Evaluation of the Scansystem method for detection of bacterially contaminated platelets

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BACKGROUND: Platelet (PLT) bacterial contamination occurs in approximately 1 in 2000 PLT units. The College of American Pathologists recommends and AABB requires procedures to detect PLT bacterial contamination. Although two methods, BacT/ALERT (bioMérieux) and Pall BDS (Pall Corporation), have FDA approval for quality control testing, additional methods are in development. One such method was evaluated, the Scansystem (Hemosystem), which has been developed for use on leukoreduced PLT components between 30 and 72 hours after collection.

STUDY DESIGN AND METHODS: Leukoreduced, single-donor apheresis PLT units (LR-SDPs) were inoculated with 10 bacterial species (low and high inocula) associated with PLT contamination. Bacterial detection was compared with the Scansystem and BacT/ALERT. Testing was initiated (10 replicates performed) when LR-SDPs were experimentally inoculated with bacteria. The Scansystem was evaluated 30 hours later, the shortest manufacturer recommended time after PLT collection.

RESULTS: All replicates were positive with the Scansystem at 30 hours and with the BacT/ALERT, at 9.3 to 24.0 hours after inoculation. The Scansystem detected bacteria in 83 of 200 replicates (42%) at the time of inoculation indicating a potential for earlier application.

CONCLUSIONS: The Scansystem, used to test LR-SDPs 30 hours after bacterial inoculation, detected all 20 replicates with a sensitivity equal to the BacT/ALERT system. Based on use of Scansystem with LR-SDPs 30 hours after collection and the BacT/ALERT being inoculated 24 hours after collection and incubated for an additional 24 hours before being determined to be negative, the Scansystem will potentially provide results at an earlier time point (32 hr) than provided by the BacT/ALERT system (48 hr).

Although there have been significant advances in reducing the risk of transfusion-transmitted infectious diseases during the past two decades, bacterial contamination of platelets (PLTs) remains a major cause of transfusion-associated morbidity and is the second most common cause of death overall from transfusion following only clerical error resulting in ABO mismatch.[1,2] Bacterial contamination of PLTs has been estimated to occur with a frequency of 1 in 2000 to 1 in 3000 PLT units, with clinical sepsis occurring in approximately 5 to 10 percent of patients.[2,3] Several techniques for detecting bacteria in PLT units, reducing bacterial contamination of PLT units, or preventing bacterial growth in PLT units have been devised, and several are in various stages of development, including gram and acridine orange staining, chemiluminescence and PCR assays, pretransfusion culture, analysis of swirling patterns, analysis of metabolic changes (particularly glucose and pH), leukoreduction, diversion of initial phlebotomy volume, and cold preservation and storage of PLTs.[1,4] Two detection methods, both based on pretransfusion culture, have been approved for quality assurance of PLT products by the FDA: BacT/ALERT (bioMérieux, Hazelwood, MO) and Pall BDS (Pall Corporation, East Hills, NY).[5]

Hemosystem (Marseille, France) has developed a rapid and sensitive new technology for bacterial detection named Scansystem, with a proprietary sample preparation method, with detection of bacteria with a laser-based, solid-phase scanning cytometry detection method. This

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method is a highly sensitive bacterial screening system and can detect very low levels of bacteria after concentration from a larger volume by filtration. We compared the utility of this system in detecting bacterial contamination of PLTs with that of the BacT/ALERT system in PLT units experimentally inoculated with strains of the 10 bacterial species most often associated with bacterial contamination of PLTs.

MATERIALS AND METHODS

The Scansystem (Hemosystem) has been developed to detect bacterial contamination of leukoreduced PLT components from 30 to 72 hours after PLT collection. The system consists of Scansystem PLT kits (Fig. 1) and the Scansystem solid-phase cytometry system. The Scansystem PLT kits are multicompartment, closed devices consisting of a 10-mL syringe containing 1 mL of the BLS1 PLT aggregation and bacterial staining solution (polyethylenimine, 60 mg/L; monoclonal antibody CD9 6B1, 30 mg/L; 1:2000 dilution of picogreen nucleic acid-binding dye) and a 15-mL pouch containing 7 mL of the bacterial cell membrane permeabilizing and labeling reagent BLS2 (EDTA, 1.86 g/L; nisin, 8 mg/L; *N*-octyl-β-D-glucopyranoside, 2.5 g/L; chlorhexidine diacetate, 150 mg/L, all in distilled water), with the two compartments separated by a 5-µm-pore-size filter in a sealed holder and a breakable Luer connection.[6] The pouch is connected with a second breakable Luer connection to a 0.4-µm-pore-size black membrane in an unsealed holder. The Scansystem solid-phase cytometry analyzer includes four modules: 1) a scan module, in which the black membrane is placed; 2) an argon laser module (488-nm excitation light), which is connected directly to the scan module to scan the black membrane; 3) an epifluorescence microscope with a suitable light source and filters, which has an automated motor-driven stage, for visual discrimination by the operator of fluorescent particles detected by the laser scan as bacteria or nonbacterial particles; and 4) a computer with proprietary software to monitor the other modules.

The Scansystem is used by drawing a 3-mL sample of PLTs into the syringe of a Scansystem PLT kit through the sample injection port (Fig. 1). The kit is then agitated in a PLT incubator at 22°C for 40 minutes to achieve PLT aggregation. The Luer lock is then broken and the contents of the syringe are expressed through the filter, which retains the aggregated PLTs and allows passage of bacteria into the second portion of the kit. The kit is then incubated at room temperature for 20 minutes to allow labeling of any bacteria present with the fluorescent stain. A black membrane in a holder attached to a vacuum pump is then attached to the bottom port, the second Luer lock is broken, and the contents of the pouch are filtered through the black membrane. Any bacteria present will be deposited on the surface of this membrane. The membrane is then removed from the holder and placed into the Scansystem scan module for detection of fluorescent particles by laser scanning. PLT aggregation has been shown to occur with this kit on sterile PLT units for up to 5 days of storage. PLT aggregation, however, occasionally fails to develop, and this is also liable to occur in bacterially contaminated units. In such instances, PLTs can block the black membrane. If this occurs, the Scansystem PLT kit is removed from the filter cartridge and the membrane is allowed to dry before being placed in the laser scanner. After scanning of the membrane, all fluorescent signals detected are discriminated by the software on the basis of their sizes

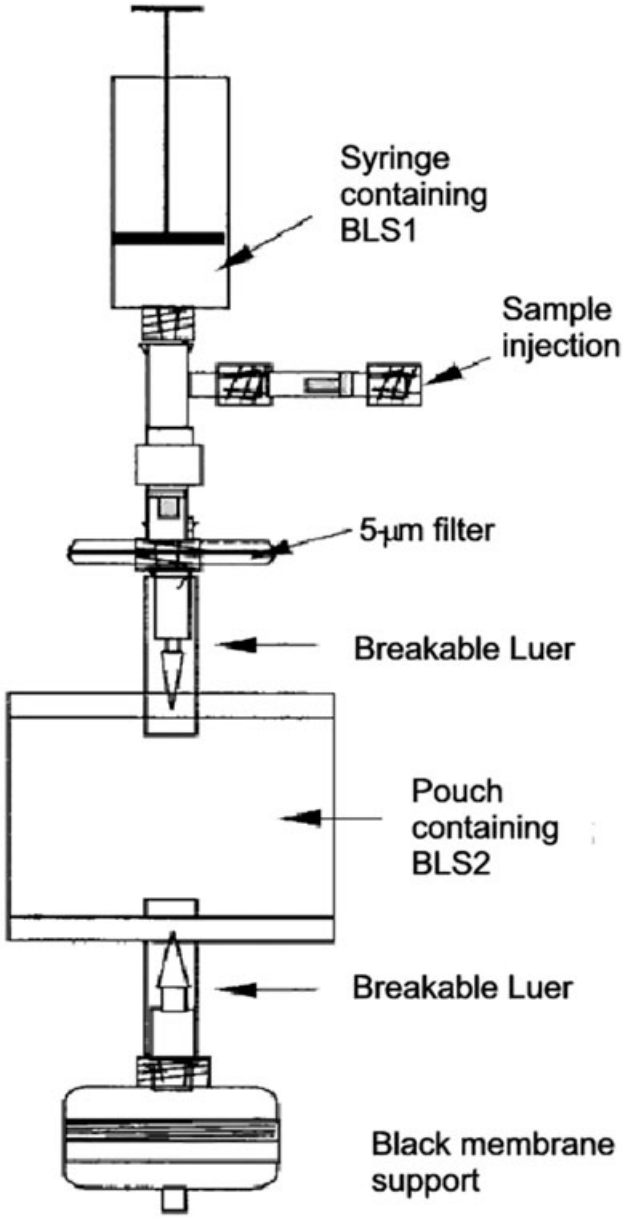

Fig. 1. Diagram of the Scansystem PLT kit (Hemosystem) design. Reproduced, with permission, from Ribault et al.[6]

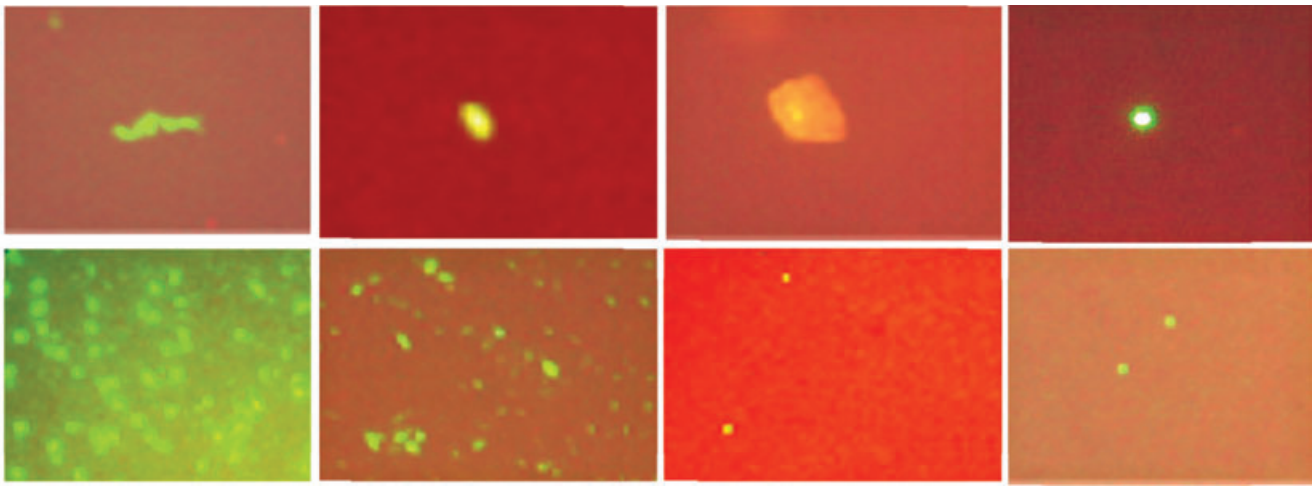

Fig. 2. Photographs of the microscopic appearance of particles and bacteria on membranes under 500 × original magnification. Top row, particles, including a 2-mm-diameter control bead (top right) used for calibration of the microscope. Bottom row, bacteria (from left to right): *E. coli, B. cereus, S. epidermidis,* and *Y. enterocolitica*. The contrast and brightness of the photographs have been enhanced in this figure.

and fluorescence intensities. The results are then displayed on the computer screen as a map showing the number of discriminated spots and their positions on the scanned membrane. If fluorescent particles are detected, the membrane is then placed on the stage of the computer-controlled microscope and examined by the operator under 500× magnification to verify the presence of bacteria. The computer locates fluorescent particles identified by the laser, and the operator examines 50 such fluorescent particles and determines whether each is a bacterium (positive) or a nonbacterial particle (negative). Examples of the microscopic appearance of particles and bacteria are shown in Fig. 2. Specimens are interpreted as positive if at least 20 percent of 50 fluorescent particles are visually confirmed to be bacteria.

Quantitative bacterial counts were performed on inoculated PLT units at time 0 and at 30 hours by making serial 10-fold dilutions of 1-mL volumes removed from PLT units and plating 0.1-mL volumes onto blood agar plates. Additionally, to detect very low inocula at Time 0 only, 1-mL volumes removed from PLT units were filtered through a 0.45-µm membrane filter, and filters were then placed on blood agar plates. All counts were performed in duplicate and results expressed as the mean of the two determinations. Plates were incubated at 35°C for up to 48 hours and counts were determined from the number of colonies present on the membrane filter at Time 0 for counts of less than 300 colony-forming units (CFUs) per mL or from plates from dilutions with 30 to 300 colonies per plate for all other determinations.

Experimental design

For our evaluation of the Scansystem, 2-day old, leukoreduced, single-donor, apheresis PLT units were inoculated with bacteria at final concentrations of 5 to 57 CFUs per mL (mean, 32 CFUs/mL—low inoculum) and 70 to 680 CFUs per mL (mean, 337 CFUs/mL—high inoculum). Although the actual number of contaminating organisms in naturally occurring cases of PLT contamination is unknown, we used the low inoculum to test the system with as low an inoculum as can be reliably produced in an in vitro spiking study. The bacterial strains used were reference strains of the 10 bacterial species that are typical PLT contaminants. Isolates were obtained from the American Type Culture Collection (ATCC), and are listed with their ATCC reference numbers: *Escherichia coli* 25922, *Staphylococcus epidermidis* 49134, *Staphylococcus aureus* 49476, *Klebsiella oxytoca* 13182, *Enterobacter cloacae* 29005, *Serratia marcescens* 43862, *Bacillus cereus* 7064, *Pseudomonas aeruginosa* 27853, *Streptococcus pyogenes* 12344, and *Salmonella choleraesuis* 8326. Inoculated PLT units were maintained in a PLT agitator at 22°C for 30 hours, which is the shortest time after collection of apheresis PLT units recommended by the manufacturer for testing with the Scansystem. Following this 30-hour incubation, a 3-mL sample of each inoculated PLT unit was pooled with 3-mL samples from 2 uninoculated PLT units (the kit allows screening of 3 units together), and 3 mL of this final pooled sample was transferred to the syringe of the Scansystem PLT kit and processed as

described above. Ten replicate tests were performed on each bacterially inoculated PLT unit. For comparison, 10 replicate aerobic and anaerobic BacT/ALERT bottles were inoculated with a 1-mL sample from inoculated PLT units immediately after contamination (Time 0) plus 3 mL of uninoculated PLTs (to provide the 4-mL volume specified for this system), and the bottles were incubated in a BacT/ALERT 3D automated microbial detection system until positive. Uninoculated PLTs were tested with each inoculated unit and served as negative controls. The Scansystem was also tested at Time 0 to determine the sensitivity of this system for detecting very small numbers of bacteria.

RESULTS

Initial (Time 0) bacterial inocula in PLT units ranged from 5 to 57 CFUs per mL (mean, 31.9 CFUs per mL) for the low inocula and 70 to 680 CFUs per mL (mean, 337.2 CFUs per mL) for the high inocula (Table 1). Bacterial counts at 30 hour increased by greater than 3 log CFUs per mL compared with counts at Time 0 for PLT units inoculated with *B. cereus, S. pyogenes, E. coli, K. oxytoca,* and *S. marcescens* and by 1 to 3 log CFUs per mL for PLT units inoculated with *S. epidermidis, S. choleraesuis,* and *E. cloacae*. Bacterial counts increased 4.8- to 7.4-fold in the PLT units inoculated with *S. aureus*, but were approximately 3-fold lower at 30 hours compared to Time 0 in the units inoculated with *P. aeruginosa*.

Bacteria were detected by the Scansystem for all 10 replicates of all 10 bacterial species in the PLTs units inoculated and stored for 30 hours (Table 1) for both low and high inocula. At Time 0, 83 of the 200 samples tested (42%) were positive, with little difference between low and high inocula. Overall, more than 70 percent of replicates at Time 0 were positive for PLT units inoculated with *E. coli, K. oxytoca,* and *S. aureus*, whereas approximately 50 percent were positive with *S. marcescens* and *S. pyogenes* and not greater than 30 percent for the other bacterial species. All uninoculated PLTs produced negative results. No instances of blockage of the black membrane occurred with any of the Time 0 experiments; however, blockage did occur in a few instances on inoculated PLTs with the 30-hour determinations, requiring use of the alternate procedure for processing the membrane (see Materials and methods). This did not affect bacterial detection because all 30-hour Scansystem tests were positive.

The BacT/ALERT system inoculated at Time 0 also detected all the organisms in all replicates, with detection times of 10.0 to 20.4 hours (mean, 14.8 hr) for the low inocula and 9.3 to 24.0 hours (mean, 13.4 hr) for the high inocula (Table 1). All uninoculated PLTs produced negative results.

TABLE 1. Scansystem results of 10 replicates of each inoculated PLT unit at 0 and 30 hours after inoculation for 10 representative PLT contaminants used*

Organism	Bacterial inoculum in PLT units at results per 10 Time 0 (CFUs/mL)		Number of positive Scansystem replicates at Time 0†		Time (hr) to positivity of BacT/ALERT for 10 replicates after inoculation at Time 0‡		Bacterial inoculum in PLT units at 30 hr (CFUs/mL)		Number of positive Scansystem results per 10 replicates at 30 hr†	
	Low inoculum	High inoculum	Low inoculum	High inoculum	Low inoculum	High inoculum	Low inoculum	High inoculum	Low inoculum	High inoculum
Bacillus cereus	5	70	2	1	10.8 (10.0-11.2)	9.9 (9.3-10.0)	2.85×10^{4}	2.85×10^{5}	10	10
Enterobacter cloacae	46	680	4	2	13.1 (12.2-13.5)	11.7 (11.1-12.1)	1.75×10^{4}	2.00×10^{5}	10	10
Escherichia coli	35	250	9	7	13.2 (12.6-13.3)	12.2 (11.6-12.4)	6.45×10^{6}	2.45×10^{7}	10	10
Klebsiella oxytoca	43	425	7	8	13.3 (13.0-13.5)	11.9 (11.4-12.3)	7.30×10^{7}	7.25×10^{7}	10	10
Pseudomonas aeruginosa	57	665	2	1	17.2 (17.0-17.4)	15.7 (15.2-16.1)	2.05×10^{1}	1.77×10^{2}	10	10
Salmonella choleraesuis	45	580	1	2	13.4 (12.4-14.1)	12.1 (11.4-12.5)	2.45×10^{3}	7.15×10^{3}	10	10
Serratia marcescens	26	250	5	6	12.2 (12.1-12.3)	11.2 (11.1-11.3)	9.65×10^{7}	1.05×10^{8}	10	10
Staphylococcus aureus	26	217	9	8	16.5 (15.5-17.1)	15.2 (14.5-15.5)	1.25×10^{2}	1.60×10^{3}	10	10
Staphylococcus epidermidis	11	120	1	0	19.1 (18.1-20.4)	17.8 (16.4-24.0)	2.25×10^{2}	3.00×10^{3}	10	10
Streptococcus pyogenes	25	115	4	4	18.8 (13.0-20.1)	16.6 (14.3-18.5)	1.20×10^{5}	3.50×10^{6}	10	10
Mean	31.9	337.2	4.4	3.9	14.8 (10.0-20.4)	13.4 (9.3-24.0)	1.76×10^{7}	2.06×10^{7}	10	10

* Strains used are identified by their American Type Culture Collection (ATCC) reference numbers as provided in the text.
† These results are expressed as number of replicates interpreted as positive (≥20% of 50 fluorescent particles visually confirmed to be bacteria) per set of 10 replicates.
‡ Results shown as mean time to detection in aerobic and anaerobic bottles, with range of detection times shown in parentheses.

DISCUSSION

This study demonstrates that the Scansystem method is able to detect 10 typical bacterial PLT contaminants in experimentally contaminated, leukoreduced, single-donor, apheresis PLT concentrates 30 hours after contamination with high and low bacterial inocula with sensitivity equal to that of BacT/ALERT. Bacterial counts at 30 hours varied considerably, but this did not affect the ability of the Scansystem to provide positive results. This is likely to be due to the fact that the Scansystem can detect both live and dead bacteria, so that the presence of bacterial species such as *P. aeruginosa* and *S. epidermidis*, which are frequently inhibited or killed in the presence of human plasma,[7,8] can still be detected provided some bacterial growth occurred. This is particularly important for contamination with *P. aeruginosa* because transfusion of small amounts of endotoxin produced by the growth of this organism can result in a fatal outcome.

Advantages of the Scansystem method are that PLTs, from 30 to 72 hours after collection can be tested for the presence of bacteria rapidly, with a total test time of approximately 90 minutes;[6] this compares to the BacT/ALERT method, which typically provides negative results 48 hours after collection (based on BacT/ALERT testing being performed 24 hr after collection and being negative after inoculation for 24 hr).[9] Thus the Scansystem allows testing of PLTs closer to time of use and allows PLT units to be used as early as 32 hours after collection, compared to typically 48 hours for the BacT/ALERT method. Moreover, the Scansystem method is able to detect both live and dead bacteria, which may prevent transfusion of endotoxin-containing products. Disadvantages of the Scansystem are the need to visually verify the presence or absence of bacteria for each test and the need to test PLT units between 30 and 72 hours after collection. Elimination of the need to visually verify the presence or absence of bacteria for each test is currently under evaluation. The Scansystem method has the potential for the sensitive and reliable detection of bacterial contaminants in PLT products. Examination in a clinical environment to validate the applicability of this method to detect and interdict bacterially contaminated PLTs is warranted.

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