Prevention of transfusion of platelet components contaminated with low levels of bacteria: a comparison of bacteria culture and pathogen inactivation methods

Walter Nussbaumer, Doris Allersdorfer, Christoph Grabmer, Margaret Rheinschmidt, Lily Lin, Diether Schönitzer, and Cornelia Lass-Flörl

BACKGROUND: This study compared the efficacy of bacterial detection with inactivation for reducing the risk associated with transfusion of platelet (PLT) components contaminated with low levels of bacteria.

STUDY DESIGN AND METHODS: Twenty-one double-dose PLTs were spiked with seven species of bacteria at three levels (0.003-0.03, 0.03-0.3, 0.3-3 colony-forming units [CFUs]/mL). After split, each PLT unit contained 1 to 10, 10 to 100, and 100 to 1000 CFUs. One unit was photochemically treated (PCT; 150 µmol/L amotosalen and 3 J/cm^2 ultraviolet A). The other unit was untreated. All units were stored and sampled on Days 1, 2, and 5 of storage for aerobic and anaerobic culture in the BacT/ALERT system (bioMérieux). PLTs were classified as sterile when no bacterial growth was detected after 120 hours of culture.

RESULTS: In all PCT PLTs, no bacteria were detected throughout 5 days of storage regardless of species, level of contamination, and sampling time. In untreated PLTs, *Staphylococcus aureus* was consistently detected by culturing. Growth of 1 to 10 CFUs per unit *Staphylococcus epidermidis*, 1 to 100 CFUs per unit of *Klebsiella pneumoniae*, and 1 to 1000 CFUs per unit *Propionibacterium acnes* was delayed and only detectable after 5, 2, and 5 days of storage, respectively. Low levels of *Streptococcus agalactiae* (1-10 CFUs/unit), *Escherichia coli* (1-100 CFUs/unit), and *Clostridium perfringens* (1-100 CFUs/unit) were not detected during 5 days of storage, although bacterial outgrowth was detected at higher levels of contamination.

CONCLUSIONS: For the seven bacterial species examined, contaminated PLTs may be released for transfusion on test-negative-to-date status. In contrast, bacterial inactivation by PCT could reduce the risk associated with transfusion of PLTs contaminated with low levels of these bacteria.

Bacterial contamination of platelet (PLT) components is the leading cause of transfusion-transmitted infection and is associated with significant morbidity and mortality.[1-4] During donation and preparation, PLT components may be contaminated with low levels of bacteria. Because PLT components are stored under conditions that favor bacterial growth, the small number of bacteria may proliferate to very high levels upon prolonged storage.[5,6] Importantly, the incidence of nonfatal transfusion-transmitted sepsis associated with substantial morbidity may be underdiagnosed in neutropenic patients receiving repeated PLT transfusions during ablative chemotherapy.[7]

The safety of PLT transfusions could be improved if blood donation services could prevent the release of bacterially contaminated PLT components or inactivate bacteria in contaminated PLT components after collection before transfusion. Blood centers have instituted rigorous donor screening, improved skin disinfection, and use of blood diversion devices. Recently, two additional approaches have been taken: bacterial detection and pathogen inactivation treatment of PLT components.[5,6]

ABBREVIATION: PCT = photochemical treatment.

From the Department of Transfusion Medicine and the Institute for Hygiene and Social Medicine, University of Innsbruck, Innsbruck, Austria; and Cerus Corp., Concord, California.

Address reprint requests to: Walter Nussbaumer, MD, Head of Apheresis Division, Department of Transfusion Medicine, University Hospital Innsbruck, Anichstrasse 35, AT-6020 Innsbruck, Austria; e-mail: walter.nussbaumer@chello.at.

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The BacT/ALERT system (bioMérieux, Vienna, Austria) for detecting bacteria in PLT components before release has been widely used. BacT/ALERT has been shown to detect 10 colony-forming units (CFUs) per mL and for some species BACTEC 9240 can detect 5 CFUs per mL.[4,6,8,9] These systems allow for the culture of aerobic and/or anaerobic organisms with continuous monitoring during the approved storage shelf life of the PLT component. Cumulative data from several centers in Europe with 2 to 4 years of routine use have shown that the bacterial testing approach prevented the release of approximately 50 percent of contaminated PLT units.[10,11] In the United States, the American Red Cross found that the rate of probable posttransfusion sepsis cases was reduced by 75 percent after the implementation of aerobic culture.[12]

The INTERCEPT Blood System (Baxter Healthcare Corp., Lessines, Belgium) for pathogen inactivation of PLT components received the CE Mark in 2002 and is currently in clinical use in several countries in Europe. By use of this system, pathogens are inactivated by photochemical treatment (PCT) with a combination of amotosalen and ultraviolet A (UVA) light.[13] A broad range of bacteria in PLT components has been shown to be sensitive to PCT and high levels of inactivation have been demonstrated.[14]

In clinical practice, bacterial contamination of blood components at time of collection and preparation is presumed to be due to low levels of bacteria from skin contamination or asymptomatic donor bacteremia. Few studies have been specifically designed to evaluate detection of low levels of bacteria. This study was designed specifically to replicate low-level bacterial contamination in PLT concentrates that may occur during donation and preparation and to compare the efficacy of bacterial detection and pathogen inactivation to prevent the release of contaminated PLT components. This study provides a controlled comparison of these two methods and examines the characteristics of bacterial proliferation in PLT components due to contamination from low numbers of bacteria.

MATERIALS AND METHODS

PLT collection

Double-dose apheresis PLT components were collected from normal volunteer donors with a blood cell separator (Amicus, Baxter Healthcare Corp., Deerfield, IL). PLT components were in-process leukoreduced to levels of fewer than 10^6 white blood cells during collection. Each PLT collection consisted of approximately 6×10^{11} PLTs suspended in 570 mL of 37 percent plasma and 63 percent Intersol (PLT additive solution, Baxter Healthcare Corp., La Châtre, France). A 10-mL sample was withdrawn through a sampling site coupler from each PLT collection before bacterial seeding. Approximately 4 to 5 mL each was inoculated into aerobic and anaerobic culture bottles and cultured to confirm baseline sterility of all units. All samples taken from PLT components were collected with aseptic techniques.

Bacterial species and preparation of bacterial inocula

Seven bacterial strains, obtained from ATCC (Rockville, MD) or from clinical isolates, were used to inoculate the double-dose PLT components. The strains used were *Staphylococcus aureus, Staphylococcus epidermidis* (ATCC 29886)*, Streptococcus agalactiae, Klebsiella pneumoniae, Escherichia coli, Clostridium perfringens*, and *Propionibacterium acnes* (Table 1). These strains were chosen to represent a variety of the most common contaminants (Gram-positive, Gram-negative, aerobic, and anaerobic), "fast-growing"[15,16] and "slow-growing,"[15,17] or because they were reported as contaminants of PLT concentrate units associated with transfusion-transmitted sepsis.[18-20] *C. perfringens* was selected because it is a spore-forming species and it has been shown to contaminate PLT concentrates.[20] All of these bacterial strains have been shown to be resistant to the bactericidal effects of plasma and have been shown to grow in plasma.

Each bacterial inoculum was prepared by suspending a single colony from an overnight blood-agar culture into approximately 5 mL of nutrient broth. Serial twofold dilutions were performed from these suspensions and bacteria were counted with a hemocytometer. The bacterial concentration was adjusted to the desired inoculum concentration with nutrient broth. In addition, the viability and final inoculum dose was confirmed from quantitative cultures of duplicate aliquots of each inoculum by colony counting of serial dilutions placed on agar plates.

TABLE 1. Characteristics and confirmed bacterial doses inoculated into double-dose PLT components*

Bacterium	Gram stain	Confirmed contamination dose for the target dose of 2-20 CFUs	20-200 CFUs	200-2000 CFUs
S. aureus	+	5	44	1000
S. epidermidis	+	10	127	1200
S. agalactiae	+	2	19	300
K. pneumoniae	–	4	50	250
E. coli	–	10	71	250
P. acnes†	+	12	61	Not measured
C. perfringens‡	+	3	16	500

* Contamination doses reflect the number of viable bacteria inoculated into each double PLT unit. The number of CFUs was measured by direct culture of the inoculum on to culture plates.
† Facultative anaerobe.
‡ Anaerobe.

Seeding of bacteria into PLT components

Immediately after PLT collection (defined as Day 0), aliquots of each bacterial strain were inoculated into individual double-dose PLT components at three different concentrations (2-20, 20-200, and 200-2000 CFUs/570 mL) to yield final bacteria concentrations ranging from approximately 0.003 to 3 CFUs per mL. Inoculation of PLT components was performed through sampling site couplers. After the bacteria were seeded into the PLTs, each double unit was divided equally into two containers with the containers connected. The PLT units were allowed to rest without agitation for 2 hours followed by agitation for 2 additional hours. After the 4-hour rest period, PLT concentrate in the two containers was again pooled into one container and mixed well to ensure even distribution of bacteria. After mixing, the contaminated double dose of PLT was split into two single doses of approximately 285 mL each with the containers separated.

Conventional treatment with bacterial detection

One of each paired contaminated PLT units received no further treatment and was stored in a PLT shaker incubator with agitation (Helmer Laboratories, Noblesville, IN) at 22 ± 2°C for up to 5 days. Bacterial growth was monitored as described below with the BacT/ALERT system.

Inactivation of bacteria by PCT

The corresponding paired contaminated PLT units were stored with agitation at 22 ± 2°C overnight before inactivation treatment. This holding period facilitated germination of spores and growth of the relevant bacteria. PCT involved the following steps. The PLT unit was connected to the PCT disposable set (Code R5628B, Baxter Healthcare Corp.) with a sterile weld. PLTs were transferred through a small pouch containing 15 mL of a 3 mmol per L amotosalen solution into an illumination container resulting in a final concentration of approximately 150 µmol per L amotosalen.[14] The PLT mixture was illuminated with a 3 J per cm^2 UVA (320-400 nm) treatment in a UVA Illuminator (R4R4007, Baxter Healthcare Corp.). After treatment, the unit was stored with agitation at 22 ± 2°C for up to 5 days. Bacterial growth was monitored in parallel with the paired untreated PLT unit as described below with the BacT/ALERT system.

Detection of contamination with bacterial culture and release criteria for transfusion

The day of PLT collection was designated as Day 0, and all sampling and storage times were based on this time scale. Bacterial spiking was performed within 3 hours after PLT collection. On Days 1, 2, and 5 of storage (correspond to 24 ± 2, 48 ± 2, and 120 ± 2 hr after bacterial spiking, respectively), approximately 10 mL of PLT concentrate was removed from each PLT container with aseptic technique in a laminar airflow cabinet. Aerobic and anaerobic culture bottles were inoculated with 4 to 5 mL of PLT concentrate each. The anaerobic bottle was inoculated first and then the aerobic bottle. Bacterial growth was assessed with the automated liquid media culture system BacT/ALERT 3D 60 (bioMérieux). Bottles were incubated in the BacT/ALERT at 35°C up to 120 hours after inoculation or until a positive signal was recorded. The amount of time required to detect bacterial contamination, when detectable, was recorded. PLT samples were considered free of bacteria (or sterile) if a negative signal was registered after 120 hours of incubation in the BacT/ALERT system. For positive signals detected in the PCT units, morphologic identification and Gram stain were performed to confirm the characteristics and morphologic consistency of the isolate with the initial inoculum. In clinical practice, because PLT components could outdate in less than or equal to 120 hours after inoculation of the culture, PLT components are released with a test-negative-to-date status.

RESULTS

Initial PLT sterility

Twenty-one double-dose PLT components (6×10^{11} PLTs in 570 mL) were used in this study evaluating the growth of seven bacterial species each at three contamination levels. The PLT samples taken from each double-dose PLT component before bacterial inoculation remained negative throughout the course of the study, confirming that all units were sterile before the inoculation of study bacteria.

Characteristics of bacterial contaminants

The target levels of bacterial contamination in the double-dose PLT components were 2 to 20, 20 to 200, and 200 to 2000 CFUs in 570 mL. The actual bacterial dose inoculated into each double-dose component was determined by quantitative culture analysis by colony counting of serial dilutions placed on agar plates and found to be within the target range with two exceptions (Table 1). Only 19 CFUs of *S. agalactiae* and 16 CFUs of *C. perfringens* were seeded in the 20 to 200 CFU target dose units. After the contaminated double-dose component was divided into two identical units, each single-dose unit (3×10^{11} PLTs in 285 mL) contained 1 to 10, 10 to 100, and 100 to 1000 CFUs of bacteria, equivalent to 0.003 to 0.03, 0.03 to 0.3, and 0.3 to 3 CFUs per mL, respectively.

Bacterial detection efficacy

In the untreated units, the length of culture time until growth was detected varied depending on the species, the

TABLE 2. Detection of positive aerobic and anaerobic cultures in BacT/ALERT system of bacterially contaminated and untreated PLT components*

	Bacterial contamination level on D0:	Time to positive signal (hr)†								
		1-10 CFUs/unit			10-100 CFUs/unit			100-1000 CFUs/unit		
	PLT sampling date:	D1	D2	D5	D1	D2	D5	D1	D2	D5
S. aureus	Aerobic	14	6	4	11	5	4	7	4	4
	Anaerobic	16	6	4	12	6	4	7	4	4
S. epidermidis	Aerobic	Negative‡	Negative	Negative	50	32	5	16	ND§	ND
	Anaerobic	Negative	Negative	33	36	27	7	17	ND	ND
S. agalactiae	Aerobic	Negative	Negative	Negative	Negative	8	4	8	5	4
	Anaerobic	Negative	Negative	Negative	13	8	4	8	5	4
K. pneumoniae	Aerobic	Negative	9	4	Negative	7	4	8	4	4
	Anaerobic	Negative	8	4	Negative	7	4	8	4	4
E. coli	Aerobic	Negative	Negative	Negative	Negative	Negative	Negative	4	4	4
	Anaerobic	Negative	Negative	Negative	Negative	Negative	Negative	4	4	4
P. acnes	Aerobic	Negative	Negative	Negative	Negative	Negative	Negative	Negative‖	Negative	
	Anaerobic	Negative	Negative	53	Negative	Negative	50	Negative‖	53	
C. perfringens	Aerobic	Negative	Negative	Negative	Negative	Negative	Negative	11	7	5
	Anaerobic	Negative	Negative	Negative	Negative	Negative	Negative	Negative	9	5

* PLT components were contaminated on day of collection (Day 0) at the specified range of CFUs per unit and stored for 5 days. Samples were withdrawn for culture on Days 1, 2, and 5 of storage. All aerobic and anaerobic cultures in the BacT/ALERT system were automatically monitored and the time to a positive signal was recorded in hours after inoculation. The inoculated sample was classified as test-negative if a positive signal had not been recorded after 120 h of culture. D = day of PLT storage the culture sample was withdrawn.
† Number of hours after inoculation into culture bottles when a positive signal was recorded in the BacT/ALERT system.
‡ Negative = no bacterial growth was detected after 120 hours of culture in the BacT/ALERT system.
§ ND = not done, culture sample not prepared.
‖ These samples were cultured on the day of collection (Day 0).

dose, and the sampling time (Table 2). At contamination levels of 1 to 10 CFUs per unit, *S. aureus* was consistently detected when the PLT unit was sampled on Days 1, 2, and 5. *K. pneumoniae* was test-negative in the PLT sample obtained on Day 1, but readily detected in the PLT samples obtained on Days 2 and 5. *S. epidermidis* and *P. acnes* were not detected when PLT units were sampled on Days 1 and 2, but these units grew out the inoculated bacteria when sampled on Day 5. The units seeded with *S. agalactiae, E. coli,* and *C. perfringens* were test-negative after 120 hours of culture with all PLT samples obtained on Days 1, 2, and 5.

At contamination levels of 10 to 100 CFUs per unit, *S. aureus* was consistently detected. *K. pneumoniae* was test-negative on Day 1 but readily detectable on Days 2 and 5. Growth of *S. epidermidis* and *S. agalactiae* was delayed in the Day 1 PLT sample but readily detected in the Day 5 PLT sample. *P. acnes* was not detected when the PLT unit was sampled on Days 1 and 2, even though this unit grew out the inoculated bacteria when sampled on Day 5. The units seeded with *E. coli* and *C. perfringens* were test-negative after 120 hours of culture with all PLT samples obtained during the 5 days of storage.

At contamination levels of 100 to 1000 CFUs per unit, the Day 1 cultures became positive readily for *S. aureus*, *S. agalactiae*, *K. pneumoniae*, and *E. coli* and positive with a slight delay for *S. epidermidis*, but were negative for the anaerobic bottle of *C. perfringens* and negative for *P. acnes*. All bacterial species, however, were detected in the samples withdrawn and cultured on Day 5 of storage.

Time to first positive bacterial detection and relevance to PLT product release

To simulate blood bank practices and logistics for the use of bacterial culture to release PLT products, the time from inoculation immediately after completion of PLT preparation to the first positive culture result (aerobic or anaerobic) was determined (Table 3). For each bacteria species and level of contamination, the PLT units that would have been released as "test-negative to date" were identified based on the time to the first positive result with a threshold of 48 hours as the minimal time to release.

At contamination levels of 1 to 10 CFUs per unit, the time to first positive culture for *S. aureus* was 14 hours for the aerobic culture inoculated on Day 1 or 38 hours (24 + 14) from the time of donation. The time to first positive culture for *K. pneumoniae* was 8 hours after sampling on Day 2 of storage or 56 h (48 + 8) from donation. *S. epidermidis* and *P. acnes* were first detected in samples withdrawn on Day 5 and only in anaerobe cultures or 153 (120 + 33) and 173 hours (120 + 53) from donation, respectively. Therefore, if the PLT products were released for transfusion on Day 2 of storage, or 48 hours after donation as customary in many blood centers, units containing viable *S. aureus* would have been detected and prevented from release but units containing viable *S. epidermidis*, *K. pneumoniae,* and *P. acnes* would have been released for transfusion on a test-negative-to-date status.

At contamination levels of 10 to 100 CFUs per unit, PLT products contaminated with *S. aureus*, *S. epidermidis*, and *S. agalactiae* were detected in samples withdrawn

TABLE 3. Time to earliest detection of bacterial growth of contaminated and untreated PLT components taking the sampling time into account

Bacterium	Bacterial contamination level on D0:	Time to earliest bacterial detection (hr)*		
		1-10 CFUs/unit	10-100 CFUs/unit	100-1000 CFUs/unit
S. aureus		38†	35†	31†
S. epidermidis		153	60	40†
S. agalactiae		No growth	37	32†
K. pneumoniae		56	55†	32†
E. coli		No growth	No growth	28†
P. acnes		173	170	173
C. perfringens		No growth	No growth	35†
Number of contaminated units test-negative-to-date status at 48 hr‖		6‡	5§	1

* PLT components were contaminated on day of collection (Day 0) at the specified range of CFUs per unit and stored for 5 days. Samples were withdrawn for culture on Days 1, 2, and 5 of storage. All aerobic and anaerobic cultures in the BacT/ALERT system were automatically monitored for 120 hours after inoculation. No growth indicated that a positive signal was not recorded after 120 hours of culture. Time to earliest detection was the time lapse in hours between the time of PLT collection or bacterial contamination and the first detection of a positive bacterial culture in BacT/ALERT.

† Indicates species that could be detected by aerobic culture alone. Not indicated were species that were detected first by anaerobic culture or only by anaerobic culture.

‡ Of the 6 units with test-negative-to-date status at 48 hours after donation, only 3 units (*S. epidermidis*, *K. pneumoniae,* and *P. acnes*) have been shown to contain viable bacteria.

§ Of the 5 units with test-negative-to-date status at 48 hours after donation, only 3 (*S. epidermidis*, *K. pneumoniae*, and *P. acnes*) have been shown to contain viable bacteria.

‖ The number of PLT units that would have been released at 48 hours after donation as test-negative-to date status.

for culture on Day 1 of storage. The times to first positive culture were 35, 60, and 37 hours after donation, respectively (Table 3). PLT products contaminated with *K. pneumoniae* were first detected in bacterial cultures taken on Day 2 of storage or 55 hours (48 + 7) from donation. PLT units contaminated with *P. acnes* were first detected in samples withdrawn on Day 5 of storage and only after 50 hours of anaerobe culture or 170 hours (120 + 50) from donation. Therefore, if these PLT products were released for transfusion on Day 2 of storage, or 48 hours after donation, units containing viable *S. aureus* and *S. agalactiae* would have been detected and discarded, but units containing viable *S. epidermidis*, *K. pneumoniae*, and *P. acnes* would have been released for transfusion on test-negative-to-date status.

At contamination levels of 100 to 1000 CFUs per unit, with the exception of *P. acnes*, bacterial growth was detected in all contaminated PLT units on cultures taken on Day 1 of storage. The times to first positive culture results were between 28 and 40 hours from time of donation. *P. acnes* was detected in the Day 5 anaerobic culture after 53 hours of incubation or 173 hours (120 + 53) from donation. Therefore, for PLT products released for transfusion on Day 2, or 48 hours after donation, these units containing viable *P. acnes* would have been released as test-negative-to-date status.

Pathogen inactivation

All units seeded with bacteria and treated with PCT for bacterial inactivation had no detectable aerobic or anaerobic bacteria during the entire storage period. No bacteria were detected at any level of contamination after 120 hours of culture regardless of the species, doses, and sampling time. Morphologic identification and Gram stain were not required for any of these units based on the study protocol.

DISCUSSION

The purpose of this study was to compare the efficacy of bacterial culture and pathogen inactivation to reduce the risk of release for transfusion of PLT components contaminated with low levels of bacteria. Low levels of bacterial contamination are the most relevant type of contamination based on recognized potential sources. Subclinical infection in blood donors, contamination during blood collection, and contamination during processing have all been implicated as sources of bacterial contamination in PLT units.[2] Skin flora has long been recognized as the major source of bacteria found in PLT units.[1,3] In spite of rigorous venipuncture site cleaning, bacterial contamination of PLT concentrates has been reported to range from 0.08 to 8 percent.[21]

A number of methods are being used to reduce the incidence of contamination. The diversion of the first 10 to 40 mL of blood reduced the prevalence of bacterial contamination by greater than 50 percent[22,23] but, even so, bacterial contamination remains a primary source of blood-borne pathogen transmission associated with PLT transfusion. Skin flora may be especially prevalent in PLT units collected from repeat donors who have developed scarring in the antecubital fossae.[6] It is widely assumed that the initial bacterial inoculation contains a very small

number of CFUs. In one study of quantitative culture from the scarred collection site of a repeat donor, the investigators found that three of four blood samples yielded at least 50 colonies.[24] The study of five such donors resulted in 0 to more than 100 CFUs per collection. Very few bacteria are initially inoculated into the blood unit during collection, and processing further reduces the numbers. Some bacteria remain in the red cell portion of whole-blood units during PLT preparation,[25] leaving fewer bacteria in the PLTs. Liters of blood are processed during the apheresis collection of PLTs and further dilute the number of bacteria remaining in the unit after contamination from skin.

Although theoretically a single bacterium inoculated into a culture bottle is sufficient to detect a contaminated unit, the lower the inoculation concentration of bacteria, the more slowly a positive culture is detected. Previous studies evaluated the detection of bacteria in PLT units inoculated with 10 to 100 CFUs per mL.[8,26] It was noted that concentrations of less than 5 CFUs per mL were often complicated by inconsistent growth. The Council of Europe recommends that small-sample-volume sterility testing (2-5 mL) be performed only after a 48-hour delay after collection (Day 2 of storage) to allow sufficient time for bacterial growth to a detectable level based on volumes sampled in bacterial culture systems.[27] Sterility testing can begin before 48 hours, but larger culture volumes are required. The larger volume increases the probability that bacteria will be detected, but at the same time reduces the final PLT dose available for transfusion. For units contaminated with low levels of bacteria, the volumes required for detection of bacteria during culture are impractically large and are a limitation of the process. In a number of studies designed to detect contamination in routine practice, samples were withdrawn from PLT components 2 to 24 hours after preparation.[1,10,11,21,28,29] The time that culture bottles are monitored before PLT release is variable ranging from immediate release after culture up to 24 hours after culture. Clinical demand for PLTs prevents many centers from waiting the 24 hours after culture and PLTs are released for transfusion on a test-negative-to-date status. It is not unusual for a bacterial detection test to become positive after the PLTs have been transfused.[1,11]

This study compared the efficacy of two different approaches to reduce the risk of releasing PLT units contaminated with low levels of bacteria: bacterial detection and bacterial inactivation. Three levels of contamination per bacterial species were targeted: 1 to 10, 10 to 100, and 100 to 1000 CFUs per PLT concentrate unit (equivalent to 0.003-0.03, 0.03-0.3, and 0.3-3 CFUs/mL, respectively). These levels closely approximate the actual contamination level in freshly prepared PLTs.[21,24] Although other previously published studies have seeded many more CFUs per mL into PLT concentrates (0.1-1000 CFUs/mL[30,31]), this study more closely simulated an actual contamination event by seeding between 1 and 1000 CFUs per unit (or 0.003-3 CFUs/mL) at the time of PLT collection. Given the limited storage time for PLT components and the preference for physicians to request fresh PLTs, this study simulated the impact of contamination on the earliest possible times for release of product.

S. aureus, S. agalactiae, K. pneumoniae, and *E. coli* are fast-growing bacterial species under optimal growth conditions. Time to detection by bacterial culture varied, however. The results of this study showed that if a PLT unit is contaminated with low levels of *S. aureus* or *K. pneumoniae,* the chance of detection before product release is excellent if sampled on Day 2 and cultured for 24 hours before product release.[28,32] Because of operation constraints, however, some European blood centers as well as most US blood centers sample on Day 1 and hold for 12 to 24 hours before release thus reducing the probability of detection. In contrast, low levels of *S. epidermidis* required a longer time to detect. Consequently, contaminated PLT units could be released for transfusion on test-negative-to-date status. *S. agalactiae* and *E. coli* were not detected in the PLT units with the lowest contamination level (1-10 or 0.003-0.03 CFUs/mL). No growth detected in the 4 to 5 mL taken for culture could be a result of sampling due to the delayed growth of the organism after overnight storage. The possibility of loss of viability, with low concentrations of bacteria as a form of "self-sterilization," cannot be ruled out. *P. acnes* is a slow-growing anaerobic species. Cultures were positive only in anaerobic bottles inoculated with samples drawn on Day 5 of storage and even then required 50 to 53 hours for detection. These results are consistent with findings by other investigators.[8,17] Most blood centers use only aerobic cultures; thus contamination by *P. acnes* would be missed, although this organism is of debatable clinical significance.

These data also address the utility of anaerobic testing. In the lowest level of inoculum, four species were detected by culture. The anaerobic bottle alone was the first indication of contamination for two of the species. Although this is less impressive for *P. acnes,* a known obligate anaerobe, the detection of the 1 to 10 CFUs inocula of *S. epidermidis* on Day 5 samples and the 10 to 100 CFUs inocula of *S. agalactiae* on Day 1 samples is instructive. The latter organisms are facultative anaerobes.

Unlike many other *Clostridium* sp., *C. perfringens* is aerotolerant and is capable of growing in PLT concentrates.[20] The highest inocula resulted in positive cultures in both the aerobic and the anaerobic bottles for PLT samples taken on Days 2 and 5 of storage; low-level contaminations were not detected, however. Self-sterilization for extreme low-level bacterial contamination is possible due to plasma factors. It is also possible, however, that due to the delayed growth of the bacteria in PLT concentrate, the 4- to 5-mL sample taken for culture may not contain viable organisms.

Although self-sterilization for low levels of bacterial contamination is possible, the use of leukoreduction before contamination reduced the sterilization effect due to phagocytosis by WBCs[33] and the use of 65 percent Inter-Sol with 35 percent plasma reduced the levels of complement and immunoglobulin, thus making self-sterilization less likely. Furthermore, previous studies have shown that low levels (1-6 CFUs/unit) of contamination for four of the bacterial species (*S. aureus, S. epidermidis K. pneumoniae,* and *E. coli*) used in this study grew to high levels after PLT storage.[14] In the prior study, the bacteria were detected by colony counting of serial dilutions placed on agar plates.

The time to first detection of a positive culture of contaminated PLT ranged from 28 to more than 240 hours (or no growth) after bacterial inoculation into PLT components on Day 0. Detection of a positive culture depended on the bacterial species, the concentration of the bacteria in the inoculum, and the duration of PLT storage before withdrawal of the sample for culture. The shelf life of a PLT is limited to 5 days and in some countries to 7 days. An effective bacterial detection system should be able to detect low levels of bacterial contaminants within 24 to 48 hours of storage from the time of preparation. The organisms must have enough time to proliferate before they can be detected by the culture system, and very-low-level inoculum or slow-growing organisms may not have adequate numbers to be detected by the bacterial culture system at the time of culture testing.[29,34-36] Given the standard 5-day shelf life of PLTs and the time required to detect low levels of bacteria, bacterial culture methods can significantly constrict the available time for PLT use. In contrast, PCT could be used to inactivate bacteria and permit the release of PLT components to inventory within 10 to 12 hours of collection.

In this study, the initial bacterial contamination levels were likely representative of the actual levels of bacterial contamination in freshly prepared PLT concentrates. These units were stored overnight under standard PLT storage conditions at 22 ± 2°C before treatment with PCT. Therefore, at the time of treatment the level of bacterial contamination could have been higher than the initial inoculum because of the bacterial growth during overnight storage. The delay in PCT treatment until Day 1 of collection provides blood donation centers the flexibility in performing the process. Concerns of potential generation of bacterial endotoxin during this storage period would push the blood center to perform the PCT process as soon as logistically feasible. Despite this delay, PCT effectively inactivated the seven bacterial species at three inoculation levels in all test PLT concentrates. These findings are consistent with earlier studies with high-titer viable bacteria (approximately 10^6 CFUs) and culture methods that confirmed 6 logs of inactivation.[14,37] Compared to the bacterial culture method, PCT inactivated all levels of bacterial contamination to below the level of detection and prevented the release of PLT units contaminated with bacteria.

Finally, it should be pointed out that the bacterial testing approach is limited only to the detection of bacteria. The pathogen inactivation of PLT has been shown to be effective in inactivating viruses,[38-40] protozoa,[41,42] and WBCs,[43] in addition to bacteria, thus providing a more comprehensive approach to transfusion safety.

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