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ORIGINAL ARTICLE

Evaluation of the 3D BacT/ALERT automated culture system for the detection of microbial contamination of platelet concentrates

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SUMMARY. Bacterial transmission remains the major component of morbidity and mortality associated with transfusion-transmitted infections. Platelet concentrates are the most common cause of bacterial transmission.

The BacT/ALERT 3D automated blood culture system has the potential to screen platelet concentrates for the presence of bacteria. Evaluation of this system was performed by spiking day 2 apheresis platelet units with individual bacterial isolates at final concentrations of 10 and 100 colony-forming units (cfu) mL^{-1}. Fifteen organisms were used which had been cited in platelet transmission and monitoring studies. BacT/ALERT times to detection were compared with thioglycollate broth cultures, and the performance of five types of BacT/ALERT culture bottles was evaluated. Sampling was performed immediately after the inoculation of the units, and 10 replicates were performed per organism concentration for each of the five types of BacT/ALERT bottles.

The mean times for the detection of these 15 organisms by BacT/ALERT, with the exception of *Propionibacterium acnes*, ranged from 9·1 to 48·1 h (all 10 replicates were positive). In comparison, the time range found using thioglycollate was 12·0–32·3 h (all 10 replicates were positive). *P. acnes*' BacT/ALERT mean detection times ranged from 89·0 to 177·6 h compared with 75·6–86·4 h for the thioglycollate broth.

BacT/ALERT, with the exception of *P. acnes*, which has dubious clinical significance, gave equivalent or shorter detection times when compared with the thioglycollate broth system. The BacT/ALERT system detected a range of organisms at levels of 10 and 100 cfu mL^{-1}. This study validates the BacT/ALERT microbial detection system for screening platelets. Currently, the system is the only practically viable option available for routinely screening platelet concentrates to prevent bacterial transmission.

Key words: BacT/ALERT, bacteria, platelets, screening, transmission.

In the 20th century, considerable success has been achieved in reducing viral transmission owing to blood transfusion. The advent of sterile disposable plastic collection bags provides closed systems and prevents the entry of bacteria from the environment, and refrigerated storage of components results in the death or stasis of the majority of contaminating bacteria. However, bacterial transmission by transfusion does still occur and remains the major component of morbidity and mortality associated with transmitted infections. Several countries have a national surveillance system to collate and monitor data on the occurrence of transfusion-transmitted infection. The French Blood Agency, Haemovigilance Surveillance System, from 1994 to March 1998, has attributed 18 deaths to blood components contaminated with

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bacteria (Morel, 1999). In the United States, from 1986 to 1991, bacterial contamination accounted for 15·9% of all transfusion-related fatalities (Hoppe, 1992). The UK surveillance system SHOT (Serious Hazards of Transfusion) between 1995 and 2000 reported 15 incidents, including four fatalities owing to bacterial contamination of blood components (Love *et al*., 2001).

Platelets are responsible for most of the bacterial transmissions to recipients. In a retrospective monitoring study performed by the National Bacteriology Laboratory on English blood components, the bacterial contamination rate was found to be 0·5% (one in 191) in pooled (pool size = four donations) and 0·2% (one in 436) in apheresis platelet concentrates (McDonald *et al*., 2000).

In UK, no routine screening of platelets is currently undertaken for bacteria. The BacT/ALERT 3D automated blood culture system (bioMérieux formerly Organon Teknika, Cambridge, UK) has the potential to allow the screening of platelets for the presence of bacteria. Evaluation was performed by spiking units with individual isolates at 10 and 100 colony-forming units (cfu) mL^{-1}. Fifteen organisms were used which had been cited in platelet concentrate transmission and in monitoring studies (Goldman & Blajchman, 1991; Sazama, 1994; Wagner *et al*., 1994; McDonald *et al*., 1998). BacT/ALERT detection times were compared with thioglycollate broth culture, and the performance of five types of BacT/ALERT culture bottles was evaluated.

MATERIALS AND METHODS

Fifteen organisms were used, comprising nine gram-positive bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Clostridium perfringens*, *Streptococcus pyogenes*, *Streptococcus viridans* group, *Propionibacterium acnes* and *Corynebacterium* sp.), five gram-negative organisms (*Escherichia coli*, *Klebsiella oxytoca*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*) and one fungal organism (*Candida albicans*). Standard aerobic, standard anaerobic, fan aerobic, fan anaerobic and Pedi-BacT (paediatric aerobic) BacT/ALERT (bioMérieux formerly Organon Teknika) bottles were tested in comparison with the thioglycollate broth culture (Cherwell Laboratories, Bicester, UK). The medium used in standard bottles was tryptic soya broth. Paediatric and fan bottles were filled with brain heart infusion broth.

Apheresis platelets (Cobe Leucocyte Reduction System™ – Cobe Laboratories, Quedgeley, Gloucestershire, UK) at day 2 after collection were inoculated with the above organisms to reach target pack concentrations of 10 and 100 cfu mL^{-1}. Inoculation and any subsequent sampling was performed using hypodermic syringes through a sterile sampling device (Baxter Health Care, Newbury, Berks, UK), which was placed into a port of platelet bags. The mean white cell count was $0{\cdot}3 \times 10^6$ per unit and the platelet count 331×10^9 per unit. Prior to the inoculation, each platelet unit was tested to confirm the absence of pre-existing bacteria by inoculating 100 mL on two Columbia blood agar plates (Mast Diagnostics, Bootle, UK) and incubating individual plates aerobically and anaerobically for 48 h at 37 °C. In duplicate, standard aerobic/anaerobic and fan aerobic/anaerobic bottles were inoculated with 4 mL, Pedi-BacT bottles with 2 mL and thioglycollate bottles with 1 mL of platelet concentrate. This resulted in a one in 10 dilution of the platelet sample in each bottle and served as negative controls.

Inocula were prepared using a 0·5 McFarland standard (approximately 10^8 cfu mL^{-1}) in sterile saline with 10-fold serial dilutions to achieve the requisite inoculum level. The inocula concentrations were quantified in duplicate by direct plating of 100 mL onto Columbia blood agar plates, incubating them under the appropriate conditions and enumerating the colonies after 48 h of incubation at 37 °C.

Post inoculation, the platelet bags were agitated on a platelet shaker for 10 min. Quantification of the bacterial contents was performed on the platelet bags as in the enumeration of the inoculum concentration (see above). The contents of the inoculated platelet bags were also inoculated into the BacT/ALERT bottles and thioglycollate broth bottles, as described, with the negative controls, except that 10 replicates were tested, rather than the duplicates. Again, a one in 10 dilution of platelet concentration to broth in each test system was maintained.

Inoculated BacT/ALERT bottles were loaded onto the operating system and incubated for up to 14 days at 37 °C. Bottles were removed when flagged positive by the system or on reaching the 14-day incubation end point, at which time they were deemed to be negative. Bottles which were flagged culture-positive were gram stained and subcultured to confirm that the bacterial growth identified was the seeded organism. Two culture bottles which were flagged as positive from each replicate were identified. All negative bottles were gram stained and subcultured at the end of the incubation period. Any growth occurring from

any of these initially negative BacT/ALERT bottles was identified.

The thioglycollate broth bottles were sampled, gram stained and subcultured at time zero and then every 12 h until positive cultures were obtained, throughout the 14-day incubation period. Broths were considered positive when at least one organism was observed in the gram stain. Culture-positive and -negative bottles were processed as for the BacT/ALERT bottles (see above).

RESULTS

At the intended 10 cfu mL^{-1} inoculation level, the mean concentration of the bacterial inocula injected was 19 cfu mL^{-1} (standard deviation, ±9·7), and the mean concentration of bacteria recovered immediately post inoculation was 22 cfu mL^{-1} (standard deviation, ±12·4). For the intended 100 cfu mL^{-1} inoculum, the mean concentration of the bacterial inocula injected was 188 cfu mL^{-1} (standard deviation, ±97·0), and the mean concentration of bacteria recovered immediately post inoculation was 233 cfu mL^{-1} (standard deviation, ±90·1).

As expected, *Corynebacterium* sp. and *P. aeruginosa* failed to grow in standard anaerobic and fan anaerobic bottles. In the thioglycollate broth with *Corynebacterium* sp. at the 10 cfu mL^{-1} inoculum, growth was detected in six of 10 replicates and at the 100 cfu mL^{-1} level growth was detected in four of 10 replicates. Also as expected, *B. subtilis* failed to grow in fan anaerobic bottles, but growth in eight of 10 replicates at the 10 cfu mL^{-1} inoculum and in seven of 10 replicates at 100 cfu mL^{-1} was obtained with the standard anaerobic bottles. Growth was obtained in nine of 10 replicates in standard aerobic bottles at the 10 cfu mL^{-1} inoculum. In the thioglycollate broth, *B. subtilis* grew in only three of 10 replicates at the 10 cfu mL^{-1} inoculum and at 100 cfu mL^{-1} in 10 of 10 replicates. *C. perfringens* was detected in four of 10 paediatric bottles at the 10 cfu mL^{-1} and two of 10 bottles at the 100 cfu mL^{-1} inoculum. Fan aerobic bottles gave no growth with *C. perfringens*. No growth was obtained in the standard aerobic bottles at the 10 cfu mL^{-1} inoculum and in one replicate at 100 cfu mL^{-1}. *P. acnes* was detected six of 10 times in paediatric bottles at the 10 cfu mL^{-1} and 10 of 10 bottles at the 100 cfu mL^{-1} level. The standard aerobic bottles at the 10 cfu mL^{-1} inoculum produced no growth with *P. acnes* and growth was seen in only seven of 10 replicates using the standard anaerobic bottles at the 100 cfu mL^{-1} inoculum. No growth with *P. acnes* occurred in fan aerobic or anaerobic bottles. *C. albicans* gave no growth with fan anaerobic bottles and no growth and false-negatives with the standard anaerobic bottles at 10 cfu mL^{-1} (growth was obtained on terminal subculture of the bottles). At the 100 cfu mL^{-1} inoculum level with standard anaerobic bottles, growth was obtained in three of 10 replicates. All organisms/concentrations not mentioned above gave 100% growth with all replicates (Table 1). No growth was obtained with any of the negative controls.

BacT/ALERT mean detection times, with the exception of *P. acnes*, ranged from 9·1 to 48·1 h overall (all replicates positive – Table 1). In comparison, the thioglycollate mean detection time ranged from 12·0 to 32·3 h (all replicates positive – Table 1). At the 10 cfu mL^{-1} inoculum level, the mean detection times for the BacT/ALERT bottles ranged from 9·6 to 48·1 h (Fig. 1), and at the 100 cfu mL^{-1} level, the mean detection time range was 9·1–35·4 h, excluding *P. acnes* (all replicates positive – Fig. 2). The thioglycollate mean time to detection at the 10 cfu mL^{-1} inoculum level ranged from 12·0 to 32·3 h and at the 100 cfu mL^{-1} inoculum, 12·0–26·0 h, excluding *P. acnes* (all replicates positive). *P. acnes*'s mean detection times with the BacT/ALERT bottles ranged from 89·0 to 177·6 h compared with 75·6–86·4 h when using the thioglycollate broth.

Differences in the mean detection times between the 10 and 100 cfu mL^{-1} inoculum were 8·6% for standard aerobic, 6·3% for standard anaerobic, 8·9% for fan aerobic, 8·1% for fan anaerobic, 12·2% for paediatric and 10·5% for the thioglycollate broth. Overall, a 1 log greater inoculum resulted in more rapid times to detection.

DISCUSSION

Our data indicate that the paediatric bottles are the preferred bottle for aerobic culture, detecting growth in 100% of such cultures and even in some of the replicates of anaerobic organisms (*P. acnes* and *C. perfringens*). In Lund, Sweden (University Hospital, Blood Centre), the paediatric bottle alone is used for screening (U. Johnson, personal communication). A one-bottle system, as opposed to a two-bottle system, has the advantage of increasing the capacity on the BacT/ALERT system and also reduces logistical problems with regard to inoculation and labelling; obviously costs would also reduce. The paediatric bottle used for this study was inoculated with 2 mL compared with 4 mL for the other bottle types, thus increasing the volume available for transfusion of this clinical product. Problems with gram staining were

Table 1. Mean detection times (hours) of the BacT/ALERT bottles and thioglycollate broth

		Mean detection time (hours)											
		Standard aerobic		Standard anaerobic		Fan aerobic		Fan anaerobic		Paediatric aerobic		Thioglycollate	
Organism	ATCC number	10 cfu mL^{-1}	100 cfu mL^{-1}	10 cfu mL^{-1}	100 cfu mL^{-1}	10 cfu mL^{-1}	100 cfu mL^{-1}	10 cfu mL^{-1}	100 cfu mL^{-1}	10 cfu mL^{-1}	100 cfu mL^{-1}	10 cfu mL^{-1}	100 cfu mL^{-1}
S. epidermidis	49 134	17·7	15·6	48·1	26·8	21·4	19·9	24·8	22·3	17·8	15·7	28·7	23·5
S. aureus	27 217	11·9	11·1	14·8	13·8	14·2	12·6	16·4	15·4	12·2	11·3	19·4	13·7
B. subtilis	6633	13·6	12·4	110·9†	48·6†	13·7	12·8	NG	NG	13·5	12·5	76·2†	12·5
B. cereus	11 772	9·9	9·5	11·9	11·1	10·0	9·5	12·1	11·6	9·6	9·1	12·0	12·0
S. pyogenes	19 615	17·9	15·2	14·6	14·5	16·4	15·1	16·7	14·2	15·0	13·8	24·0	24·0
S. viridans group	Wild type	40·2	35·0	24·2	22·6	18·1	16·4	20·0	18·4	19·5	18·0	29·8	26·0
Corynebacterium sp.	Wild type	24·0	7	NG	NG	37·3	35·4	NG	NG	25·8	21·3	87·0†	111·0†
E. coli	25 922	11·1	10·3	10·8	10·1	11·3	10·5	10·8	10·0	11·1	10·2	14·0	14·0
K. oxytoca	Wild type	11·7	10·6	11·8	10·9	12·4	11·2	11·9	10·9	11·8	10·7	13·0	13·0
S. marcescens	43 862	11·6	10·4	12·9	11·5	12·2	11·0	13·0	11·7	11·9	10·7	13·0	13·0
P. aeruginosa	27 853	16·2	14·5	NG	NG	16·9	15·0	NG	NG	16·0	14·6	24·0	24·0
E. cloacae	Wild type	10·9	10·5	11·5	11·0	11·9	11·3	11·5	11·0	11·0	10·3	12·5	12·5
C. perfringens	13 124	NG	14·9†	10·9	10·3	NG	NG	10·9	10·1	18·7†	12·7†	22·0	20·1
P. acnes	11 827	NG	163·7	89·0	104·9†	NG	NG	NG	NG	177·6†	126·0	86·4	75·6
C. albicans	10 231	23·0	19·2	NG‡	19·0†‡	34·2	27·3	NG	NG	23·1	19·9	32·3	26·0

*ATCC, American Type Culture Collection;
NG, no growth;
†Not all bottles in the set were positive.
‡False-negatives were found on terminal subculture.

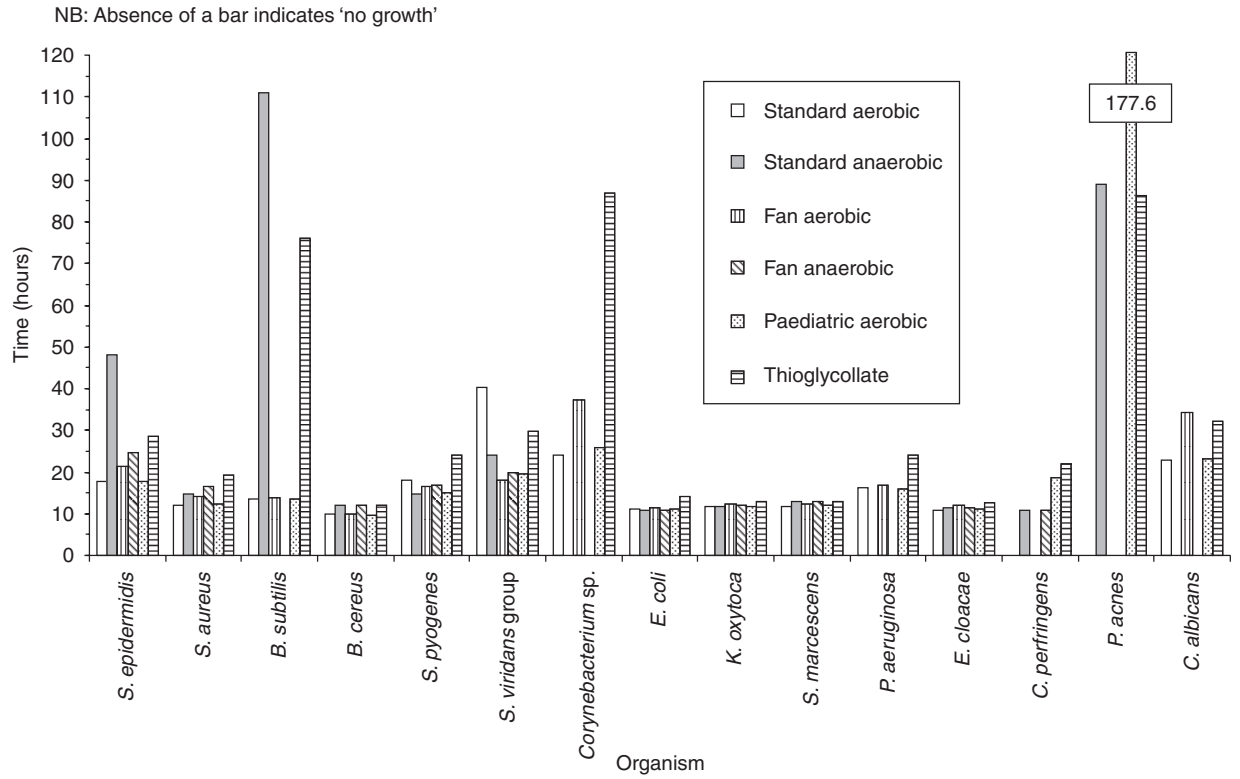

Fig. 1. Mean time to detection in the BacT/ALERT bottles and thioglycollate broth from an initial inoculum of 10 cfu mL^{-1}.

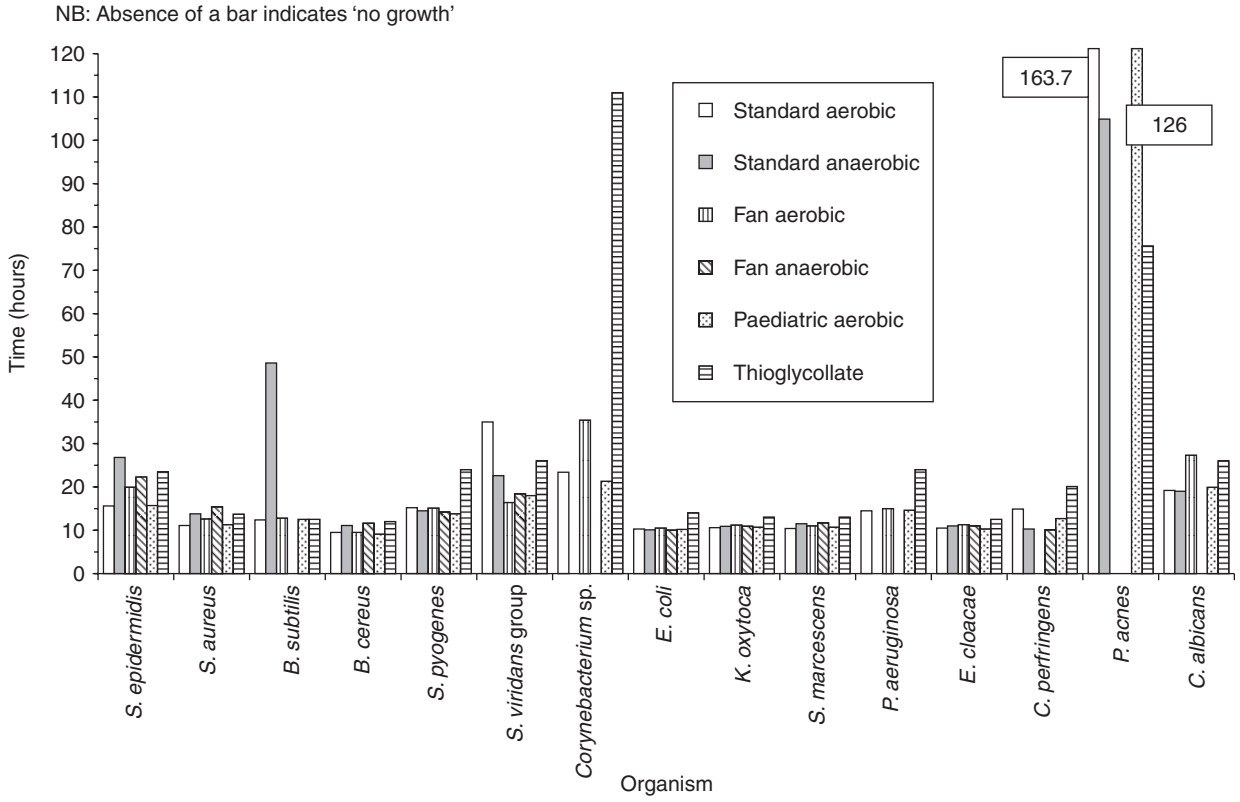

Fig. 2. Mean time to detection in the BacT/ALERT bottles and thioglycollate broth from an initial inoculum of 100 cfu mL^{-1}.

found with fan aerobic and anaerobic bottles owing to the dark colouration of the activated charcoal used to absorb antibiotics. Interestingly, the fan anaerobic bottle did not detect *P. acnes*. The preferred anaerobic bottle was the standard one, although growth was not detected in all 10 bottles inoculated with *P. acnes* at the 100 cfu mL^{-1} level. Opinion as to the clinical significance of *P. acnes* in a blood transfusion context is divided.

A critical factor in the screening of platelet concentrates is the sampling time. Bacteria, unlike viruses, can continue to replicate in stored platelet concentrates. Our own experiments have shown that after spiking the platelet concentrates with the inocula of between 2 and 10 cfu mL^{-1} at day 0 of platelet shelf-life, by day 2, the bacterial number was 10^5 cfu mL^{-1} or greater (McDonald *et al*., 1999). It has been postulated that the level of bacterial contamination found in the whole blood would be in the order of <10 cfu mL^{-1} (Wagner *et al*., 1995). Therefore, it seems prudent to allow an incubation period pre inoculation for the bacterial concentration to increase to a level sufficient enough to fall within the detection range of the BacT/ALERT system and not to test for bacteria on day 0 of platelet shelf-life. Wagner and Robinette in their evaluation of the BacT/ALERT system concluded that sampling times of 24 h or more post preparation would be necessary to provide the confidence of detecting *E. coli* and *S. epidermidis* (Wagner & Robinette, 1998). Blajchman *et al*. performed a prospective study using an automated blood culture system in which the platelet units were screened at day 1 and also at day 3. On days 1 and 3, four units were found to be positive, with an additional three units (43%) found to be positive on day 3 only (Blajchman *et al*., 1996). This provides further evidence that an incubation period is required prior to sampling, in order to increase the probability of detection, potentially reducing the incubation times of the inoculated bottles. Interestingly, the Hong Kong Red Cross Transfusion Service are screening platelets using the BacT/ALERT system at day 2 after collection with release after 24 h, but continuing monitoring for a further 24 h (Liu *et al*., 1999). In this system, five donations are pooled per aerobic culture bottles, which increases the testing capacity of the BacT/ALERT system. A 1–1·2 mL inoculum per platelet concentrate is introduced into culture bottles. In our study, the BacT/ALERT system detected inocula with a concentration of 10 cfu mL^{-1} or greater. Theoretically, the system will detect one viable organism if inoculated into a BacT/ALERT culture bottle.

In USA in 1983, the platelet concentrate shelf-life was extended from 5 to 7 days (Simon *et al*., 1983), with a resulting increase in reactions owing to bacterially contaminated platelet concentrates. In 1983 alone, four fatal transfusion reactions were reported to the Food and Drug Administration (FDA) (Heal *et al*., 1987). In response, in 1986, the FDA reduced the storage time back to 5 days (Heal *et al*., 1987; Punsalang *et al*., 1989). If bacterial screening is undertaken using the BacT/ALERT, it might be possible to return the shelf-life to 7 days, reducing the number of time-expired units, thereby alleviating supply problems and also reducing overall costs. Shelf-life has been currently increased in Lund in Sweden (University Hospital, Blood Centre) to 6 days, by screening at day 3 (U. Johnson, personal communication). The testing costs are recouped, and additional cost savings are generated owing to the reduction in time-expired platelet units.

In conclusion, the study validates the BacT/ALERT microbial detection system for the screening of platelet concentrates. The BacT/ALERT system gave equivalent or reduced detection times compared with the thioglycollate broth system, with the exception of *P. acnes*. BacT/ALERT was able to detect a range of organisms at the inoculum levels of 10 and 100 cfu mL^{-1}. Currently, the system is the only practical option for routine screening to detect bacterial contamination and avoid transmission. The BacT/ALERT system could enable large numbers of platelets to be screened, with specific sample identification, affordable labour costs, electronic transfer of data and rapid detection times. This is one option that merits serious consideration as part of possible strategies to significantly reduce bacterial transmission by transfusion.

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