collections, because the single implicated WBP component in the pool is generally not identified. Limiting the initial bacterial contamination of each WBP component in the pool therefore is critical and expected to occur with optimized skin preparation techniques and initial whole blood diversion at collection.[3,4]

In this report we describe the baseline risk of sepsis associated with transfusion of WBPs, as measured by the rate of septic transfusion reactions reported to the American Red Cross Hemovigilance Program in 2003 through 2006.[5] After successful implementation of PSPs at four American Red Cross regional blood centers, we assess the impact of sample diversion on the risk of contamination during collection and document the ability of culture testing to identify contaminated products and prevent their release into inventory.

MATERIALS AND METHODS

Whole blood was collected into collection sets with or without a sample diversion system (Leukotrap-RCPL, Pall Medical) from routine blood donors in four American Red Cross Blood Services Regions (A-D). The time frame for the introduction of PSPs and whole blood collection utilizing sample diversion strategies overlapped, such that the first 5211 PSPs were manufactured with WBP that lacked sample diversion. The phlebotomy site was prepared by our standard, FDA-recommended, skin disinfection protocol utilizing povidone-iodine scrubs (or chlorhexidine gluconate in 70% isopropyl alcohol scrubs for donors allergic to iodine)[4] throughout the study. Leukoreduced WBP concentrates were prepared and stored at 20 to 24°C. After a minimum of 24 hours, five ABO-identical WBP concentrates were pooled into a 1.5-L CLX storage bag (Acrodose PL System, Pall Medical) by means of multiple sterile connections (TSCD or SCD sterile tube welders, Terumo Medical Corp., Elkton, MD). Pools were labeled D+ if there was one or more D+ WBP unit in the pool.

An 8-mL sample for bacterial testing was removed from the storage bag using a platelet (PLT) sampling device (SampLok sampling kit, ITL, Reston, VA; or accessory sampling assembly, Gambro BCT, Lakewood, CO). An additional 1.5 to 2 mL was used for product qualification testing (PLT count, pH). PLT yield was calculated from the PLT concentration and the volume of the PSPs.

At the start of the evaluation of the Acrodose PL system, process control testing was performed. PLT yield, concentration and product volume, sterility by bacterial culture, and pH were determined and success was defined by our standard process control procedures. This entailed testing the first 60 pools with no failures or 91 pools with one allowable failure. pH testing was performed for 60 pools by determining the pH of 40 PSPs at issue, 10 PSPs on Day 5, and 10 PSPs at outdate; for 90 pools: by determining pH of 61 PSPs at issue, 15 PSPs on Day 5 and 15 PSPs at outdate. After successfully completing process control, monthly QC testing of 1 percent of PSPs was initiated.

Detection of bacterial contamination

An automated microbial detection system (BacT/ALERT 3D, bioMérieux) was used for aerobic cultures, with bottle inoculation performed in a laminar-flow hood, as previously described.[6-8] PSPs were released into inventory for distribution after at least 12 hours with negative culture results, and cultures were monitored until product outdate on Day 5. All components associated with positive initial culture results were quarantined or retrieved if already distributed to transfusion services. A second 8-mL sample was taken from the initially positive PSPs and/or cocomponents and inoculated into a new aerobic bottle for confirmatory culture. The initial and subsequent positive culture bottles were sent to independent microbiology laboratories for bacterial isolation and identification. Bacterial culture test results were classified according to AABB Bulletin 04-07[9] with further subcategorization as follows: A confirmed-positive result is a true-positive result with the growth of the *same organism* in the initial and confirmatory sample; a false-positive result is a positive bottle signal but a negative result on subsequent culture. False-positive results were further characterized as either possible sampling contamination, if bacteria were detected in the initial bottle but not the confirmatory culture, or instrument error, if the initial culture bottle was found to be sterile.[6] If PLT components were not available for confirmatory culture because they were transfused or destroyed during the manufacturing process, the initial-positive signal could not be resolved and the results were classified as "indeterminate." Occasions where an initial-positive culture was detected but the subsequent confirmatory culture revealed a different bacterial isolate were labeled "discrepant." In the AABB classification, these cases would be included in the "true-positive" category.

Septic transfusion reactions

All septic transfusion reactions reported to the 35 regional American Red Cross blood centers were investigated and compiled in a centralized database through the American Red Cross Hemovigilance Program, as previously described.[5] Clinical criteria for a possible septic transfusion reaction to apheresis PLTs were any of the following symptoms within 4 hours of transfusion: fever of 39°C or greater or a change of 2°C or more from pretransfusion value, rigors, tachycardia greater than 120 bpm or a change of 40 bpm or more from pretransfusion value, or a change of more than 30 mm Hg in systolic or diastolic blood pressure.[10] A definite septic transfusion reaction

fulfilled the clinical criteria and yielded the same bacterial species from the residual PSP component and the patient. A probable septic transfusion reaction fulfilled the clinical criteria but was associated with a positive culture on the residual component without a matching positive blood culture result in the recipient. Transfusion reactions that did not meet the criteria of probable or definite septic reactions were excluded.

Statistical analysis

Odds ratios (ORs) and 95 percent confidence intervals (CIs) were computed to compare the odds of contamination before and after the implementation of sample diversion strategies.[11] Differences between groups were significant at p value of less than 0.05. The American Red Cross Institutional Review Board determined that the operational trial was exempt under 45CFR46, 21CFR50.

RESULTS

Baseline risk of bacterial sepsis from WBPs before implementation of PSPs

We assessed the baseline risk of bacterial sepsis with WBPs as reported to the American Red Cross Hemovigilance Program, before the introduction of PSPs and sample diversion. Between January 1, 2003, and December 31, 2006, the 35 American Red Cross blood regions distributed 2,535,043 WBP units and 20 septic reactions were reported, including 2 fatalities, for an overall rate of 7.9 per million (1:126,752) reactions and 0.79 per million (1:1,267,522) fatalities per distributed component (Table 1). Products involved in septic reactions were pooled at the transfusion service in a median pool size of 5 components (mean, 4.75; range, 2-8 components). The median calculated risk per transfused product, assuming that 5 products were pooled, was therefore 39.4 per million (1:25,350) for sepsis and 3.94 per million (1:253,504) for fatality.

Eight of the reported reactions fulfilled the criteria for definite septic reactions and 12 were probable septic reactions, because the incriminated organism was not cultured from the patient, either due to lack of blood culture testing (5 cases) or due to negative culture results for patients on antibiotic therapy (7 cases). The most common bacterial contaminants were likely commensal skin organisms (16 cases; 80%); 4 (20%) were likely enteric organisms (Table 1). The components that comprised each pool of WBPs were of uniform storage age, and 15 reactions occurred on Day 5 (75%), 3 on Day 4 (15%), 1 on Day 3 (5%), and 1 on Day 2 (5%) after collection. The two fatalities implicated *Staphylococcus aureus* and *Escherichia coli* and were transfused on Day 5 of storage.

TABLE 1. Bacteria implicated in septic transfusion reactions to WBPs distributed between January 1, 2003, and December 31, 2006

Bacterial isolate	Number of septic reactions
Coagulase-negative *Staphylococcus*	11
*S. aureus**	2
Staphylococcus spp. (mixed culture)†	1
Bacillus spp.	1
Corynebacterium spp.	1
Likely skin contaminants (subtotal)	16
S. bovis	1
*E. coli**	2
Multiple (*Klebsiella pneumonia, E. coli*)	1
Likely enteric organisms (subtotal)	4
Total reactions	20
Number of WBP units distributed	2,535,043
Septic reactions, rate per 10^6	7.9
Fatalities, rate per 10^6	0.79

* Associated with a fatality.
† Coagulase-negative and coagulase-positive *Staphylococcus*.

Operational trial and implementation of PSPs

Because of the potential safety benefit of QC bacterial detection and other operational advantages, we implemented the Acrodose PL PSP system. In an initial operational trial, four regions of the American Red Cross manufactured 7628 PSP units, of which 5211 were produced before and 2417 after the implementation of whole blood collection sets with initial sample diversion. A pool size of 5 units was necessary to produce a consistent component containing more than 3×10^{11} PLTs, as losses due to pooling and sampling for QC tests and bacterial culture amounted, on average, to 16.1 percent (range, 8.3%-31.5%) of the PLT yield. The pooling and sampling procedure was accomplished on average in 17.5 minutes (range, 13.5-21.3 min) once staff were fully trained and familiar with the technique.

All four regions successfully completed the phase of process control testing. Two regions had no failures in their first 60 pools and two regions had one failure in their first 91 pools (see *Guidance for Industry and FDA Review Staff Collection of Platelets by Automated Methods*, December 2007 for current criteria[12]). One failure was due to a high PLT concentration greater than 2300×10^3 per µL, and the other, to PLT yield less than 2.2×10^{11} per pool. Once process control was established, monthly QC testing was then initiated, involving pH testing between Day 2 and outdate, of at least 1 percent of PSPs or 4 PSP units per month if fewer than 400 pools were prepared. A total of 131 pools (all four regions) were tested over 2 to 6 months and 100 percent had pH values of 6.2 or greater within a range of 6.58 to 7.70.

The vast majority of PSPs had PLT yields equivalent to the minimum yield for plateletpheresis (i.e., $\geq 3.0 \times 10^{11}$; Table 2). The Acrodose PL system incorporates acceptance product qualification criteria that are determined by

TABLE 2. Product qualification data (±1 standard deviation) during the operation trial for the Acrodose PL system for PLTs pooled in four regions of the American Red Cross

	Region					
Total pools: 7628	A	B	C	D	Mean	Requirement
Number of pools	2224	4313	604	487		
Volume (mL)	253 ± 8	266 ± 10	232 ± 9	250 ± 6	258 ± 13	180-420
PLTs ($\times 10^3/\mu L$)	1547 ± 206	1530 ± 224	1647 ± 240	1548 ± 193	1546 ± 220	≤2300
Yield ($\times 10^{11}$)	3.9 ± 0.5	4.1 ± 0.6	3.8 ± 0.6	3.9 ± 0.5	4.0 ± 0.6	2.2-5.8
Pools ≥ 3.0×10^{11} (%)	97.10	98.10	94.50	98.80	97.60	
pH ≥ 6.2* (%)	100	100	100	100	100	100
PLT qualification success (%)	99.96	99.56	98.68	100	99.60	

* Includes process control and monthly QC.

TABLE 3. Bacterial detection in PSPs before and after the implementation of sample diversion (SD)

	Before SD		After SD		
Variable	Number	Rate per 10^6	Number	Rate per 10^6	After SD vs. before SD OR (95% CI)
Pools	5,211		20,725		
Confirmed-positive	11	2111	20	965	0.46 (0.22-0.95)
False-positive (machine error)	3	576	8	386	0.67 (0.18-2.53)
False-positive (contamination)	5	960	6	290	0.30 (0.09-0.99)
Indeterminate	2	384	4	193	0.50 (0.09-2.75)
Discordant	0	0	2	97	ND
Total	21	4030	40	1930	0.48 (0.28-0.81)

ND = not determined.

the manufacturer for PLT yield ($2.2 \times 10^{11} - 5.8 \times 10^{11}$ per pool), pool volume (180-240 mL), PLT concentration ($<2.3 \times 10^6/\mu L$), pH at the time of issue (≥6.2), and sterility (bacterial culture–negative). The product qualification success rate was 99.6 percent for pools manufactured during the operational trial (Table 2). Twenty-eight of 7628 pools failed, due to a concentration of greater than 2.3×10^6 per mL (11 cases), a yield of less than 2.2×10^{11} per pool (8 cases), or a yield of more than 5.8×10^{11} per pool (14 cases; 5 pools failed on multiple criteria).

Sample diversion during collection of WBPs

The first 5211 PSP units were manufactured from collection sets lacking sample diversion and 21 products yielded an initial-positive result (4030 per million [1:248]; Table 3). In each case, the PSPs and all cocomponents (RBCs and plasma) were retrieved and destroyed if they had not yet been transfused. After the introduction of sample diversion, 20,725 pools were manufactured and tested by culture (during the operational trial and subsequent routine production until December 31, 2007). Only 40 of the 20,725 PSPs yielded an initial-positive culture result (1930 per million [1:518]) indicating a significant 52 percent reduction in the rate of bacterial contamination (OR, 0.48; 95% CI, 0.28-0.81). This reduction reflected a significant decrease associated with sample diversion in the rates of both confirmed-positive culture rates (2111 vs. 965 per million; OR, 0.46; 95% CI, 0.22-0.95) and false-positive (contamination) rates (960 vs. 290 per million; OR, 0.30; 95% CI, 0.09-0.99), but not in the rates of indeterminate or false-positive samples due to machine error (Table 3). Most of the bacterial isolates in confirmed-positive cultures were likely skin flora (Table 4), and the rate of detection was significantly decreased after implementation of sample diversion (1727 vs. 724 per million; OR, 0.42; 95% CI, 0.18-0.96). The rate of detection for likely enteric organisms, however, did not change with sample diversion (384 vs. 241 per million; OR, 0.63; 95% CI, 0.12-3.24). All organisms detected as false-positive samples due to contamination were likely skin commensal organisms (Table 4).

PSPs with confirmed-positive or false-positive cultures were all successfully removed from inventory and not distributed for transfusion. Four pools with indeterminate culture results were transfused on Day 3 or Day 4 of storage, before the initial-positive culture was detected, but no adverse reactions to these components were reported.

DISCUSSION

Retrospective passive surveillance studies performed before the advent of screening cultures, initial sample diversion, and standardized skin preparations, revealed that the risk of a septic reaction with transfusion of WBPs ranged from 1:14,000 to 1:95,000.[10,13,14] The data presented herein from the American Red Cross Hemovigilance

TABLE 4. Bacteria detection and time to initial culture-positive in PSPs before and after the introduction of sample diversion (SD)

	Confirmed-positive				False-positive (contamination)			
	Before SD* (5,211 pools)		After SD (20,725 pools)		Before SD (5,211 pools)		After SD (20,725 pools)	
Bacterial isolate	Number (rate per 10^6)	Mean time to positive (hr)	Number (rate per 10^6)	Mean time to positive (hr)	Number (rate per 10^6)	Mean time to positive (hr)	n (rate per 10^6)	Mean time to positive (hr)
Coagulase-negative *Staphylococcus*	8	17.5	13	17.5	3	22.7†	4	30.6
S. aureus	1	10.5	1	10.5				
Streptococcus spp. (β-hemolytic)			1	7.0				
Bacillus spp.							2	28.6
Corynebacterium spp.					2	44.1		
Likely skin contaminants	9 (1727)		15 (724)		5 (960)		6 (290)	
S. bovis			2	9.3				
E. coli			1	7.7				
Serratia marcescens	2	7.5						
Proteus mirabilis			1	14.5				
Enterococcus faecalis			1	4.9				
Likely enteric organisms	2 (384)		5 (241)		0		0	
All isolates	11 (2111)		20 (965)		5 (960)		6 (290)	

* SD = initial sample diversion.
† Mean time to a positive culture for all (before SD and after SD) coagulase-negative *Staphylococcus* confirmed-positive cultures was 17.5 hours and for all false-positive cultures was 27.2 hours.

Program provide an estimate derived from 2,535,043 distributed WBP units between January 1, 2003, and December 31, 2006, with 20 reports of sepsis after transfusion, including 2 fatalities. Assuming a mean pool size of 5 WBP components, we calculated a rate per distributed product of 39.4 per million (1:25,350) for sepsis and 3.94 per million (1:253,504) for septic fatality. A limitation of this approach is that the actual number of transfused PLT doses is not known with certainty. The 2005 National Blood Collection and Distribution Survey records that a mean of 18.1 percent of WBPs were discarded before use, further suggesting that the actual septic transfusion rate per transfusion was higher than documented here.[15] Furthermore, several small studies and a large single-institution active surveillance study performed over 15 years have established that active surveillance may detect considerably more contaminated products and septic reactions than passive surveillance, supporting a higher actual contamination rate, currently estimated at 1:1000 to 1:3000 transfused products.[16,17] For comparison, our published estimates of the risk of plateletpheresis samples tested by bacterial culture and distributed by the American Red Cross during the same period (2004-2006) was 1:74,807 for septic reactions and 1:498,711 for fatalities, suggesting that WBPs may have been associated with a greater bacterial risk than apheresis PLTs for each transfused dose.[5] Similarly, the organisms involved in these reactions were mostly skin organisms; fatalities occurred predominantly on Day 5 of storage and frequently involved major pathogens.[16]

In an effort to improve the safety profile of WBPs, we successfully implemented and validated PSPs with the Acrodose PL system and sample diversion and demonstrated reduced contamination at the time of collection. Our preliminary experience suggested that the Acrodose PL system requires a significant investment in staff training and hands-on experience to optimize yields and to minimize QC loses (data not shown). We therefore performed an operational trial in four regions that routinely distribute WBPs. After successful production and distribution of 7628 PSPs over a 5-month period, we implemented PSPs for routine manufacture. We demonstrated the ability to consistently meet all of the manufacturers' quality variables for PSPs; indeed, 97.6 percent contained more than 3×10^{11} PLTs and only 0.4 percent (28 of 7628 pools) of pools failed QC testing, either on yield or on PLT concentration variables.

For bacterial culture, we selected the bioMérieux BacT/ALERT 3D system rather than the Pall eBDS system, because the former approach allows greater culture sensitivity by testing 8-mL rather than 3- to 4-mL samples, allows product release into inventory at 12 hours rather than 24 to 36 hours after culture inoculation, and is consistent with our laboratory approach with apheresis PLTs.

Bacterial culture of 5211 PSPs before and 20,725 PSPs after the introduction of sample diversion at the time of collection revealed a significant reduction in the overall rate of initial positive samples, which was accounted for by a significant decrease in the rate of confirmed-positive and false-positive (contamination) cultures. In contrast, the rate of indeterminate cultures and false-positive cultures due to machine error were unchanged. Similar decreases in confirmed-positive cultures with sample diversion have been previously documented in WBPs.[18,19] In our hands, sample diversion reduced the confirmed positive rates by 54 percent, from 2111 per million to 965 per million pools (OR, 0.46; 95% CI, 0.22-0.95) and the false-positive (contamination) rate by 70 percent from 960 per million to 290 per million pools (OR, 0.30; 95% CI, 0.09-0.99; Table 3).

Sample diversion technology likely reduces bacterial contamination by skin commensal bacteria mobilized during phlebotomy.[3,20] Our finding (Table 4) that confirmed-positive cultures derived from skin commensal bacteria decreased from 1727 to 724 per million after the implementation of sample diversion (OR, 0.42; 95% CI, 0.18-0.96), while the rates of enteric contaminants were unchanged (384 vs. 241 per million: OR, 0.63; 95% CI, 0.12-3.24), supports this concept. The finding that false-positive cultures (contamination) were also significantly reduced supports our previously published hypothesis that some false-positive samples are likely due to components contaminated by low levels of bacteria that do not proliferate on PLT storage.[21] In this view, a truly contaminated product that gives rise to an initial-positive culture is labeled a false-positive culture due to inadequate sampling during reculture of the product, such that the sample is sterile. Further evidence to support the hypothesis is provided by analyzing the time between culture inoculation and the initial positive test result. The most frequent species involved in false-positive (contamination) cultures is *Staphylococcus* (coagulase-negative), which took significantly longer to grow in false-positive than confirmed-positive cultures (mean, 27.2 hr vs. 17.5 hr; $p = 0.02$, t test; Table 4). This delay in growth may be ascribed to lower initial concentrations, prolonged lag phase of growth, or longer doubling times in culture, although the latter is less likely for a given species of bacteria.

PSP implementation required appropriate management of cocomponents associated with initial-positive cultures, directed by the culture results. After implementation of sample diversion, there were 40 initial-positive cultures of 20,725 pools tested (1930 per million [1:518]), and these products and their cocomponents were placed into hold quarantine; 14 of the initial-positive samples were shown to be false-positive samples and their cocomponents were released into inventory. The other positive culture results were associated with 130 RBCs and 130 plasma cocomponents that were retrieved and discarded if they had not been transfused. Because only a single WBP component likely contaminated the pool but could not be isolated in retrospect as the source by the current procedure, approximately 80 percent of the destroyed cocomponents were likely sterile and acceptable for transfusion. The direct and indirect cost of these component losses represent a disadvantage of the Acrodose PL system and should be accounted for in the cost analysis of PSP manufacturing. Of the 20 confirmed-positive cultures after implementation of sample diversion, 15 (75%) were still skin commensal organisms, raising the possibility that further improvements in skin preparation may be effective at lowering contamination and mitigating the loss of cocomponents with PSP production. Two of the four enteric organism contaminants were *Streptococcus bovis*, a species that has been linked to colonic carcinoma, and the donors of these products were counseled to seek further medical investigation.[22]

The confirmed-positive rate of 965 per million (1:1036) bacterial cultures of PSPs after the implementation of sample diversion is 5.2-fold greater than our published rate for apheresis PLTs of 185 per million (1:5399) collected between 2004 and 2006. In that report, the BacT/ALERT cultures were inoculated with 4-mL samples and only a proportion (~39%) of the products were collected with inlet line sample diversion strategies.[5] We recently implemented universal inlet line sample diversion for plateletpheresis, doubled the BacT/ALERT sample volume to 8 mL, and report a confirmed-positive culture rate of 167 per million (1:5,922) after testing 431,490 collections (Table 5 and A.E. Eder et al., submitted for publication).

These data suggest that when utilizing a standardized protocol of skin preparation, sample diversion, and culture, a unit-per-unit comparison of PSPs and plateletpheresis reveals a 5.8-fold (OR, 5.8; 95% CI, 3.5-9.5) greater risk of confirmed-positive contamination for PSPs, suggesting that individual WBP and plateletpheresis collections carry a similar risk of contamination (given the pooling of 5 WBP products in PSPs). These data raise the possibility that PSPs may pose a greater risk of sepsis to transfusion recipients due to false-negative bacterial culture results, after pooling five components. Despite this theoretical risk, PSPs offer to supplement the available PLT inventory at relatively low cost and may mitigate the risk of some transfusion reactions (e.g., transfusion-related acute lung injury, immune hemolysis), due to lower plasma volume derived from individual donors. The counterargument that there may be greater risk from increased donor exposure will need careful evaluation.

Direct comparison of absolute PLT contamination rates assessed by different institutions is not possible, due to variations in sampling time, volume of sample, and conditions of culture (e.g., aerobic/anaerobic conditions). Nevertheless, in those institutions where WBP and