Analysis of Bacterial Detection in Whole Blood–Derived Platelets by Quantitative Glucose Testing at a University Medical Center

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Key Words: Platelets; Bacterial contamination; Bacterial detection; Transfusion medicine

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Upon completion of this activity you will be able to:
• discuss the scope of the problem of transfusion-transmitted bacterial infection and its clinical ramifications.
• list several strategies and methods currently employed to abrogate the risk of platelet bacterial contamination.
• compare various methods of bacterial detection in terms of threshold of detection, test sensitivity, and false-negative rates and correlate this clinically.

Abstract

After the March 2004 implementation of American Association of Blood Banks standards regarding platelet bacterial detection, we began quantitative glucose screening of whole blood–derived platelets (WB-P). The glucose level was measured immediately before component release—often storage day 4 or 5—using the Glucometer SureStep Flexx Meter (LifeScan, Milpitas, CA), with a positive cutoff of less than 500 mg/dL; failing units were cultured and not transfused. During 29 months (March 1, 2004–July 31, 2006) 93,073 units of WB-P were tested. Initially, 929 units (0.998%) screened positively. Bacterial growth was culture-confirmed in 6 units, for a bacterial contamination incidence of 0.006% and a true-positive rate of 6.4/100,000. Three additional culture-confirmed contamination cases were detected in transfused units causing febrile nonhemolytic reactions, for a false-negative rate of 3.2/100,000. Our overall contamination prevalence was 9.6/100,000 units of platelets transfused, lower than ordinarily cited, and showed a false-negative rate remarkably congruent to that of culture: 3.2/100,000. A low-sensitivity screening test applied late in platelet shelf-life can be comparable to culture in preventing bacterial-related morbidity.

Bacterial contamination of blood components is a longstanding problem in transfusion medicine that is only partially controlled by modern phlebotomy practices and improving materials for and technology of transfusion product collection and storage. Transfusion-associated sepsis remains a significant risk of blood transfusion and, in fact, is increasing in relative importance as the efficiency of viral testing improves.1–5 Whereas the risk of transfusion-related transmission of viral disease has steadily decreased during the past couple of decades, the risk of bacterial infection transmission has remained unchanged and now poses a greater threat than the combined risks of receiving a blood product contaminated with HIV-1, HIV-2, hepatitis C virus, hepatitis B virus, or human T-cell lymphotropic virus I or II1 and now surpasses the incidence of viral transmission by 2 orders of magnitude.2–5 Bacterially contaminated platelets have become the most common source of transfusion-transmitted infection and the consequent sepsis, with high associated morbidity and mortality.6–8 Bacterial contamination of blood components is the third most frequent cause of transfusion-related deaths reported to the US Food and Drug Association (FDA), following transfusion-related acute lung injury and hemolytic reactions, accounting for 12.0% of transfusion-related deaths (15/125) in 2005 and 2006 combined.9 The risk of sepsis-related mortality due specifically to platelet transfusion reportedly ranges from 1 in 20,000 to 85,000 donor exposures, with a potential of 100 to 150 cases of serious morbidity annually.5

As this risk was gaining attention through the 1990s, in the wake of publicity surrounding transfusion-associated viral transmission in the 1980s, the FDA sponsored several workshops with the intent to elucidate salient issues and to
foster development of technological interventions to mitigate this risk, while the Centers for Disease Control and Prevention undertook a national surveillance study of bacterial contamination in blood products.\textsuperscript{10-12} What followed was a proliferation of studies that built a body of data striving to characterize the clinical impact and influence of bacterial contamination.

The present storage requirement mandated to preserve platelet survival and function (22°C ± 2°C) provides an effective milieu for bacterial incubation and growth vs the refrigerated (1°C-6°C) or frozen (<18°C) parameters for RBC and plasma components, respectively.\textsuperscript{13} Consequently, not only are the complications of bacterial contamination and sepsis seen more commonly in platelets than in other whole blood components but also older platelet units are at increased risk of developing clinically significant bacterial loads compared with their fresher counterparts.\textsuperscript{14,15} Therefore, transfusion safety strategies during the past several years have focused on implementing new regulatory standards for and development of platelet testing methods directed at pretransfusion identification of bacterial contamination and after-the-fact identification and confirmation that patient sepsis indeed arose from a given platelet product.

The reported prevalence of bacterially contaminated platelet products varies significantly in the extant literature, with estimates ranging from 1 in 1,000 to 3,000 platelet products by differential centrifugation to separate platelets from other components, and each unit was leukocyte-reduced before storage. At the time of this study, SDP units were infrequently used, and it was decided to maintain the platelet product mix used before the study period.

Materials and Methods

Patient Population

The patients herein described were treated at the University of Minnesota Medical Center, Fairview, a 1,700-bed academic tertiary-care center, between March 2004 and July 31, 2006. Throughout the 29-month study period, patients received transfusions as medically indicated with pooled, WB-P concentrate (5 concentrates per pool), and single-donor apheresis platelets. The majority of platelets were given to adult hematology-oncology patients, particularly patients with acute leukemia having induction, reinduction, or consolidation chemotherapy or patients undergoing allogeneic or autologous marrow or peripheral hematopoietic progenitor cell or umbilical cord progenitor cell transplantation.

Platelet Sources and Preparation

Our institution is a transfusion service, and as such, we receive platelet products from an outside supplier as a mix of apheresis and WB-P. At the time of the study, two thirds of our use was pooled WB-P and the balance SDP units, and our per annum use was approximately 60,000 platelet equivalents, with 1 equivalent to a single WB-P unit. Platelets were obtained from the American Red Cross (ARC) North Central Blood Region, St. Paul, MN, ordinarily on a standing-order basis and disbursed oldest units first. As mandated by the American Association of Blood Banks (AABB), the outdate time was 5 days for all platelet units.\textsuperscript{24} SDP units were prepared via AABB plateletpheresis guidelines and standards, while WB-P concentrates were produced from single whole blood units by differential centrifugation to separate platelets from other components, and each unit was leukocyte-reduced before storage. At the time of this study, SDP units were infrequently used, and it was decided to maintain the platelet product mix used before the study period.

Surveillance Methods

Active Surveillance (Prospective)

The St Paul, MN, American Red Cross implemented culture of apheresis platelet units to comply with the AABB standard, while we elected to use quantitative glucose measurement of WB-P concentrates. Because platelet pooling is done immediately before issuing the product for transfusion, culture-based tests are logistically difficult. As an alternative, we chose the Glucometer SureStep Flexx Meter (LifeScan, Milpitas, CA), a point-of-care, automated monitoring system, frequently used for home monitoring by people with diabetes, that measures plasma glucose via the glucose oxidase method.

To cover the span of glucose concentrations expected in platelet concentrates prepared with plasma and anticoagulant solution, the glucometer was operated in its “linearity” mode, which enables numeric glucose determinations in a range of 0 to 800 mg/dL, compared with the range of 0 to 500 mg/dL ordinarily reflected in the manual mode.

The glucose measurement method was validated for accuracy and precision, and linearity was confirmed for the range in which the glucose concentration was measured. A
positive screening cutoff of less than 500 mg/dL was chosen on the basis of bacterial spiking studies before implementation and correlated with the appearance of bacterial growth during those studies.

Each of 5 WB-P concentrates was tested before pooling within 12 hours of transfusion. A small drop of platelets from the segment is expressed onto the reagent strip reaction pad, which is inserted into the SureStep Flexx Meter, and the results are reported on the instrument screen within 2 minutes. Units with a glucose concentration measured at more than 500 mg/dL had affixed to them stickers indicating suitability for issue within 12 hours. Those measuring less than 500 mg/dL were cultured and not approved for issuance. Maintenance and quality control of the Flexx Meter was performed daily and when questionable or spurious test results occurred.

Passive Surveillance (Transfusion Reaction–Triggered)

Transfusion reactions reported to the transfusion medicine service were investigated by examination and, in some cases, culture of residual platelet product, if any, and return of the transfusion set to the blood bank; review of clinical staff observations and patient findings recorded in the filed report of suspected transfusion reaction; speaking with and examining the patient when possible and indicated; and interview of clinical staff present during the reaction.

Microbiologic Procedures

Gram Stain

Gram stains were routinely performed on residual platelet product returned to the blood bank for transfusion reaction investigation unless not enough component remained to produce a stainable specimen. Dried smears of platelet specimens were prepared from 10 to 15 μL of product on a glass slide, stained per standard methods, examined microscopically at ×1,000 magnification under oil, and interpreted as negative or positive on the basis of the absence or presence of bacteria, respectively. When bacteria were found, a categorization of gram-positive or gram-negative was accordingly determined.

Culture for Platelet Concentrates Screening Positively

By using aseptic technique under a laminar flow hood, 4 to 5 mL of platelets were withdrawn by syringe from the component bag, and standard aerobic bottles were inoculated. An automated microbial detection system (BacT/ALERT, bioMérieux, Durham, NC) was used, and bottles were incubated (36°C ± 2°C) for 7 days or until a positive reaction was bated (36°C ± 2°C) for 7 days or until a positive reaction was indicated by the monitor unit of the BacT/ALERT system.

Culture for Transfusion Reaction

Transfusion reactions were investigated by aseptically inoculating an automated aerobic liquid culture bottle with residual platelet concentrate, incubated at 35°C. When the returned component bag and transfusion set are essentially empty, a specimen for culture is obtained by aseptically rinsing 5 mL of sterile normal saline through the set, retrieving it, and inoculating this rinse into an automated liquid aerobic culture bottle. Specimens are incubated for 7 days or until positive, whichever occurs first. Bottles are uniformly discarded 12 days postinoculation.

Results

From March 1, 2004, to July 31, 2006, samples were collected from 93,073 WB-P units Table 1. A total of 929 samples (1.0%) initially screened as positive. Of these units, confirmatory culture was positive in just 6 cases (0.65%; 0.006% of the total), whereas 923 showed no growth. The false-positive rate was 0.99% (923 of 93,073 concentrates). None of the platelet units with positive screening tests was ultimately transfused. The organisms detected were consistent with skin flora, ordinarily associated with nonfatal septic transfusion reactions; the majority were coagulase negative Staphylococcus (CONS) species, with 1 case each of α-hemolytic Streptococcus and Propionibacterium acnes Table 2. None of these concentrates had a positive Gram stain.

Detecting only 6 cases of bacterial contamination in more than 93,000 platelet concentrates seemed unduly low in context of a mean platelet contamination rate of 33.9 per 100,000
reported in the US literature.\textsuperscript{20,25-28} In an attempt to capture cases of infused contaminated products, reported transfusion reactions concomitant with the study period were reviewed. During the 29-month period, 383 total reactions were reported, with approximately one third (132 cases) associated with platelet transfusion; the majority of these were allergic reactions. Investigation of these reactions included culture of the residual platelet concentrate or transfusion set in 234 cases (61%); reactions that were clearly allergic—with isolated hives or rash—were not subjected to Gram stain and culture. Cultures are performed in the transfusion reaction investigation by inoculation of aerobic bottles with residual platelet concentrate when it remained, and if the entire component volume had been infused, a culture specimen is derived by rinsing the transfusion set with a small amount of normal saline, which is then used to inoculate the culture bottles.

Although 9 products were positive by culture, only 3 were truly positive due to contamination of the component infused; 6 false-positives were inadvertently contaminated during the culture process.\textsuperscript{11} The suspected transfusion reactions linked to the positive cultures were judged to be truly contaminated; all had positive Gram stains of saline rinses of the respective transfusion sets, all patients had chills and rigors as their predominant symptoms with documented temperature elevations of 3.4°F to 6.1°F (1.9°C-3.4°C), and positive subsequent blood cultures had results identical to those from the transfusion sets (except in the case of \textit{Bacillus cereus}, discussed subsequently).

None of the 6 cases of positive cultures judged to be contaminated during the culture process had positive Gram stains; in 4 cases, blood cultures were not done, and in the 2 patients whose samples were cultured, the results were negative. Clinically, 2 reactions included chills and rigors as predominant symptoms, but in the context of a temperature decrease in one of 0.6°F (0.3°C) and an increase of 0.3°F (0.1°C) in the second, neither was ruled febrile or septic. In both cases, the symptoms were judged as resulting from the patients’ underlying conditions (in the first case, the chills occurred with nausea, and the patient was admitted for nausea and diarrhea; the second patient had neutropenic fever). In 1 case, fever was the only symptom, but the documented temperature increase was from 98.7°F (37.1°C) to only 100.7°F (38.2°C) in the absence of systemic inflammatory response, after which the patient remained afebrile. In the other 4 cases, the principal complaints were more allergic in nature, and temperature changes during the platelet infusion ranged from a decrease of 0.4°F (0.2°C) to an increase of 0.1°F (0.0°C-0.1°C). The organisms detected by culture of components involved in reactions were consistent with those reported in the literature in connection with nonfatal septic reactions associated with contaminated platelets, in particular, gram-positive organisms in platelets issued on days 4 or 5 of storage (Table 3).\textsuperscript{12}

In the first true septic reaction, 5 ABO-identical, prestorage leukocyte reduced platelet concentrates were pooled and transfused to a 46-year-old patient with thrombocytopenia and myelodysplastic marrow syndrome, following allogeneic bone marrow transplantation. During the infusion, his temperature increased from 96.3°F (35.8°C) to 100.8°F (38.3°C) with accompanying rigors and shortly thereafter to 101.7°F (38.8°C); resolution of the infection occurred during the next several days with oral antibiotic treatment. Subsequent blood culture grew CONS, and a Gram stain of residual platelets demonstrated gram-positive cocci in clusters that on culture revealed CONS with the same biochemical profile as found in the patient’s blood.

The second reaction occurred in a pancytopenic 41-year-old patient whose transfusion occurred on days 4 or 5 of storage (Table 3).\textsuperscript{12}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Organism} & \textbf{Gram Stain} & \textbf{Specimen} & \textbf{Preinfusion} & \textbf{Postinfusion} \\
\hline
\textbf{True septic reactions}\textsuperscript{1} & & & & \\
CONS & GPC & Saline rinse & 96.3 (35.8) & 102.3 (39.1) \\
\textit{Bacillus cereus} & GPR & Saline rinse & 100.1 (37.9) & 103.5 (39.8) \\
\hline
\textbf{Contaminated cultures}\textsuperscript{1} & & & & \\
\textit{Corynebacterium} & GPC & Saline rinse & 99.0 (37.3) & 104.2 (40.1) \\
CONS & Negative & Negative & 98.6 (37.0) & 98.2 (36.8) \\
\textit{Bacillus cereus} & Negative & Saline rinse & 100.0 (37.9) & 98.4 (36.9) \\
CONS & Negative & Saline rinse & 99.0 (37.3) & 103.4 (36.7) \\
\hline
\textbf{Blood Culture Result} & CONS & CONS & CONS & CONS \\
\textbf{Clinical Symptoms} & Chills, rigors & Chills, rigors, hypoxia, tachycardia, tachypnea, dyspnea & Chills, rigors, nausea, vomiting, headache & Chills, rigors, nausea, vomiting, headache \\
\hline
\end{tabular}
\caption{Analysis of False-Negative Reactions Found in Transfusion Reaction Review}
\end{table}

CONS, coagulase-negative \textit{Staphylococcus} species; GPC, gram-positive cocci; GPR, gram-positive rods; ND, not done.

\textsuperscript{1} Both the residual component/transfusion set culture and patient blood culture grew identical organisms, and clinical signs and symptoms were strongly suggestive of sepsis.

\textsuperscript{2} Falsely positive cultures thought to be contaminants occurring during the culture process.
old patient 18 days after myeloablative double umbilical cord blood transplantation for acute myelogenous leukemia. During infusion of a pool of 5 units of WB-P, chills and rigors, dyspnea, worsening hypoxia, tachyplea (to 56 respira
tions per minute), and tachycardia (to 150 beats per minute) developed; his temperature increased from 100.1°F (37.9°C) to 103.5°F (39.8°C). Culture of the transfusion set saline rinse grew B c
erus with broad sensitivity to antibiotics, including the antibiotics he was receiving at the time. Despite blood cultures negative for B c
erus, the patient septic shock developed, requiring subsequent intubation; the clinical picture was complicated owing to concomitant fungal pneumonia that pre
dated the transfusion reaction. The patient later recovered to his pretransfusion baseline, with continued CONS bacteremia and severe fungal pneumonia.

The third reaction occurred in a 34-year-old patient who had previously received extensive chemotherapy and radiotherapy and natural killer cell infusion and was receiv
ing maintenance therapy with biweekly platelet infusions for thrombocytopenia. She received a pool of 5 platelet concentra
tes without acute sequelae, but rigors, nausea, headache, and fever (temperature to 105°F [40.6°C]) developed 1 hour following the infusion. A Gram stain of the saline rinse from the infusion set and platelet bag showed gram-positive cocci in clusters; culture of the saline rinse from the transfusion set and platelet bag and from the patient’s blood both grew CONS. The patient was treated with broad-spectrum oral antibiotics.

**Discussion**

Bacterial contamination of blood components persists as a significant clinical complication of transfusion, primarily of platelet therapy. In the literature, the most commonly reported estimates of the prevalence of contaminated platelets, before the implementation of required screening, included as many as 1 in 1,000 to 3,000 concentrates of SDP or WB-P units as contaminated.\(^7,8,29\) Since the institution of screening, the American Red Cross has reported a decrease to approximately 1 in 5,000 units of SDP.\(^30\)

The relationship between infusion of a contaminated component and subsequent clinical ramifications is not well defined, with perhaps 17% to 25% of contaminated infusions yielding clinically reportable sepsis and 1 in 3 to 5 of these cases resulting in death.\(^12,20,31\) This discrepancy is frequently attributed to loose criteria for septic reactions, broad variations in reporting, masking of reactions by concomitant treat
m ent with antimicrobials and antipyretics for other issues, and underrecognition of adverse events. It is, in any case, clear that morbidity and mortality arising from this therapeutic intervention are not trivial, with an associated death rate esti
mated in the BaCon study of 1 in 500,000 units for SDP and pooled platelets,\(^12\) which should be considered in the context of platelet transfusions nearing 3 million units annually in the United States (on the basis of 2004 data, with 1.4 million apheresis units and 1.5 million random donor units).\(^32\) Eder et al\(^30\) recently reported a death rate of 1:498,711 per distributed apheresis platelet unit in a 2-year period, supporting the BaCon estimate. More concerning are reported death rates 10-fold higher than this estimate in the university hospital setting, ranging from 1 in 17,000 to 1 in 50,000.\(^5,33,34\)

In consideration of these statistics highlighting the conundrum of contaminated platelets, a new AABB standard was implemented March 1, 2004, regarding bacterial detection in platelets. The AABB’s standard requires member blood banks and transfusion medicine services to effect measures to limit and detect bacterial contamination in all platelet components: “5.1.5.1 The blood bank or transfusion service shall have methods to limit and detect bacterial contamination in all platelet components.”\(^35\) Similarly, the College of American Pathologist’s Transfusion Medicine Checklist (TRM 4955, Phase II) asks “Does the laboratory have a validated system to detect the presence of bacteria in platelet components?” indicating the necessity of such a measure.\(^36\)

Strategies implemented in attempts to decrease the morbidity and mortality associated with transfusion-associated bacteremic and septic episodes coalesce around 1 of 3 pri
mary approaches: avoidance, detection, and decontamination. Abrogating the risk of bacterial contamination at collection has been addressed with improvements in donor screening and skin disinfection, which reduces bacterial load, but cannot entirely sterilize the phlebotomy site. When combined with diversion of the initial aliquot of blood, enhanced donor arm skin preparation has been shown to reduce bacterial contamination from 47% to 77% of residual levels.\(^37\) Barring prevention of introduction of bacteria into the component, optimization of platelet processing and storage may contribute to mitigation of the involved risk, as septic events linked to platelets occur most often with units stored 3 or more days (permitting organism proliferation), for example.\(^38\)

Widely diverse methods have been applied to the task of pretransfusion detection of bacterial contamination in platelet concentrates since regulations calling for such measures were implemented. Fortunately, the standard enacted is quite open
dended, permitting use of any one or more of the broad mea
sures available. The least sensitive methods, those generally requiring a threshold of organisms in concentrations of 10³ or more colony-forming units (CFU)/mL for detection, rely on gross visual inspection of the product for evidence of bacterial contamination, such as a change in product color or loss of the “swirling” quality of platelets (a characteristic, pearly wave
like light diffraction by the movement of platelets seen with unit inversion when discoid platelets align with fluid flow that
is lost when platelets become spherical in presumably infected units).  Of slightly more sensitivity are methods detecting metabolic evidence of bacterial presence, such as qualitative pH and glucose indicators (on reagent urine dipsticks), and direct visualization with Gram stain, with detection thresholds approximating 10^7 CFU/mL.  More sensitive methods, such as ribosomal RNA probes based on detection of highly conserved regions of bacterial ribosomal RNA–labeled antibiotic probes that capitalize on the capacity of antibiotics to bind specific bacteria and microscopy with acridine orange staining, become feasible with inoculum exceeding 10^5 CFU/mL, but they may take up to day 4 of storage to attain this level.  Of course, the most sensitive methods are the bacteriologic gold standard of sterility—culture (automated liquid-based systems can reliably detect as few as 10 CFU/mL) and the newer and more rapid molecular technique, real-time polymerase chain reaction (enabling detection of 50 CFU/mL within 4 hours).

Ideally, a bacterial sterility test should be inexpensive, specific, noncomplex, sufficiently sensitive, and expeditious.  The problem of bacterial contamination in blood components is dynamic because bacteria grow during the storage period, amplifying the signal(s) to be detected so that tests applied later in the storage period can be less sensitive and potentially equally as effective as more sensitive tests applied earlier in the storage period—this is a unique characteristic of the blood component testing situation. Based on this hypothesis, we chose to evaluate a quantitative glucose assay as a screening test for bacterial contamination in WB-P concentrates immediately before pooling. The glucose measurements were performed by using a handheld point-of-care testing device, the SureStep Flexx Meter, chosen for its ease of use and ready incorporation into the day-to-day activities of a busy hospital blood bank and because our point-of-care laboratory already had expertise not only in use, maintenance, and validation, but also in the operational parameters, limitations, and linearity of the instrument.

Our study detected a contamination rate of 0.006% for WB-P concentrates (6/93,073, or 1/15,512; 6.44/100,000), which seems quite low, compared with the historically quoted 1 in 1,000 to 3,000 units. We then reviewed concomitant transfusion reactions linked to platelets reported during the study period and identified 3 cases of culture-confirmed bacterial contamination. Including these 3 cases of contamination missed by our screen changes the overall prevalence of contaminated platelet concentrates in the study to 0.009% (9/93,073 or 1/10,341; 9.66/100,000) that, while approximately half that reported by Eder et al in 2007 for the American Red Cross study (1/5,000 units), is congruent with the range of 7 to 80 per 100,000 units (mean, 37.1/100,000 units) found in a review of available prospective studies evaluating the frequency of bacterial contamination of WB-P components.

Consequently, by implementing glucose screening, we prevented the transfusion of 6 units of WB-P concentrates that were bacterially contaminated. As would be expected, the organisms detected were all gram-positive bacteria, consistent with skin flora ordinarily associated with nonfatal septic transfusion reactions.  The majority were CONS (4 of 6 cases), with 1 case each of α-hemolytic Streptococcus and P. acnes. None of the concentrates had a positive Gram stain. Although these microorganisms are not considered highly virulent, they have been linked to severe and occasionally fatal septic transfusion reactions, and their detection is always clinically relevant.

The organisms missed by glucose screening (false-nega-

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**Table 4**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Positives/ No. of Units Tested</th>
<th>Prevalence of Contamination/100,000 Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klein et al77</td>
<td>2006</td>
<td>1/13,579</td>
<td>7</td>
</tr>
<tr>
<td>Morrow et al76</td>
<td>1991</td>
<td>6/74,598</td>
<td>8</td>
</tr>
<tr>
<td>Barrett et al77</td>
<td>1993</td>
<td>1/4,272</td>
<td>23</td>
</tr>
<tr>
<td>Rock et al46</td>
<td>2004</td>
<td>4/12,062</td>
<td>33</td>
</tr>
<tr>
<td>Yomtovian et al20</td>
<td>1993</td>
<td>6/15,705</td>
<td>38</td>
</tr>
<tr>
<td>Chiu et al28</td>
<td>1994</td>
<td>10/21,503</td>
<td>46</td>
</tr>
<tr>
<td>Yomtovian et al2</td>
<td>2006</td>
<td>31/64,305</td>
<td>48</td>
</tr>
<tr>
<td>Blachman et al49</td>
<td>1994</td>
<td>16/31,610</td>
<td>51</td>
</tr>
<tr>
<td>Blachman et al50</td>
<td>1997</td>
<td>7/10,065</td>
<td>70</td>
</tr>
<tr>
<td>Schrenzeiier et al51</td>
<td>2007</td>
<td>16/22,044</td>
<td>73</td>
</tr>
<tr>
<td>Leiby et al52</td>
<td>1997</td>
<td>4/4,995</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>102/275,238</td>
<td>37.1</td>
</tr>
</tbody>
</table>

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**Figure 1**

Graphic summary of prospective data evaluating the prevalence of bacterial contamination in whole blood–derived platelet concentrates.

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**Figure 2**

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tives), detected instead by transfusion reaction investigation, were, likewise, consistent with skin commensals seen in nonfatal platelet-associated septic reactions and corresponded with microbes linked in case studies to false-negatives occurring after screening by culture techniques.†Table 5‡§⁵,³⁰,⁵⁴-⁵⁷⁸

Of the initial 929 platelet concentrates that screened positively, 923 were not confirmed by subsequent culture, for a false-positive rate of 0.99%, which is very close to that previously reported using urine reagent strips (0.90%) and comparable to the rate for culture reported by AuBuchon et al⁵⁹ of 0.6%, but is nearly an order of magnitude higher than the false-positive rate reported for culture in other studies of 0.03% to 0.12%.³⁰,⁵⁵,⁵⁹ Variation in observed false-positive rates may be attributed to lack of standardization of the definition, with questionable distinction between “reactive signal” and “positive signal.” Other contributing factors may include the actual contamination rate, differences in test sensitivities (detection threshold) between methods, and vagaries in sampling protocols between studies. Ideally, the screening test for bacterial contamination should minimize the false-positive rate, the importance of false-positives being, of course, the unnecessary wastage of uncontaminated components. A finding of zero false-positives corresponds to perfect specificity, 100%. The specificity of culture methods has been reported up to 100% in the literature.⁶⁰ Our specificity was 99%.

In the present study, meticulous review of reported transfusion reactions during the study period revealed 3 culture-confirmed cases of sepsis linked to platelet infusions. In each case, cultures derived from residual platelet concentrate or saline rinse of the transfusion set grew bacteria identical to that detected in respective blood cultures. Thus, by using quantitative glucose measurement, our false-negative rate was 0.003% (3/93,073; 3.2/100,000). There are sparse data available in the literature relating false-negative rates of bacterial detection methods, although the continuing occurrence of septic reactions and rare deaths linked to bacterially contaminated platelet products tested and cleared by culture methods suggests a tangible rate of false-negative bacterial culture results. The available data and case studies addressing ongoing septic complications of platelet transfusion that persist despite widespread, largely culture-based, bacterial screening programs reveals a range of false-negative rates of culture-based screening from 0.002% to 0.009% (2.0-9.1 per 100,000 units transfused) or about an order of magnitude below the rate of true-positive detection.†Table 6‡§⁶,³⁰,⁵⁴-⁵⁷ These studies largely approached false-negative detection in a manner similar to that used in the present study, via review of documented transfusion reaction investigations, although Larsen et al⁴⁵ undertook the repeated culture of outdated units.

Another clinically relevant issue in critical consideration of our study’s findings is that of sensitivity, with a 2-fold definition: sensitivity refers to the detection threshold of a test in this application, ie, the number of bacterial CFU per milliliter of platelets that need to be present to trigger a positive result, and to the test statistic, describing the probability of test positivity when bacterial contamination is truly present (true-positives divided by all positives). This study did not address the issue of detection threshold for quantitative glucose screening; we accepted that the method most likely required an inoculum similar in scale to other methods relying on metabolic surrogates to detect the presence of bacteria, likely approximating 10⁷ CFU/mL (as addressed earlier). In terms of threshold of detection, culture is one of the most sensitive methods, if not the most sensitive method, for bacterial detection, capable of discerning contamination.

†Table 5‡
Organisms Reported in Conjunction With Septic Transfusion Reactions Resulting From False-Negative Bacterial Screening

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organisms</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eder et al⁵⁷</td>
<td><em>Staphylococcus lugdunensis</em> (CONS)</td>
<td>Fatal reaction</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em> (2 cases)</td>
<td>Fatal reaction, 2 cases</td>
</tr>
<tr>
<td></td>
<td>7 additional cases of <em>Staphylococcus spp</em></td>
<td>Nonfatal septic reactions</td>
</tr>
<tr>
<td>Fang et al⁵⁴</td>
<td><em>S. lugdunensis</em></td>
<td>Fatal reaction</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>Clinical sepsis, nonfatal</td>
</tr>
<tr>
<td></td>
<td>CONS</td>
<td>Clinical sepsis, nonfatal</td>
</tr>
<tr>
<td>Larsen et al⁴⁵</td>
<td><em>Bacillus spp</em></td>
<td>Component not transfused</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>Component not transfused</td>
</tr>
<tr>
<td>Ramirez-Arcos et al⁶</td>
<td><em>Serratia marcescens</em></td>
<td>Fatal reaction</td>
</tr>
<tr>
<td>Schmidt et al⁵⁶</td>
<td><em>Salmonella spp</em></td>
<td>Clinical sepsis, nonfatal</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> in 1 apheresis collection that was split and transfused to 2 patients*</td>
<td>Fatal reaction, 1 case; sepsis, nonfatal, 1 case</td>
</tr>
<tr>
<td>te Boekhorst et al⁵⁷</td>
<td><em>Bacillus cereus</em></td>
<td>Sepsis, nonfatal</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>Sepsis, nonfatal</td>
</tr>
</tbody>
</table>

CONS, coagulase-negative *Staphylococcus* species.

* Reported 1 additional cases.

† Reported 5 additional cases.
at levels as low as 10 CFU/mL.\textsuperscript{43}

Two culture methods approved by the FDA are used in bacterial detection in platelet concentrates, the BacT/ALERT system, an automated system based on detection of carbon dioxide produced by bacteria, and the Pall eBDS approach (Pall, East Hills, NY), an automated system that detects the percentage of oxygen in the headspace gas of a sample pouch. The observed sensitivity of these methods in validation study settings exceeds 96% (true-positives divided by all positives, detecting the presence of bacteria if there are any actually present), although sensitivity in the context of a test statistic was not overtly addressed.\textsuperscript{60,61} Given the infrequency of analysis of false-negative results in studies reporting field implementation of these automated culture systems, their true sensitivities are not precisely clear.

Data documented in the reviewed studies disclosing false-negative cases reveal sensitivities for culture-based bacterial screening range from 75.0% (Ramirez-Arcos et al\textsuperscript{4}) to 98.9% (te Boekhorst et al\textsuperscript{5}) (Table 6). This range may be attributed to numerous factors, including differences in culture protocol (timing of culture, volume of platelet concentrate inoculated), study size, and even vagaries of the contaminating bacterial species. A recent article by Murphy et al\textsuperscript{62} specifically addressed the issue of automated culture sensitivity in platelet screening, finding an overall sensitivity of 29.2% (true-positives divided by all positives, detecting the presence of bacteria if there are any actually present), their study detected false-negatives by retesting platelet concentrates on storage days 4 and 7, after initial screening on day 1. This active pursuit of false-negative units in the study by Murphy et al\textsuperscript{62} may account for the large disparity between the sensitivity reported for the present study and the values calculated in Table 6. Our sensitivity was 66.7%, which seems to overlap the expected and reported ranges for automated culture methods.

Despite the modest sensitivity in our study in comparison with FDA-approved automated culture bacterial screening technologies (with sensitivities ranging from 75.0% to 98.9% in published studies, Table 6), the prevalence in our study is congruent with the range detected in practice by culture screening methods. This finding suggests that quantitative glucose screening approximates well the performance of screening culture in the field. Supporting this supposition is our retrospective transfusion reaction review that revealed only 3 true cases of transfusion-transmitted sepsis from contaminated platelet products owing to false-negatives. Our false-negative rate was, likewise, comparable to that of culture methods. Our data support that application of a test of lower sensitivity than the purported gold standard of culture applied late in the shelf-life of platelets can be comparably effective in detecting bacterial contamination and in preventing sepsis-related morbidity and mortality compared with culture methods currently in use.

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References


55. Larsen CP, Esligini F, Hermansen NO, et al. Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. *Vox Sang*. 2005;88:93-97.


