

Detection of bacterial contamination in prestorage culture-negative apheresis platelets on day of issue with the Pan Genera Detection test

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BACKGROUND: Bacterial contamination is currently the most important infectious risk associated with transfusion of platelet (PLT) products. Prestorage culture has reduced but not eliminated this problem.

STUDY DESIGN AND METHODS: Eighteen hospitals studied the Pan Genera Detection (PGD) test, a rapid, lateral-flow immunoassay for the detection of Gram-positive and Gram-negative bacteria. The PGD test was performed on day of issue on apheresis PLTs released by collection centers as culture negative. Confirmatory bacterial culture was performed when PGD tests were repeatedly reactive, with three sites performing culture on all doses studied.

RESULTS: PGD tests on nine of 27,620 (1:3069, 95% confidence interval [CI] 1:6711 to 1:1617; or 326 per million, 95% CI 149-618 per million) apheresis PLT doses were repeatedly reactive and verified as bacterially contaminated by confirmatory culture. Bacterial species isolated included coagulase-negative staphylococci ($n = 6$), *Bacillus* sp. ($n = 2$), and *Enterococcus faecalis* ($n = 1$). The ages of these contaminated doses were Day 3 ($n = 4$), Day 4 ($n = 2$), and Day 5 ($n = 3$). Two contaminated doses with nonreactive PGD tests were detected among 10,424 doses at hospitals where concurrent culture was performed, and one other was identified via a transfusion reaction investigation. There were 142 PGD false positives (0.51%).

CONCLUSIONS: The PGD test detected bacterial contamination in 1:3069 (9 of 27,620) doses released as negative by prestorage culture in PLTs as young as 3 days old. Three contaminated doses, two clinically insignificant, had nonreactive PGD tests, while 0.51% of tests were false positives. Application of this test on day of issue can interdict contaminated units and prevent transfusion reactions.

ABBREVIATIONS: ARC = American Red Cross; CFU = colony-forming units; CoNS = coagulase-negative staphylococci; FDA = US Food and Drug Administration; FN = false negative; LTA = lipoteichoic acid; LPS = lipopolysaccharide; PGD = pan-genera detection; PLT = platelet; SDP(s) = single-donor apheresis platelet(s); TP = true positive.

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Careful donor screening and expanded and improved testing for detection of blood-borne viruses have greatly advanced blood transfusion safety as infected blood and donors have been excluded from the transfusion process. While the risk of transmitting hepatitis B, hepatitis C, and human immunodeficiency viruses, currently estimated at 1:350,000, 1:1,149,000, and 1:1,467,000 transfusions, respectively,¹⁻³ has decreased considerably in recent years, bacterial contamination and its associated risk of septic reactions remains a serious clinical problem in transfusion medicine. Bacterial contamination of platelet (PLT) products is currently regarded as the most important infectious risk associated with transfusion therapy.^{4,5} Between 2005 and 2009, microbial infection was the third leading cause of transfusion-related fatalities reported to the FDA, exceeded only by transfusion-related acute lung injury and hemolytic transfusion reactions.⁶ These microbial infections accounted for 12% (33/267) of transfusion-related fatalities, with PLT products being associated with 61% of cases (20/33); 16 of these 20 PLT products were apheresis units. It is likely, however, that these reports are an underestimate as transfusion-associated septic reactions, which often occur in patients at high risk for sepsis, are well known to be underrecognized and underreported.^{7,8}

To preserve function and survival, PLTs are stored at room temperature (20 to 24°C), which provides an excellent environment for bacterial growth. During the past decade, several processes have been implemented by blood centers to minimize bacterial contamination of PLTs, including improved skin disinfection before donation, diversion of the first 15 to 30 mL of the collection, and culture of donated PLT components early in the storage period.^{4,9} Per the 2003 AABB Standard 5.1.5.1, AABB-accredited blood centers are required to employ a method to limit and detect bacterial contamination in all PLT components.^{10,11} To meet this standard for single-donor leukoreduced apheresis PLTs (SDP), many blood centers use one of the two bacterial detection systems (bacT/ALERT, bioMérieux, Durham, NC; or eBDS Pall Corp., Port Washington, NY), which have been cleared by the FDA for quality control (QC) testing. These qualitative, bacterial growth-based tests are typically initiated at least 24 hours after collection, with SDP products typically released for use if tests are negative after 12 to 24 hours, with monitoring usually continuing until outdate with the BacT/ALERT system. Use of these prestorage, culture-based tests has been associated with a reduction in fatalities associated with bacterial contamination of apheresis PLTs reported to the FDA, decreasing from a mean of 7 (range 6-8) per year in 2001 to 2003 to 2.5 (range, 2-4) per year in 2006 to 2009.⁶ The decrease in fatal Gram-negative cases has been particularly notable, with only four such cases during the 5-year period 2005 to 2009 compared to 3.7 cases per year from 1995 to 2004.^{6,12}

Several reports also document decreases in the incidence of septic transfusion reactions after implementation of these early culture systems. A 70% decrease in septic transfusion reactions associated with apheresis PLTs after the introduction of prestorage culture was noted in The Johns Hopkins Medical Institutions.¹³ A 29% decrease in septic transfusion reactions associated with apheresis PLTs was reported by the American Red Cross (ARC); these reactions decreased from 1.7 in 100,000 for the period March 2004 to May 2006 to 1.2 in 100,000 for the period December 2006 to July 2008.^{4,14} This reduction also was attributed to increasing the sample volume of PLTs cultured from 4 to 8 mL and increased use of diversion of the first 15 to 30 mL during apheresis. Nevertheless, although lower in recent years, the problem of bacterial contamination of apheresis PLTs persists despite application of early culture techniques and diversion.

The severity of transfusion reactions associated with PLTs contaminated with bacteria is, in general, related to the virulence of the bacterial species and the concentration of bacteria contained in the PLT units.¹⁵ Bacterial titers of 10^5 colony-forming units (CFU)/mL or greater in PLT units were associated with septic and fatal transfusion reactions, while lower titers were associated with mild or no reactions. Recognition of transfusion reactions is also related to surveillance methods, with the assessed rate of sepsis from transfused, contaminated units reported to be 11-fold greater by active than by passive surveillance, illustrating the underassessment of this problem using passive surveillance.

The main factor associated with failure of culture to detect bacterial contamination near the time of collection is sampling error, which can occur if bacteria are not at a sufficient concentration in PLT units to be consistently present in the culture sample.¹⁶ Using the BacT/ALERT system, five studies of both apheresis and whole blood-derived PLTs showed that sampling of units after Day 1 detected bacteria that were not detected upon initial culture.¹⁷⁻²¹ These studies showed that detection rates on Day 1 were 14.9% to 40.0% of detection rates on Days 6 and 7.

In view of the demonstrated limitations of prestorage culture methods to adequately detect bacterial contamination of PLTs,^{22,23} alternate assays have been pursued. In 2007, the Platelet Pan Genera Detection (PGD) test (Verax Biomedical, Inc., Worcester, MA), a qualitative, rapid, lateral-flow immunoassay for the detection of Gram-positive and Gram-negative bacteria, received FDA clearance as an adjunct test for QC testing of SDP when used in conjunction with another FDA-cleared QC test. The PGD test was subsequently also FDA cleared as a stand-alone QC test for the detection of bacterial contamination in pools of up to six random-donor PLTs, both leukoreduced and nonleukoreduced. The goal of this study was to determine if the PGD Test could detect bacterial contami-

nation in apheresis PLT units, previously released as culture negative by collection centers, at hospitals on the day of transfusion.

MATERIALS AND METHODS

Participating hospitals and sources of PLT units

Eighteen hospitals, including academic, community and specialty cancer hospitals, participated in the study (Table 1). PLT doses were SDP units obtained from FDA-licensed and/or -registered collection centers. Approximately 8% of doses were derived from in-house collection and the remainder almost evenly split between regional ARC centers and other blood centers. SDP units had been tested at collection centers using the BacT/ALERT system (bioMérieux) or the eBDS system (Pall Corp.) and released as culture negative. Positive BacT/ALERT results occurring after release of units were interpreted according to AABB Bulletin 04-07.²⁴ A PLT dose was defined as a unique SDP containing at least 3×10^{11} PLTs, available for administration to a patient and included in this study, with multiple doses obtained from one collection process considered to be different doses. Each site obtained approval or waiver to conduct the study from its institutional review board.

Verax PLT PGD test

The Verax PLT PGD test is a dual-sandwich immunoassay, carried out in a small, single-use, disposable, lateral-flow device that includes a Gram-positive assay on its left side and a Gram-negative assay on its right side. The test is performed on a 500- μ L sample obtained from the PLT

dose and processed according to the manufacturer's instructions with reagents that release highly conserved antigens that are expressed on the surface of any contaminating bacteria: lipoteichoic acid (LTA) on Gram-positive bacteria and lipopolysaccharide (LPS) on Gram-negative bacteria. The processed sample is applied to the center of the test device and materials are drawn into the device in opposite directions by wicks at each end of the device. The moving materials from the sample initially pass through conjugate pads that contain gold-conjugated detection antibodies that are specific to either LTA or LPS; detection antibodies bind specifically to LTA or LPS if they are present in the sample. As the detection antibody-antigen complexes move toward the ends of the test device, they are captured and concentrated by specific anti-LPS or anti-LTA that are bound in discrete areas on the nitrocellulose substrate at opposite ends of the device. Binding of these complexes to the bound capture antibodies results in color formation in one or two discrete vertical lines on either side of the device if bacteria are present in the sample. The color of the line ranges from light pink to dark purple, with any discrete line within either test result window considered reactive regardless of the intensity of the line. There are two procedural controls on each test device, one each for the Gram-positive and Gram-negative assays. For a test result to be considered valid, both procedural controls must be positive, indicated by the color changing from yellow to blue or purple. Typically, the assay runs to completion in less than 30 minutes. The test can be performed in batches of up to six PLT samples; a testing session may comprise several batches.

TABLE 1. Participating sites and numbers of SDP doses tested and PGD TP doses

Site no.	Institution	Number of doses tested	Number of PGD TP doses
1	Adventist Hinsdale Hospital, Hinsdale, Illinois	72	
2	The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania	98	
3	Indiana University Health, Indianapolis, Indiana	4989	
4	Cornell University, New York, New York	333	
5	Johns Hopkins Hospital, Baltimore, Maryland	563	*
6	Lahey Clinic, Burlington, Massachusetts	1273	2
7	North Shore-Long Island Jewish Health System, Manhasset, New York†	2039	
8	Oregon Health and Science University, Portland, Oregon	1700	
9	University of Colorado Hospital, Aurora, Colorado	355	
10	University of California, San Diego Medical Center, Thornton Hospital, San Diego, California	566	1
11	University of California, San Francisco Medical Center, San Francisco, California	1377	
12	University Hospitals Case Medical Center, Cleveland, Ohio‡	7672	3‡
13	University of Illinois at Chicago, Chicago, Illinois	1730	1
14	University of Minnesota, Minneapolis, Minnesota	2296	
15	University of North Carolina Hospital, Chapel Hill, North Carolina	497	1
16	Los Angeles County/University of Southern California Medical Center, Los Angeles, California	657	1
17	University of Virginia, Charlottesville, Virginia	746	
18	Vanderbilt University Medical Center, Nashville, Tennessee†	719	
	Total	27,682	9

* One contaminated dose detected by passive surveillance.
 † Concurrent culture sites.
 ‡ Two additional contaminated doses detected by concurrent culture.

Confirmatory testing of doses with initially reactive PGD test results

In the event of an initial reactive result with the PGD test, a second, freshly drawn sample was tested in duplicate with the PGD test. The PGD test was interpreted as repeatedly reactive if the initial and at least one of the repeat tests was positive based on interpretation protocols typically used for immunoassays used to test blood donors.²⁵ In addition, the fresh sample was cultured for bacteria under aerobic as well as anaerobic conditions by plating 100- μ L aliquots onto two blood agar plates or into appropriate broth culture systems. Plates and broths were incubated for up to 5 days, and any bacteria that grew were identified following standard microbiologic methods. A Gram stain also was performed on the freshly drawn sample at most sites.

Testing schedules and management of doses with repeatedly reactive PGD tests

SDP doses were tested with the PGD test on day of issue, predominantly by batch testing part or all of the SDP inventory during one testing session per day using existing staff. Testing was performed before doses were issued at 16 sites, with doses with repeatedly reactive PGD tests (see above) removed from inventory; initially reactive doses that were negative on retesting were not removed from inventory. At the remaining two sites (Sites 12 and 18), testing was performed shortly after issue to accommodate concurrent culture, with reactive doses therefore not interdicted.

Concurrent culture testing of SDP doses

At the time of PGD testing at three of the study sites (Sites 7, 12, and 18) culture of all SDP doses was performed by plating 100- μ L aliquots onto blood agar plates that were incubated under aerobic conditions for at least 48 hours. At Site 12, if the culture was positive, quantitative cultures were performed on serially diluted aliquots from the original sample that had been retained at 4°C.

Study design

Because not all bacterially contaminated PLT units are detected by prestorage culture testing,^{3,21} the goal of this population study was to determine if the PGD test could detect and interdict such breakthrough cases. The study was therefore designed as a multihospital study of the PGD test used on the day of PLT transfusion to identify bacterially contaminated SDP doses that had been released as culture-negative after testing for bacterial contamination using a prestorage growth-based system. The goal of the study was to detect approximately 10 PGD true-

positive (TP) doses. The sample size required was based on data generated during the period when early culture of apheresis units was performed (2004 onward). These data sources included estimates of residual rates of bacterial contamination of 200 to 400 per million apheresis units^{4,5,13-15,19-21} and on findings from active surveillance at University Hospitals Case Medical Center, Cleveland, Ohio.²⁶ The latter showed a bacterial contamination rate of 638 per million units, with the rate for units with bacterial loads of at least 10⁵ CFU/mL (the detection limit of the PGD test) being 438 per million units (1:2283). Based on these data sources, the sample size required to detect 10 positives therefore was estimated to be between 22,830 and 50,000 doses. A proportion of the total SDP doses tested would also be evaluated by concurrent culture at the time of PGD testing to assess the sensitivity of the PGD test, with the goal of testing 10,000 doses in this arm of the study. Analysis of results when 25,000 doses had been tested showed that the study targets had been met, and the study was therefore terminated at this point.

RESULTS

A total of 27,682 individual SDP doses, derived from 22,371 apheresis collections, were tested with the PGD assay at 18 hospitals, where more than 160 technologists were trained, during 2008 to 2010, with hospitals testing between 72 and 7672 doses (Table 1). Doses had been tested for bacterial contamination at the collection centers by BacT/ALERT (81%) or eBDS (16%) systems (method not available for the remaining 3%) and released as culture negative (see Materials and Methods). All doses tested at collection centers with BacT/ALERT remained negative after release, with one exception. One of the sites was notified that, on Day 5, a BacT/ALERT bottle had become positive, with growth of coagulase-negative staphylococci (CoNS) and *Streptococcus mitis* subsequently documented. This unit was PGD negative and had been transfused on Day 3 with no adverse reaction, and the positive BacT/ALERT culture was therefore interpreted as indeterminate.

Thirteen of the sites tested doses in one session per day, four as multiple sessions per day, and one switched from two to one session per day during the study. Valid results were obtained with the PGD test from 27,620 doses (Table 1 and Fig. 1). Nonreactive PGD results were obtained for 27,469 doses. Repeatedly reactive results, defined as positive initially and in at least one repeat test (see Materials and Methods), were found for 151 doses (0.55%; 95% CI, 0.46%-0.64%). Bacteria were isolated at six sites from nine doses with repeatedly reactive PGD results (see TP results below; Tables 1 and 2). Of the 27,469 doses with nonreactive PGD tests, 108 had had initial reactive PGD test results that were nonreactive by PGD test on retesting in duplicate; these doses were treated the same as other doses with nonreactive by PGD test results and

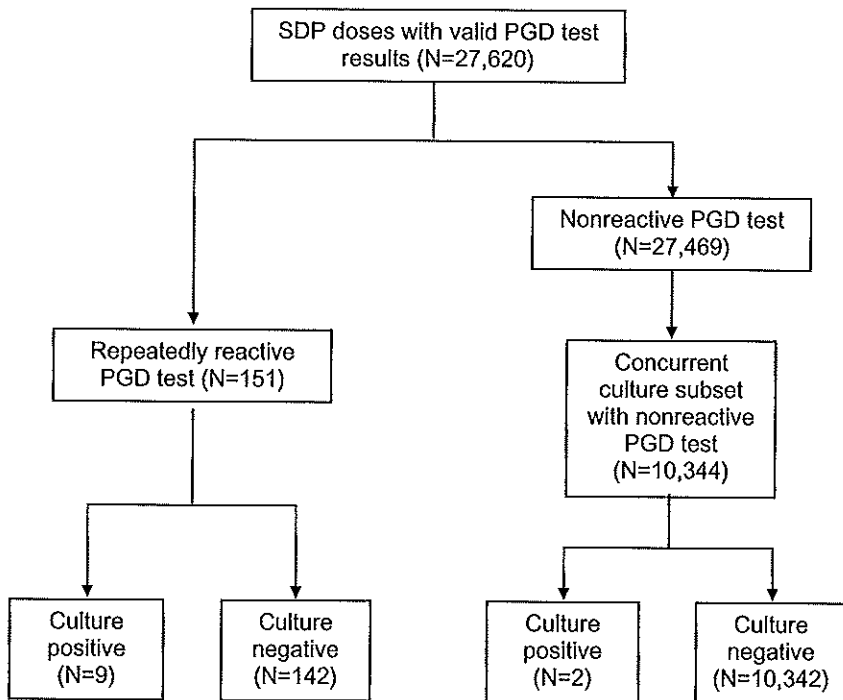


Fig. 1. Flow chart showing study design and results of PGD tests and cultures.

were not associated with transfusion reactions or positive cultures for the subset of 68 in the concurrent culture arm.

TP results

Nine of 27,620 (1:3069, 95% CI 1:6711 to 1:1617; or 326 per million, 95% CI 149-618 per million) doses were repeatedly reactive in the PGD test and confirmed as bacterially contaminated (i.e., TP) by confirmatory culture. The nine TP doses were derived from eight collections (two were from the same collection), with these units collected in house ($n = 1$) or supplied by three different regional ARC ($n = 4$) or other blood centers ($n = 4$). Seven of these units were prestorage tested by BacT/ALERT and no collection center subsequently reported a change in bacterial culture status from negative to positive for any of these doses. Two of the 9 units were prestorage tested and released by a blood center using eBDS. All bacterial species isolated from these TP doses were Gram-positive and included CoNS ($n = 6$), *Bacillus* species ($n = 2$), and *Enterococcus faecalis* ($n = 1$) (Table 2). Two TP doses (Cases 2 and 3 in Table 2) were from a split collection. One of the doses contaminated with a *Bacillus* species also contained *Propionibacterium acnes* (Case 1 in Table 2), and another dose with CoNS also contained *Peptostreptococcus* sp. (Case 5 in Table 2). Samples from seven of these nine TP PLT bags revealed the presence of bacteria by Gram stain indicating bacterial loads of at least 10^5 CFU/mL. Quantitative culture, performed on the three contaminated doses detected at Site 12, showed bacterial counts of 1.3×10^6 and

1.2×10^7 CFU/mL for the doses containing CoNS and 1×10^7 CFU/mL for the dose containing *Bacillus* species. Two contaminated doses containing CoNS from Site 12 were transfused before PGD testing had been performed, with no transfusion reaction in one case, while in the other case transfusion resulted in a severe septic transfusion reaction. The other contaminated doses were not transfused.

False-positive results

A total of 142 false-positive results were obtained (0.51%).

Frequency of contaminated PLT units with PLT age

Of the nine TP doses, four were 3 days old, two were 4 days old, and the remaining three were 5 days old (Table 3). All nine TPs were Gram-positive contaminations and were detected at their first and only PGD testing time point. There was no obvious relationship between the prevalence of bacterial contamination and PLT age.

Results of concurrent culture testing of SDP doses

Among the 10,424 doses tested with concurrent culture five doses yielded positive cultures (1:2085). The PGD test was repeatedly reactive for three of these doses (TP, Cases 7-9 in Table 2) and nonreactive for two (false negative [FN], Cases 10 and 11 in Table 2). The initial PGD test result for the first of these FN doses was invalid, and repeat testing showed one invalid and one nonreactive result. This dose was a 5-day-old PLT unit transfused at an outpatient location, and the patient experienced a septic transfusion reaction. Gram stain of this dose was positive and concurrent culture yielded *Streptococcus oralis* (viridans group streptococcus) at 1.2×10^7 CFU/mL. The second FN dose was contaminated with 4×10^2 CFU/mL of CoNS, and no transfusion reaction occurred in the recipient. One of the two FN doses was supplied by a regional ARC center using BacT/ALERT and the other by an independent blood center using eBDS.

Detection of transfusion reactions associated with bacterial contamination at sites not performing concurrent culture

Of the 17,196 doses not studied with concurrent culture, one contaminated unit was detected (1:17,196;

TABLE 2. Prestorage culture-negative SDPs identified as bacterially contaminated by active surveillance using the PGD test on day of transfusion (cases 1-9), by concurrent culture (cases 10-11) or by passive surveillance (case 12)

Case number	Bacterial species isolated	Age of dose (days)	PGD result	Confirmation method for bacterial contamination*	Bacterial load (CFU/mL)	Transfusion status
1	<i>Bacillus</i> sp. and <i>P. acnes</i>	3	Reactive	BC	†	Not transfused
2‡	CoNS	3	Reactive	PC; GS	†	Not transfused
3‡	CoNS	3	Reactive	PC; GS	†	Not transfused
4	<i>E. faecalis</i>	3	Reactive	PC; GS	†	Not transfused
5	CoNS and <i>Peptostreptococcus</i> sp.	4	Reactive	PC; BC; GS	†	Not transfused
6	CoNS	4	Reactive	PC		Not transfused
7	CoNS	5	Reactive	PC; GS	1.3 × 10 ⁶	No transfusion reaction
8	<i>Bacillus</i> sp.	5	Reactive	PC; GS	1 × 10 ⁷	Not transfused
9	CoNS	5	Reactive	PC; GS	1.2 × 10 ⁷	Severe septic shock
10	<i>S. oralis</i>	5	Nonreactive	PC; GS	2 × 10 ⁷	Septic reaction
11	CoNS	5	Nonreactive	PC	4 × 10 ²	No reaction
12	<i>S. sanguinis</i>	5	Nonreactive	BC; GS§		Allergic, nonfebrile reaction

* BC = broth culture; GS = Gram stain; PC = plate culture.

† Not determined.

‡ Doses obtained from split SDP collection.

§ Initial Gram stain negative; repeat Gram stain positive.

TABLE 3. Frequency of contaminated doses detected by PGD test by age of SDP doses

Number of doses	PLT age (days)				Total
	≤2	3	4	5	
Number (%) tested	4036 (15)*	8375 (30)	6660 (24)	8549 (31)	27,620†
TP PGD test	0	4	2	3	9

* Percentage of SDP doses tested.

† Doses with valid PGD results.

58/million doses) by passive surveillance as a result of investigation of an allergic, nonfebrile transfusion reaction at Site 5 and was associated with contamination with *Streptococcus sanguinis* (Case 12 in Table 2). This dose was supplied by a regional ARC center using BacT/ALERT.

Reproducibility in cocomponents

PGD performance within an apheresis collection was examined in a total of 10,014 SDP doses represented by 4720 apheresis collections. Two collections each comprising two doses had one contaminated dose and one sterile dose (Table 2, Cases 4 and 7), illustrating that all cocomponents from a collection should be tested as the bacterial status of one dose does not necessarily predict the status of its cocomponent. Excluding these two cases, there were 19 discordant results among doses split at or postcollection, so agreement in this group was 99.6% (4699/4718 collections).

DISCUSSION

We herein report results of a multisite study of the detection of bacterial contamination with the PGD rapid test

on day of issue in apheresis PLT doses collected in house or supplied by external collection centers and released as negative for bacterial contamination after prestorage culture. Of the 27,620 apheresis doses studied, nine doses (1:3069 or 326/million) were found to be contaminated with Gram-positive bacteria using the PGD test. Two additional doses, also contaminated with

Gram-positive bacteria, were detected in the subset of 10,424 doses that were assessed with concurrent culture, and one additional contaminated dose was detected as a result of investigation of an allergic transfusion reaction. Of the 12 bacterially contaminated doses identified, six came from five different ARC regions, five came from three other blood centers, and one was a hospital self-collected unit. Among these contaminated doses, three had been tested using eBDS and nine using BacT/ALERT. Thus, the prevalence of bacterial contamination detected in this study in spite of prestorage testing was 1:2302 or 434/million doses (Table 2). These findings document the continued presence of bacterial contamination in the apheresis PLT supply despite prior testing by early culture. Further, as bacterial levels of at least 10⁵ CFU/mL have previously been reported to be associated with significant transfusion reactions in 69.6% of cases,¹⁵ quantitative culture and Gram stain results suggest that bacterial titers in eight of these 12 doses were high enough to pose a significant risk of a severe transfusion reaction. In fact, serious transfusion reactions indeed occurred with two (Cases 9 and 10) of the three contaminated doses with high bacterial titers (Cases 7, 9, and 10) that were transfused.

While the PGD test detected nine of the 12 contaminated doses reported in this study, the clinical significance of the cases found is an important consideration in evaluating the effectiveness of the PGD test in interdicting contaminated units. Seven of the nine doses with TP PGD results had high bacterial titers based on quantitative culture or positive Gram stain results (Table 2), with severe septic transfusions likely.¹⁵ In fact, a severe septic reaction did occur in the recipient of one of the two doses in this group that were transfused (Cases 7 and 9 in Table 2). In contrast, in the cases not detected, no reaction occurred in the recipient of a dose containing a very low inoculum of CoNS (Case 11 in Table 2), while only an allergic, nonfebrile reaction occurred in Case 12. It is likely that a few other instances of contamination with low inocula of CoNS were present in the doses not studied by concurrent culture. The only notable reaction in the FN group was in Case 10, where the PGD test did not detect *S. oralis* due to the rare LTA class^{27,28} of this uncommon PLT contaminant,^{2,6,15} and it is highly improbable that other doses with high inocula of this or other pathogens were present in the doses not studied by concurrent culture. Thus we believe that the PGD test detected all but one of the contaminated doses with the potential to result in significant septic reactions.

In addition to the bacterially contaminated doses detected in doses included in the study cohort, two additional contaminated doses were detected at one of the study sites that tested only a portion of the PLT doses during the study. These two bacterially contaminated doses not included in the study were detected by passive surveillance after septic transfusion reactions. In both cases, Gram stains were positive and CoNS was identified as the contaminant. The PGD test, performed on one of these doses when the transfusion reaction was reported, was repeatedly reactive. The other dose was not PGD tested.

Our findings are consistent with several other published studies. Isolation of only Gram-positive bacteria in contaminated PLT doses here is consistent with recent reports of high prevalence of Gram-positive bacteria identified in early culture as well as at time of issue of apheresis PLT units.^{13,14,22,23} Results from this study are similar to recent US surveillance studies, which showed a prevalence of bacterial contamination by active surveillance of around 500/million apheresis units that were released from collection centers.^{9,15} Based on the 2008 US PLT utilization of 1.7 million apheresis units,²⁹ this can be extrapolated to an annual incidence of approximately 850 bacterially contaminated units per year, with approximately 40% of these likely to result in septic reactions.¹⁵

Our findings also demonstrate the superiority of active surveillance of SDP doses on the day of transfusion using the PGD test compared to passive surveillance. Active surveillance with PGD detected nine contaminated

doses in a population of just 27,620 doses released by collectors as culture negative. In comparison, in a far larger study of 781,936 apheresis PLT donations by the ARC, only nine (1:86,882 or 12/million) culture-negative units issued were detected by passive surveillance.⁴ Our findings provide further confirmation that prestorage culture of apheresis units has only limited success in detecting units contaminated with bacteria.

Bacterial contamination of doses derived from the same apheresis donation also present a challenge as the probability of one dose being contaminated is much higher if a dose derived from the same collection is contaminated.^{4,8,14} Quarantining of all doses derived from the same SDP donation should be considered.

Previous studies of apheresis PLTs retested by culture at outdate provide limited insight into when contaminated units grew to high titers and what ultimate titer was attained. In this study, in seven of the nine cases detected by PGD, contaminating bacteria were shown to be at concentrations that were at least 10^5 CFU/mL based on detection by Gram stain.^{8,30} These findings suggest that PLT doses had been held for sufficient lengths of time to allow low levels of contaminating bacteria to grow to dangerous titers, occurring in four units that were 3 days old and two that were 4 days old. These data indicate that the risk of high levels of contamination is significant and relatively constant throughout PLT shelf life at the hospital.

As discussed above, two contaminated units were not detected by the PGD test in the subset of doses where concurrent culture was performed. One of these doses was contaminated with *S. oralis* (a viridans group streptococcus) at the level of 2×10^7 CFU/mL and transfusion resulted in a septic reaction. The PGD test was developed to detect bacterial species that have been implicated in PLT transfusion reactions. The LTA structure of Gram-positive bacteria can be broadly divided into four types.^{27,28} Most Gram-positive bacteria pathogenic to humans are categorized as Type 1 LTA (e.g., *Bacillus* species, staphylococci, and most streptococci) or Type 3 LTA (e.g., *Clostridium* species). Type 2 LTA (e.g., *Bifidobacterium* species) is not considered a human pathogen. Type 4 LTA is found in a few species of viridans group streptococci (e.g., *S. mitis* and *S. oralis*). Viridans group streptococci are rare PLT contaminants,^{2,6,15} and *S. oralis* and *S. mitis* are uncommon members of this group.^{27,28} The current version of the PLT PGD test uses antibodies designed to react with Types 1 and 3 LTA as the other types had not been reported as PLT contaminants at the time the original PGD antibodies were developed. Type 4 LTA antibodies currently are in development for inclusion in the assay.

The second of these doses not detected by the PGD test was contaminated with low levels of CoNS (400 CFU/mL), which is below the limit of detection of the PGD test. The reason for failure of the PGD test to detect the

additional dose contaminated with *S. sanguinis* that was detected by passive surveillance is not known as the bacterial inoculum was not determined; however, the original Gram stain of the unit was negative so it is likely that the bacterial load was below the limit of detection of the PGD test as further testing showed that the isolate was detectable by the PGD test in inoculated PLTs (data not shown).

Before this study the only published report of testing with PGD in a routine setting on day of transfusion was by Yazer and colleagues²³ who studied the PGD test on 7731 whole blood-derived, random-donor PLT pools. In that study testing was performed at time of issue, with two TPs (1:3866 or 259/million) and 10 false-positives (0.13%) found. One pool was contaminated with CoNS and the other with *Streptococcus agalactiae* (a Group B streptococcus). Other investigators have published studies of the analytical sensitivity of the PGD test and the results of growth studies after single apheresis units were spiked with low titers of various bacteria.^{31,32}

In this study, the rate of false-positive results from the PGD tests was 0.51% (142 false-positive results). Yazer and coworkers²³ found a false-positive rate of 0.13% among 7731 whole blood-derived PLT pools tested with the PGD test. The reason for the lower rate with whole blood-derived PLT pools may be related to dilution of interfering substances such as rheumatoid factor present in one of the units included in a pool. At a false-positive rate of 0.51%, 8670 apheresis doses could potentially be discarded per year in the US apheresis PLT supply. However, in practice, this may not be the case as the rate of outdate for apheresis units is as much as 5% in many institutions, and losses from positive PGD tests may be ameliorated by replacement from existing inventory that is likely to outdate. Surrogate tests of bacterial contamination, such as glucose and pH levels, in addition to having very poor sensitivity, had much higher false-positive rates.^{8,9,13} It is unfortunately the case with rare conditions, such as bacterial contamination of PLTs, that FP rates will inevitably be higher than TP rates.

Pathogen inactivation, which is not currently approved for use in the United States, has been proposed as a solution to the presence of a wide range of contaminants, including bacteria.⁵ This could obviate the need for prestorage culture and at-issue testing and possibly the need for testing for microbial antigens, nucleic acid sequences, or antibodies directed against many infectious agents. Further developments with this technology are eagerly awaited.

Limitations of our study include the limited number of contaminated doses encountered, the limited spectrum of bacterial species detected, and the limited number of concurrent cultures performed. Detection of Gram-negative species could not be assessed because none were encountered during the study, a finding consistent with the rarity of contamination with

Gram-negative species.^{6,15} While one report indicates that the analytic sensitivity of the PGD test was lower for some strains of *Klebsiella pneumoniae* and *Escherichia coli*,³¹ this is unlikely to have had an impact on the results of this study. Only four fatal instances of apheresis PLT transfusions resulting from Gram-negative bacteria were documented by the FDA over 2005 through 2009.⁶ As transfusion of Gram-negative bacteria generally results in severe and immediate septic reactions,^{7,8,15} it is unlikely that any such instances occurred but were not recognized in our study. Furthermore, ARC data indicate that few Gram-negative bacteria are missed by early culture.⁴ Additional limitations include a lack of site-specific information on use of measures taken by collection centers to limit bacterial contamination, such as method of skin disinfection, use of diversion pouches, and volume of product cultured and use of anaerobic bottles with the BacT/ALERT system. However, all SDP studied met current AABB Standards, including those for sterility.

In conclusion, this large-scale, multisite study of the PGD test, applied to more than 27,000 apheresis PLT doses on day of issue, detected nine bacterially contaminated units that were negative by prestorage culture. If the PLT doses studied are representative of the US apheresis PLT supply of 1.7 million doses per year, this detection rate can be extrapolated to detection of 326 contaminated units per million doses or approximately 550 contaminated units per year. Based on transfusion reactions that occur in approximately two-thirds of recipients with transfusion of at least 10⁵ CFU/mL of a variety of Gram-positive bacteria¹⁵ and recent FDA fatality data,⁶ interdiction of such contaminated units in the US apheresis PLT supply has the potential to prevent more than 300 transfusion reactions and several fatalities per year, and use of the PGD test has the potential to detect the majority of these cases.

CONFLICT OF INTEREST

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