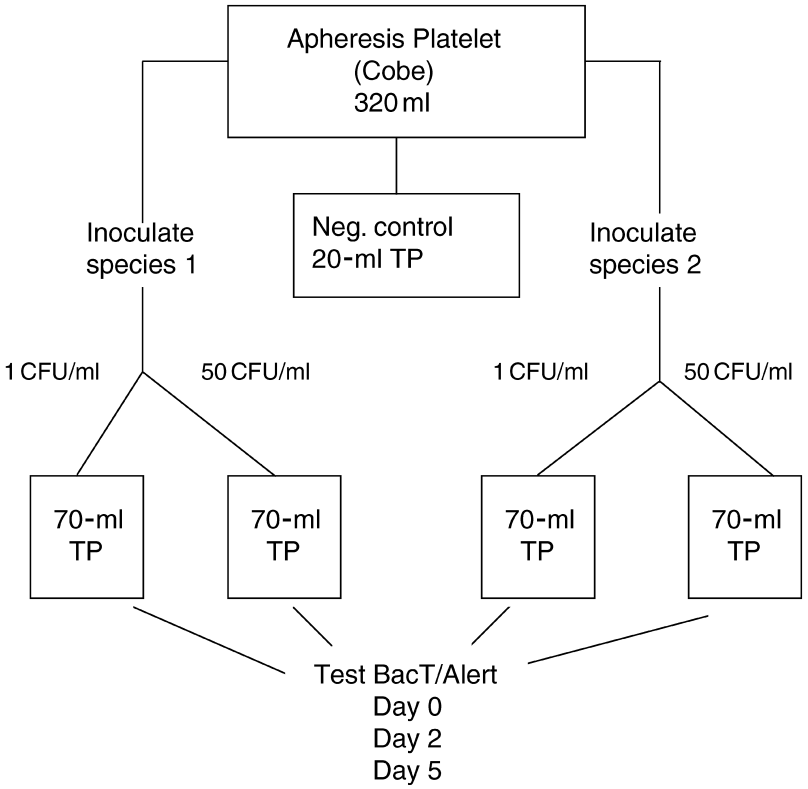

Fig. 2 Flow diagram of apheresis platelet preparation for the BacT/Alert study. All transfer packs (TPs) were incubated at 22 °C in a platelet shaker. CFU, colony-forming units; Neg., negative.

(4) *Staphylococcus epidermidis* (American Type Culture Collection [ATCC] 12228).

(5) *Staphylococcus aureus* (ATCC 27217).

(6) *Escherichia coli* (ATCC 25922).

Platelet preparation for Optipress™ units is described in Fig. 1 and for apheresis units in Fig. 2. A Pall LRP65E filter (Pall Medical, Portsmouth, Hampshire, UK) was used for Optipress™ leucodepleted units. A sterile connection device (Terumo SCD™ 312; Terumo, Knowsley, Merseyside, UK) was used to dock Baxter PL 1240 (Baxter Health Care) transfer packs onto the primary pack within a closed system and for any other manipulations required. A final transfer pack volume of 70 ml was derived, which acted as a sampling bag in the study. The transfer packs had the same 'sample to surface' ratio as did routine platelet packs. A sample site coupler spike with needle injection device (Baxter FMC 1401; Baxter Health Care) was inserted aseptically into a port of the transfer packs for inoculation and sampling. Inoculation and sampling were performed using sterile hypodermic syringes and needles.

BacT/Alert evaluation

Performance of the BacT/Alert with standard anaerobic and aerobic bottles was evaluated. Testing was performed on days 0, 2 and 5 of platelet shelf-life. BacT/Alert bottles were inoculated with a 5-ml fill volume and incubated for 7 days at 36 °C. A negative-control transfer pack, taken prior to bacterial inoculation, was tested on day 5. All isolates were identified and all BacT/Alert bottles subcultured and Gram stained at the end of incubation. Subculture was performed on duplicate Columbia blood agar plates (Mast Diagnostics, Bootle, Merseyside, UK) which were incubated, one aerobically and the other anaerobically, for 48 h at 37 °C.

Growth kinetics

Enumeration of bacteria in the platelet bags was also performed at the BacT/Alert sampling times. Serial twofold dilutions were made in one-quarter strength Ringers, 0·1 ml of which was directly plated onto Columbia blood agar and spread with a 'hockey stick'. Incubation was at 37 °C for 48 h under aerobic or anaerobic conditions, as appropriate.

Results

BacT/Alert determination times are listed in Table 1. Results for aerobic bottles are given except with *Cl. perfringens* in which the results for anaerobic bottles are shown.

Growth kinetics for the six organisms that were spiked are shown in Table 2 at the sampling times of days 0, 2 and 5.

Table 1 BacT/Alert detection times

Organism	Pack type	Mean detection times (hours)[a]											
		Day 0				Day 2				Day 5			
		Low inoculum		High inoculum		Low inoculum		High inoculum		Low inoculum		High inoculum	
		(Mean)	(± SD)	(Mean)	(± SD)	(Mean)	(± SD)	(Mean)	(± SD)	(Mean)	(± SD)	(Mean)	(± SD)
Staphylococcus aureus	OPTI-PC	12·8	0·0	11·2	0·2	3·3	3·8	3·8	0·5	5·2	0·0	5·9	0·4
	OPTI-PC-LD	13·2	0·2	11·6	0·3	4·2	4·8	3·9	0·1	4·0	0·9	5·9	0·7
	COBE-LRS	12·9	0·1	11·5	0·2	3·7	3·5	4·2	0·2	5·0	0·5	5·1	0·1
Staphylococcus epidermidis	OPTI-PC	18·0	0·8	15·1	0·1	31·8	43·5	9·6	0·4	7·2	0·1	6·7	0·2
	OPTI-PC-LD	17·6	0·1	15·0	0·2	12·3	14·8	9·3	0·1	7·2	0·2	7·0	0·3
	COBE-LRS	18·9	0·1	15·9	0·1	16·9	11·7	28·3	13·5	6·8	0·3	7·2	0·4
Escherichia coli	OPTI-PC	11·4	0·2	10·5	0·2	5·0	6·2	3·6	0·8	3·2	0·0	3·2	0·0
	OPTI-PC-LD	11·5	0·0	10·5	0·2	4·9	5·8	3·4	0·4	3·3	0·0	3·3	0·3
	COBE-LRS	11·5	0·3	10·7	0·0	7·3	8·2	4·2	0·7	3·2	0·0	3·3	0·0
Clostridium perfringens	OPTI-PC	8·3	0·3	7·4	0·2	8·8	10·8	4·6	0·3	4·5	0·0	4·9	0·9
	OPTI-PC-LD	8·7	0·2	7·0	0·3	4·4	4·5	5·0	0·3	5·8	0·3	4·6	0·4
	COBE-LRS	8·4	0·1	11·3	0·3	5·4	6·3	4·2	0·0	5·3	0·0	5·1	0·1
Group B Streptococci	OPTI-PC	13·2	1·5	8·7	0·2	3·9	3·8	3·5	0·2	4·0	0·4	4·7	0·4
	OPTI-PC-LD	11·9	0·9	8·8	0·1	4·0	3·8	3·6	0·3	3·8	0·2	4·8	0·5
	COBE-LRS	11·6	0·1	8·7	0·0	4·8	4·8	3·9	0·1	5·2	0·1	5·3	0·0
Bacillus cereus	OPTI-PC	22·2[b]	NA	8·7	0·2	3·3[b]	NA	3·4	0·1	3·8[b]	NA	3·8	0·0
	OPTI-PC-LD	NG	NA	9·2	0·2	NG	NA	3·4	0·1	NG	NA	3·5	0·3
	COBE-LRS	10·5[b]	NA	8·8	0·0	3·3[b]	NA	3·5	0·0	4·2[b]	NA	4·3	0·1

LD, leucocyte depleted; LRS, leucocyte reduction system; NA, not applicable; NG, no growth; PC, platelet concentrate; SD, standard deviation.

[a]Aerobic bottles, except *Clostridium perfringens* (anaerobic bottles).

[b]Result of a single bottle.

Table 2 Growth kinetics

		Mean number of organisms/ml					
		Day 0		Day 2		Day 5	
Organism	Pack type	Low inoculum	High inoculum	Low inoculum	High inoculum	Low inoculum	High inoculum
Staphylococcus aureus	OPTI-PC	2	75	$7{\cdot}3 \times 10^{10}$	$6{\cdot}2 \times 10^{10}$	$8{\cdot}3 \times 10^{11}$	$4{\cdot}6 \times 10^{11}$
	OPTI-PC-LD	2	75	$1{\cdot}4 \times 10^{11}$	$1{\cdot}6 \times 10^{11}$	$9{\cdot}0 \times 10^{11}$	$1{\cdot}6 \times 10^{12}$
	COBE-LRS	2	75	$2{\cdot}7 \times 10^{11}$	$2{\cdot}2 \times 10^{11}$	$2{\cdot}2 \times 10^{12}$	$1{\cdot}1 \times 10^{12}$
Staphylococcus epidermidis	OPTI-PC	4	200	$2{\cdot}0 \times 10^{9}$	$1{\cdot}8 \times 10^{11}$	$2{\cdot}6 \times 10^{10}$	$4{\cdot}8 \times 10^{10}$
	OPTI-PC-LD	4	200	$7{\cdot}0 \times 10^{9}$	$2{\cdot}6 \times 10^{11}$	$3{\cdot}2 \times 10^{10}$	$2{\cdot}5 \times 10^{10}$
	COBE-LRS	4	200	$1{\cdot}8 \times 10^{5}$	$4{\cdot}7 \times 10^{11}$	$6{\cdot}4 \times 10^{9}$	$1{\cdot}2 \times 10^{10}$
Escherichia coli	OPTI-PC	2	75	$3{\cdot}5 \times 10^{10}$	$4{\cdot}5 \times 10^{11}$	$2{\cdot}2 \times 10^{10}$	$3{\cdot}7 \times 10^{10}$
	OPTI-PC-LD	2	75	$2{\cdot}5 \times 10^{10}$	$1{\cdot}7 \times 10^{11}$	$4{\cdot}4 \times 10^{9}$	$6{\cdot}5 \times 10^{9}$
	COBE-LRS	2	75	$6{\cdot}5 \times 10^{7}$	$1{\cdot}4 \times 10^{8}$	$9{\cdot}5 \times 10^{9}$	$9{\cdot}5 \times 10^{10}$
Clostridium perfringens	OPTI-PC	10	500	$1{\cdot}0 \times 10^{5}$	$3{\cdot}0 \times 10^{5}$	$1{\cdot}6 \times 10^{5}$	$1{\cdot}3 \times 10^{5}$
	OPTI-PC-LD	10	500	$4{\cdot}5 \times 10^{5}$	$4{\cdot}5 \times 10^{5}$	$1{\cdot}3 \times 10^{5}$	$7{\cdot}5 \times 10^{4}$
	COBE-LRS	10	500	$5{\cdot}0 \times 10^{5}$	$8{\cdot}5 \times 10^{5}$	$4{\cdot}5 \times 10^{4}$	$4{\cdot}0 \times 10^{4}$
Group B Streptococci	OPTI-PC	5	50	$1{\cdot}0 \times 10^{10}$	$1{\cdot}6 \times 10^{8}$	$3{\cdot}4 \times 10^{9}$	$1{\cdot}6 \times 10^{8}$
	OPTI-PC-LD	5	50	$1{\cdot}3 \times 10^{10}$	$1{\cdot}8 \times 10^{8}$	$4{\cdot}7 \times 10^{9}$	$2{\cdot}2 \times 10^{8}$
	COBE-LRS	5	50	$1{\cdot}6 \times 10^{9}$	$1{\cdot}7 \times 10^{8}$	$3{\cdot}0 \times 10^{9}$	$4{\cdot}6 \times 10^{8}$
Bacillus cereus	OPTI-PC	10	100	$3{\cdot}3 \times 10^{7}$	$3{\cdot}5 \times 10^{7}$	$1{\cdot}5 \times 10^{7}$	$4{\cdot}5 \times 10^{7}$
	OPTI-PC-LD	10	100	0	$3{\cdot}0 \times 10^{7}$	0	$5{\cdot}0 \times 10^{7}$
	COBE-LRS	10	100	$2{\cdot}3 \times 10^{7}$	$4{\cdot}5 \times 10^{7}$	$1{\cdot}0 \times 10^{7}$	$4{\cdot}5 \times 10^{7}$

LD, leucocyte depleted; LRS, leucocyte reduction system; PC, platelet concentrate.

All platelet packs were found to be negative prior to bacterial inoculation. The mean white blood cell counts for the leucodepleted apheresis and pooled platelets were $0{\cdot}2 \times 10^{6}$/unit and $0{\cdot}3 \times 10^{6}$/unit, respectively. The mean white blood cell count in the non-leucodepleted pooled platelets was 4×10^{9}/pool.

On day 0, the BacT/Alert automated blood culture system detected all spiked units within 24 h (range 7·0–22·2 h, Table 1). Overall, on day 2, as the inoculum concentration increased, the detection time was more rapid than on day 0 (range 3·3–31·8 h). The mean detection times for *Staph. epidermidis* in non-leucodepleted pooled platelets (low inoculum) and Cobe LRS (high inoculum) on day 2 were considerably longer in comparison with other organisms tested at this time-point. The low inoculum with the non-leucodepleted pooled platelet units resulted in a mean determination time of 31·8 h and the high inoculum with the Cobe LRS gave a mean determination time of 28·3 h. On day 2, all other inoculated organisms gave determination times of less than 12 h (range 3·3–8·8 h). Determination times for all organisms on day 5 were extremely rapid, ranging from 3·2 to 7·2 h. Overall, the BacT/Alert automated blood culture system detected all cultures scoring positive within 48 h, and 98·1% were detected within 24 h.

Discussion

Our data are in accordance with a similar study performed by Wagner & Robinette, who also found that as the inoculum concentration increased, the detection time decreased [9].

Cl. perfringens is the only causative anaerobic organism which we have isolated in many years of follow-up of post-transfusion infections. The *Cl. perfringens* isolate from the fatal transmission in North London [7] grew in both aerobic and anaerobic bottles with the exception of one aerobic test bottle (Optipress™ non-leucodepleted unit on day 2). It is unclear as to why there was no growth in just this one bottle, except that oxygen levels may have been particularly high. As this organism was detected in all but one aerobic bottle, the question arises of whether both aerobic and anaerobic culture is necessary or whether aerobic culture is adequate. Detection times for aerobic and anaerobic bottles were comparable for *Cl. perfringens* and all other organisms tested.

Bacterial testing using a single aerobic bottle would have the advantage of reducing cost by 50% and doubling testing capacity on the automated blood culture system. It would also reduce the volume that is taken from the platelet bag, so that more of the product would be available for clinical use.

Results from our study indicate that a 'single bottle' test system may be adequate, particularly as the majority of organisms isolated in screening and monitoring studies are aerobic [10,11].

Rapid growth was obtained with all organisms tested except for *B. cereus*, which failed to grow on four occasions (Tables 1 and 2). Bacterial numbers on day 2 ranged from 10^5 to 10^{11} CFU/ml and on day 5 the range was 10^4 to 10^{12} CFU/ml. These data clearly illustrate that PCs are an excellent bacterial growth medium and corroborate other reported spiking experiments [12,13]. On day 5, the organisms appeared to be entering the stationary phase of growth. Growth was not significantly greater in leucodepleted units than in non-leucodepleted units. Therefore, leucodepletion appears not to enhance bacterial proliferation as a result of any postulated reduction in bacterial activity consequent upon the removal of phagocytic polymorphonuclear and mononuclear cells. Although this contrasts with reports from two other studies indicating that leucodepletion results in increased bacterial growth [14,15], a number of studies have found that white cell reduction does not affect the rate or extent of bacterial growth [9,12,16,17]. It has been suggested that these differences may be a result of the strain of organism used, although three of the organisms in our study were isolated from actual transmissions and were not simply culture collection strains as used by other workers. In interesting contrast, a marked reduction in growth was observed with *Staph. epidermidis* and *E. coli* in Cobe LRS™ on day 2 compared with Optipress™ leucodepleted and non-leucodepleted platelets. As yet we have no explanation for this phenomenon. By day 5, growth was comparable.

With regard to the screening of platelets using the automated blood culture system, although the BacT/Alert detected all spiked organisms on day 0 of platelet inoculation, in a routine situation actual bacterial numbers of contaminants may be considerably lower; it has been postulated that initial contamination level is in the order of < 10 CFU/ml at the time of whole-blood collection [13]. Therefore, it would be prudent (until data is obtained to the contrary) to screen platelets on day 1, or subsequently, after platelet preparation. This would allow bacterial numbers to increase, as demonstrated in our study, therefore increasing the probability of detection and reducing determination time. This would also offer the potential to decrease the sample volume and allow pooling of samples prior to culture, significantly reducing costs. Wagner & Robinette, in their evaluation of the BacT/Alert system, concluded that sampling times of ≥ 24 h postpreparation would be necessary to provide confidence when detecting *E. coli* and *Staph. epidermidis* in PCs. In their study, over 30% of all spiked units were not detected on day 0 of testing when inoculated with 10, 1·0 and 0·1 CFU/ml [9]. Further evidence is indicated in a prospective study using an automated blood culture system in which units were screened on day 1 and also on day 3. Four units were found to be positive on day 1 and day 3, but an additional 3 units were found to be positive on day 3 only (43%) [18]. Indeed, day-1 cultures could lead to a false sense of security with regard to bacterial safety, particularly if the shelf-life of units was extended to 7 days.

In conclusion, this study demonstrates that PCs are an excellent growth medium for bacteria. Rapid and substantial growth was obtained with all organisms under test. Results from the study indicate that leucodepletion does not enhance bacterial proliferation. The BacT/Alert automated blood culture system demonstrated its ability to rapidly detect contaminated units. Bacterial contamination with its associated morbidity and mortality is a significant problem in transfusion medicine. Bacterial screening of platelets is technically feasible using an automated blood culture system. Such approaches should be considered with the same energy and enthusiasm as is nucleic acid testing (NAT) for viral markers, which, unlike bacterial screening, offers little yield in extra viral safety, most of which has already been achieved.

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