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REVIEW

Detecting Bacterial Contamination in Platelet Products

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SUMMARY

Bacterial contamination of platelets is an important cause of transfusion-associated morbidity and mortality. It is currently the most frequent infectious complication of transfusion therapy, with between 1 in 1,000 and 1 in 3,000 platelet units being bacterially contaminated at time of transfusion. Several factors have contributed to the persistence of this problem including lack of sensitive detection methods, lack of recognition of the frequency of the problem, inadequate recognition of septic reactions by clinicians treating patients receiving platelet transfusions, differences in transfusion reactions between bacterial species and bacterial inocula transfused, and differing methodologies and time of testing for detection of bacteria in platelet units. There are also important correlations between the receipt of bacterially contaminated platelet units and the development of transfusion reactions and bacteremia. In the last few years the recognition of the importance of platelet bacterial contamination prompted the College of American Pathologists (CAP) and the American Association of Blood Banks (AABB) to set new standards requiring the screening of platelets for bacterial contamination. In the wake of these standards, an increasing number of approaches have been and are being developed to deal with this problem. The clinical sensitivity, specificity and predictive value of these detection methods vary considerably and need to be defined for routine laboratory practice. In this review, we focus on the practical aspects and feasibility of implementing FDA-cleared detection methods for identifying bacterially contaminated platelet units. We also present details of a number of methods under development for at-issue use. (Clin. Lab. 2006;52:443-456)

BACKGROUND

Platelets are currently administered as single donor plasmapheresis (SDP) and whole-blood derived, random donor platelet (RDP) units, with the latter typically being administered in pools of 4-6 units. Bacterial contamination of these platelet products is an ongoing problem associated with significant transfusion-associated morbidity and mortality.[1-10] Currently, platelet-transfusion-associated sepsis is the most frequent infectious complication of transfusion therapy, with between 1 in 1,000 and 1 in 3,000 platelet units being bacterially contaminated at time of transfusion.[1, 8, 11] Transfusion of contaminated units is estimated to cause life-threatening sepsis in between 10% and 40% of recipients receiving a bacterially contaminated platelet unit. Based on passive-reporting studies from the United States, the United Kingdom and France, the risk of death from a platelet transfusion due to bacterial contamination is between 1 in 7,500 and 1 in 100,000 transfusions.[8] These risks are up to two orders of magnitude higher than the incidence of transfusion-associated viral transmission.[1, 6, 7] Platelet units are more susceptible to contamination than other blood products because of the requirement to store platelets at room temperature to preserve platelet function.[12] At 22-24 °C, a small bacterial inoculum can grow into very high numbers within a short time period and therefore, older ("at-risk") units are most likely to be contaminated with a large number of organisms and to cause a septic reaction in the recipient.[11, 13, 14] In recognition that older platelets are at risk for significant bacterial contamination, the storage period of platelets was shortened by the United States Food and Drug Administration (US-FDA) from seven to five days in 1986 in response to an increased number of reports of transfusion-related sepsis in older platelet units.[9, 15] Only in

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Table 1: Bacteria associated with 60 cases of fatalities resulting from transfusion of platelet products as reported to the US-FDA, 1995-2004.[17]

Bacterial species	No. of cases
Gram-positive organisms (n = 22)	
Staphylococcus epidermidis	11
Staphylococcus aureus	4
Streptococcus group G	2
Clostridium perfringens (anaerobe)	1
Enterococcus spp	1
Streptococcus group B	1
Streptococcus group F/Eikenella	1
Gram + rods (unidentified)	1
Gram-negative organisms (n = 38)	
Klebsiella pneumoniae	10
Escherichia coli	9
Serratia marcescens	5
Enterobacter aerogenes	2
Enterobacter cloacae	2
Pseudomonas aeruginosa	2
Salmonella spp	2
Bacillus spp	1
Enterobacter agglomerans	1
Klebsiella oxytoca	1
Morganella/Providencia	1
Pasteurella multocida	1
Gram-negative rods (unidentified)	1

2005, with the advent of licensed storage containers for single donor apheresis platelets combined with aerobic and anaerobic culture testing under an US-FDA-approved post-market surveillance protocol, did it become permissible once again to store platelets (SDP only) for up to seven days following collection.[16]

The predominant organisms implicated in platelet bacterial contamination are the skin flora, including staphylococci (*Staphylococcus aureus* and coagulase-negative staphylococci), *Corynebacterium* species, and *Propionibacterium* species. Other contaminants include streptococci, Gram-negative bacilli and *Bacillus* species. Although Gram-positive organisms cause the majority of septic reactions, Gram-negative organisms are implicated in the majority of fatalities.[1, 3, 6] Among the Gram-negative organisms reported to contaminate platelet units are *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* species, *Enterobacter* species, *Morganella morganii*, and *Serratia* species.[9, 17] Indeed, *Klebsiella pneumoniae* was responsible for the majority of platelet transfusion-associated deaths related to Gram-negative organisms reported to the US-FDA from 1995 to 2004 (Table 1).[17] Although some of these organisms have a preference for anaerobic conditions, all are able to grow in aerobic conditions, with the exception of *Propionibacterium* spp., which grows only under anaerobic conditions. A single case of contamination of a platelet unit with *Clostridium perfringens*, also a strict anaerobe, has been reported.[17]

PREVALENCE OF BACTERIAL CONTAMINATION OF PLATELETS

The reported prevalence of bacterial contamination of platelets is highly variable and, in many cases, is difficult to assess due to differences in surveillance and testing methodologies and in case definitions. However, it is generally accepted that approximately 1 in 1,000 to 1 in 3,000 platelet units are contaminated with bacteria.[1, 8] Based on 1999 data, over 4 million platelet units are transfused per year in the USA, in the form of 1 million SDP units and 3 million RDP units, the latter administered in 0.5 to 0.75 million pools of 4-6 units.[10, 18] On this basis, it is likely that 1,300 to 4,000 platelet units and 500 to 1,500 platelet transfusions per year are bacterially contaminated. Projected fatalities from these transfusions, based on extrapolation from surveillance and other studies, vary from 15 to over 200 per year. Our nearly 15-year experience with bacterial contami-

nation surveillance in a university hospital using passive (transfusion-reaction triggered) and active (culture at issue) surveillance demonstrated that, while active surveillance detected 38 instances of bacterial contamination (1:2,090 RDP units and 1:2,213 SDP units), only one instance of contamination was detected by passive surveillance (0:48,067 SDP units and 1:168,216 RDP units).[11] The failure of passive surveillance to detect instances of bacterial contamination is attributed to the widespread lack of recognition of septic versus nonseptic reactions, and failure to associate signs of bacterial sepsis with the transfusion of a platelet unit. Additionally, transfusion of platelet units contaminated with bacterial species of low virulence such as coagulase-negative staphylococci frequently does not elicit signs and symptoms of a transfusion reaction.

Thus, there remain important outstanding questions relating to the clinical significance of detecting, defining and quantitating bacterial contamination in platelet units. Although the search for the perfect method is elusive, in the last few years a variety of different methodologies have been developed and evaluated for detection of bacteria in platelet units. Currently, three commercial methods have been cleared by the US-FDA for screening of platelets for bacterial contamination, BacT/ALERT (bioMerieux, Inc, Durham, NC), Pall eBDS (Medsep Corporation, Covina, CA), and Scansystem (Hemosystem, Marseilles, France). Based on the published information from centers that have used one or more of these systems for screening single donor platelet products,[19-23] these methods are sensitive and specific, but the culture-based systems have not detected all contaminated units in the clinical setting.[10, 24] Therefore, patients are still at risk for being transfused with bacterially contaminated platelet units due to false negative results. In addition to these US-FDA-cleared methods, there are several additional methods in various stages of development. It is our goal to present the feasibility and logistics of the application of various methods – both cleared and under development – for bacterial detection testing in platelet units and to describe their advantages and disadvantages.

CORRELATION OF TRANSFUSION OF BACTERIALLY CONTAMINATED PLATELET UNITS, BACTERIAL LOAD AND TRANSFUSION REACTIONS

The critical factor for the clinical use of any detection method is defining the number of bacteria, expressed as colony-forming units per mL (CFU/mL) that will result in a clinically significant transfusion reaction. Transfusion reactions may occur with as few as 10^2 to 10^3 CFU/mL, even with organisms of low virulence such as *S. epidermidis*.[1, 11, 25]

Transfusion of a contaminated platelet unit is often not recognized or directly associated with subsequent sepsis. Over a 3-year period, 1998 through 2000, from suspected cases reported to the CDC during the BaCon study, a voluntary reporting study with very restricted criteria for case definition, the rate of transfusion-transmitted bacteremia (in events/million) was estimated to be 9.98 for SDP and 10.64 for pooled RDP units. The rates of fatal reactions were 1.94 and 2.22 respectively.[6] Overall, the BaCon study estimated a fatality rate of 1 in 500,000 units for both SDP and RDP units.[6]

In France, over a 2-year period, of 16 cases of bacterial contamination associated with platelet transfusion 9 (56%) resulted in severe sepsis or shock. Estimates of the incidence of life-threatening reactions were 9.4 per million (1:106,000) units for RDP units and 17.7 per million (1:56,500) for SDP units.[26] A more recent report from the French Haemovigilance System identified seven confirmed cases of transfusion-transmitted bacterial contamination. Of these two were related to SDP and two to RDP pools, while the other cases were related to transfusion of red blood cells.[27] In the United Kingdom during six years of voluntary participation of blood services, the SHOT (Serious Hazards of Transfusion) study identified 26 episodes of bacterial contamination out of 40 transfusion-transmitted infection incidents reported. Twenty-two of these episodes were related to the transfusion of platelets, accounting for 5 of 6 deaths.[28]

A major problem in determining the magnitude of the problem of platelet bacterial contamination is the lack of recognition and reporting of cases, due in large measure to the failure to associate chills, rigors and fever – signs and symptoms common in patients receiving platelet transfusion therapy – with the possibility of transfusion of a bacterially contaminated platelet unit.[11, 25] Based on the reported prevalence of transfusion reactions and the detection rate of contaminated platelet units, however, it is estimated that a severe episode of transfusion-associated bacterial sepsis occurs in connection with about 17% of contaminated units transfused.[1] More serious clinical events, such as shock and death occurring in immunocompromised platelet transfusion recipients, are likewise often not linked to transfusion of a bacterially contaminated platelet unit.[11]

The data obtained by our group during more than 14 years of surveillance for bacterial contamination of platelets showed, for the first time, the correlation between the organism load in bacterially contaminated units, transfusion reactions and positive blood cultures.[11] From this study, it was clear that bacteremia did not correlate with transfusion reactions, with bacteremia not being documented in the three patients with fatal outcomes, while two patients showing no signs of a transfusion reaction were bacteremic. Absence of bacteremia was likely related to patients receiving antimicrobial agents and delays in obtaining blood cultures following transfusion. Correlation of organism load with occurrence of transfusion reactions showed that a system with a sensitivity of 10^5 CFU/ml would detect 10/11 (91%) serious reactions and 15/19 (79%) of all reactions, while one with a sensitivity of 10^4 CFU/ml would detect all 11

serious reactions and 16/19 (84%) of all reactions.[11, 29] Additionally, our study showed that rates of bacterial contamination, transfusion of contaminated units, transfusion reactions and bacteremia for RDP pool transfusions were significantly higher than those for SDP unit transfusions. These differences, however, were associated with the use of pools of 5 RDP units per transfusion as these rates were not statistically different based on the number of individual platelet units transfused. Our study also demonstrated the superiority of active surveillance over passive surveillance in detecting platelet bacterial contamination, with 38 of the 39 instances of platelet bacterial contamination being detected only by active surveillance. While our experience also highlights the finding that the vast majority of transfusion reactions with signs and symptoms consistent with sepsis are not due to transfusion of bacterially contaminated platelet units, paradoxically it demonstrates that the majority of patients who received bacterially contaminated platelet units and developed signs and symptoms of sepsis were not recognized or reported to the Blood Bank or Transfusion Service for further evaluation.

In 2004, the Infectious Diseases Society of America conducted a survey to assess clinician experience with transfusion-associated bacterial infections among infectious diseases specialists who are members of the Emerging Infections Network in the United States.[10] Among the 399 members that responded, 48 (12%) recalled consulting on 85 reactions to blood transfusions potentially caused by bacterial contamination, with 10 of these cases being fatal. A total of 143 (36%) respondents were aware that bacterial contamination of platelets is now the most common infectious risk of transfusion therapy while only 78 (20%) were aware of the new AABB standard for testing platelets for bacterial contamination. It is clear from this survey that efforts should be made to communicate with clinicians not only the new requirements from AABB and CAP, but also to recognize and request the appropriate testing to confirm the diagnosis of sepsis related to transfusion of potentially bacterially contaminated platelet units.[10]

STRATEGIES TO REDUCE OR PREVENT CONTAMINATION OF BLOOD PRODUCTS

In March of 2004, the AABB, an accrediting organization for Advancing Transfusion and Cellular Therapies Worldwide, added a new standard that requires members of this organization to implement measures to detect and limit bacterial contamination in all platelet components.[30] In an effort to assist blood banks and transfusion services, the AABB, in a supplemental guideline, suggested various strategies for reducing transfusion of bacterially contaminated platelet units and described several methods for detecting bacterial contamination,[31] which will be described in more detail below. In addition to the AABB, in 2002 the CAP added a requirement for laboratories to have a system in place to detect the presence of bacteria in platelet components.[32, 33] Thus, in order to be in compliance with the CAP Laboratory Accreditation Program, blood banks and transfusion medicine services need to have a system to detect the presence of bacteria in platelet components.

While bacterial detection methods garner most of the attention, additional strategies have been developed to reduce the risk of transfusion of bacterially contaminated platelets. These strategies include bacterial contamination avoidance methods, and bacterial growth inhibition or inactivation methods.[15] In order to accomplish the goal of eliminating bacterial contamination of platelets, these strategies likely will need to be used in combination with a detection method. Ideally, the responsibility for limiting bacterial contamination is shared between the collection center, which needs to insure suitable donor selection, proper aseptic collection technique and absence of measurable bacteria by using a sensitive detection method shortly after product collection, and by the transfusion service, which should ideally use a rapid test for detecting bacterial contamination at the time the platelet unit is issued for transfusion.

Bacterial Contamination Avoidance Methods

Platelet units are most commonly contaminated at the time of collection when bacteria from the skin or from bacteremia in the donor gain entry into the collection set during the phlebotomy process. Exhaustive donor screening and deferral if a history or symptoms of possible bacteremia is elicited has reduced contamination of blood products. Additional measures to minimize the entrance of bacteria into the blood collection system include improved disinfection of the skin, and diversion or removal of the first 20-30 mL of blood collected.[34, 35] Overall, rates of bacterial contamination of SDP and RDP units have been similar, but the rate of transfusion of contaminated products is proportionately higher in RDP pools than in SDP units.[11, 25, 36]

Bacterial Growth Inhibition and Inactivation Methods

While bacteria may gain entry into a platelet unit during collection, the number of bacteria entering the collection system is usually very small and it is only during room temperature platelet storage that the organisms may grow into large numbers. This problem can potentially be prevented by using a substance that inactivates bacteria or that inhibits bacterial growth during storage. The use of substances that inhibit or inactivate organisms present within a platelet bag should be implemented by the collection center. In order for this strategy to be feasible and practical, these substances should not cause substantial damage to platelet function, and the process should be automated and linked to the collection instrument. These

inhibitory substances need to be added in a particular concentration, and the instrument should be able to calculate and dispense the pathogen inhibitory reagent in a closed system and according to the volume collected. Although the cost is substantial, and safety concerns about the additive solutions remain, the potential application of inactivation methods seems to be a promising approach to avoiding bacterial contamination of blood products.[37]
Two inhibitory substances are being evaluated for use in platelets: amotosalen S59, which is being developed as the Intercept PLT System (Cerus, Concord, CA), and riboflavin, which is being developed by Navigant (Lakewood, CO). When activated by light, amotosalen S59 binds to the nucleic acid of pathogens and prevents replication.[38, 39] A pilot study assessing the use of 7-day-old buffy-coat platelets treated photochemically with amotosalen S59 to transfuse patients with thrombocytopenia showed that treated platelets produced acceptable efficacy and safety compared to 7-day-old untreated platelets.[40] The Intercept PLT System obtained regulatory approval in 2002 in Europe to treat platelets for transfusion, and regulatory approval is being pursued in the United States. The other pathogen inhibitory system under development for platelets is a photochemical decontamination process utilizing riboflavin, and preliminary reports are encouraging.[41, 42]

Bacterial Detection Methods

At present, the focus in identifying and preventing bacterial contamination of platelet components by the collection centers as well as the transfusion services is on detection methods. The ideal detection method should be sensitive and specific, have a rapid turn-around time, require a small sample volume for testing and be inexpensive.
For SDP platelets, the CAP and AABB recommend the use of one of the commercial systems that have been cleared by the US-FDA for in-process quality control testing of platelet units. For RDP units, where application of testing with an US-FDA-cleared method may be impractical unless applied to pooled units, a variety of less sensitive testing methods are permissible. These include detection of decreased pH, detection of decreased glucose concentration, microscopic examination of dried smears stained with acridine orange or Gram stain, plate culture, and broth culture. These methods should be validated by the user.
As discussed above, the methods that have been cleared for bacterial contamination testing of platelet products by the US-FDA are two culture-based instruments, the BacT/ALERT and PALL eBDS. More recently, the US-FDA has also cleared the Scansystem, a scanning cytometry method that uses an automated microscopic analysis of particles stained with a fluorescent stain. Both culture-based methods (BactT/ALERT and eBDS) may potentially delay the use of platelets and shift the inventory to the use of older platelets because they require a 24-h holding period prior to sampling and a generally 24-h incubation period after sampling.

Description of US-FDA Cleared Bacterial Detection Methods for Quality Control Testing of SDP units

BacT/ALERT

A sample of platelet rich plasma from a leukocyte-reduced SDP unit is inoculated into aerobic (BPA) and anaerobic (BPN) culture bottles containing broth media and then placed into an instrument that automatically monitors the production of CO_2 as a result of growth of bacteria. This and similar systems have been used for many years for the routine culture of blood for clinical purposes. The manufacturer strongly recommends using one aerobic and one anaerobic bottle, each inoculated with 4 ml of the platelet product. For optimal bacterial detection, the manufacturer recommends that the platelet specimen should be taken at least 24 hours after collection to allow for natural proliferation in the platelet product. During this time bacteria, if present at the time of collection, would have an opportunity, in most instances, to reach a level adequate for sampling and subsequent growth in the culture system. Although procurement of the aliquot from the platelet unit is achieved with the use of a sterile docking device, the subsequent inoculation of the sample into the culture bottle involves an open system using a needle and syringe. Therefore, strict adherence to the manufacturer's instructions for antisepsis of the septum of the blood culture bottle and inoculation instructions are critical factors to prevent the occurrence of false positive results due to contamination during inoculation of the platelet unit itself. Distinguishing false positive culture results from true contamination is often confounded by the fact that the same bacterial species are commonly detected in both circumstances. According to the manufacturer's directions, the inoculated culture bottles are held for the shelf-life of the platelet unit unless a signal indicating bacterial growth is generated before this time period has elapsed.
Once the system identifies a bottle as being "positive", indicating that the CO_2 content is above the baseline, an aliquot is removed for Gram stain and subculture to agar media. Most collection centers send positive bottles and a sample from the original unit to a microbiology laboratory for further testing. Absolute proof that a true positive result is correct requires that the same organism be isolated from original platelet unit and positive culture bottle. If the culture from the original platelet unit is negative, it is likely that the positive culture bottle is a false positive.
In the USA, the BacT/ALERT culture bottles are accompanied by product inserts indicating that BacT/ALERT System (3D and 240) and culture bottles may be used for quality control testing of platelets and that the laboratory should follow its own quality control pro-

cedures for this use.[43] The product inserts also state that the BacT/ALERT System, including the culture bottles, should not be used in determining suitability for release of platelets for transfusion and that users considering such release testing should first consult the Center for Biologics Evaluation and Research (CBER) of the Food and Drug Administration (FDA) for the appropriate clinical studies. A report of "negative" should not be interpreted as meaning that the original product is sterile. The negative status could be due to underinoculation of the bottle, no organisms present in the inoculum, the number of organisms were too small for detection, or a culture bottle/medium that does not support the growth of the organism.[43]

eBDS

This method is a novel culture-based system, which assesses the reduction of the O_2 content in the head space of a sample pouch containing an enriched medium, compared to ambient O_2, as a marker for bacterial growth.[23] This method involves docking an eBDS sample pouch onto the tubing of a platelet bag, transfer of an aliquot of 2-3 mL of the platelet product into the sample pouch, which is then separated from the platelet unit using a heat sealer. The eBDS pouch is then incubated at 35 ºC for 24 hrs. Pouches are then removed from the incubator, kept at room temperature for at least 2 minutes and the probe of the oxygen analyzer inserted into the sample port of the pouch. The oxygen analyzer automatically reads the oxygen concentration in the sample pouch. The instrument then gives a reading with a positive or negative interpretation for bacterial contamination.

Scansystem

This method detects bacteria using a laser-based, solid-phase scanning cytometry detection method. This method is a highly sensitive bacterial screening system, and can detect very low levels of bacteria after concentration from a larger volume by filtration.[60] The Scansystem solid-phase cytometry analyzer includes four modules: (i) a scan module, in which the black membrane is placed; (ii) an argon laser module (488 nm excitation light), which is connected directly to the scan module to scan the black membrane; (iii) an epifluorescence microscope with a suitable light source and filters, which has an automated motor-driven stage, for visual discrimination by the operator of fluorescent particles detected by the laser scan as bacteria or nonbacterial particles; and (iv) a computer with proprietary software to monitor the other modules.

The Scansystem is used by drawing a 3 ml sample of platelets into the syringe of a Scansystem Platelet kit through the sample injection port. The kit is then agitated in a platelet incubator at 22 °C for forty minutes in order to achieve platelet aggregation. The contents of the syringe are then expressed through a filter, which retains the aggregated platelets and allows passage of bacteria into the second portion of the kit. The kit is then incubated at room temperature for twenty minutes to allow labeling of any bacteria present with the fluorescent stain. A black membrane in a holder attached to a vacuum pump is then attached to the bottom port, and the contents of the pouch are then filtered through the black membrane. Any bacteria present will be deposited on the surface of this membrane. The membrane is then removed from the holder and placed into the Scansystem scan module for detection of fluorescent particles by laser scanning. The final step is visual determination, using the epifluorescence microscope, of the identity of fluorescent particles as bacteria or as other particles.

Other Detection methods

Although traditional culture and culture-based methods are far more sensitive than staining and measurement of pH or glucose, the latter methods have the advantage of having a rapid turn-around time and therefore the testing can be done at or near the time of releasing the platelet unit, while final culture results may not be available until after transfusion. For this reason, and because these non-culture based methods are among the suggested methods in the current AABB and CAP guidelines for testing of RDP units, many transfusions services and blood banks have adopted them for routine testing; and, as is discussed below, despite poor sensitivity for detection of bacterially contaminated platelet units, their use may reduce the fatalities associated with transfusion of bacterially contaminated platelet units.

Staining Methods

Among the staining methods recommended by AABB and CAP are the Gram stain and the acridine orange stain.

Gram stain procedure: A smear is prepared using a sterile pipette or syringe to transfer 1 or 2 drops from a platelet unit to a clean glass slide. The drop is spread into an even film and allowed to either air dry or heat dry on a slide warmer. Smears can also be prepared using a cytospin slide centrifuge to concentrate the sample. Cytospin preparations deposit the concentrated sample within a limited area for viewing.[44] The Gram stain procedure includes sequential staining steps with crystal violet, alcohol decolorization, and counterstaining with safranin or neutral red. Bacteria stain either Gram-positive (deep violet) or Gram-negative (red) on the basis of differences in their cell wall composition. Gram-positive bacteria have a thick peptidoglycan layer and they are unaffected by the decolorization step and retain the initial stain, appearing deep violet. Gram-negative bacteria have a thin peptidoglycan layer attached to an outer membrane containing lipids. This membrane is damaged by the decolorizer, allowing the initial stain to leak out and be replaced by the red counterstain. The smear is examined microscopically using a light microscope with a 100X oil objective. The sensitivity of the

Gram stain is 10^5 CFU/mL, or 10^4 CFU/mL if the specimen has been prepared with a cytocentrifuge.[44] Use of the Gram stain has been successful in interdicting platelet units heavily contaminated with bacteria prior to transfusion.[5]

Acridine orange stain procedure: Acridine orange is a fluorochromatic dye that binds to nucleic acids of bacteria and other cells. Under UV light, bacteria stain bright orange, whereas human cells and background debris stain pale green to yellow, although nuclei of activated leukocytes may also stain orange or red. The smear is prepared as for a Gram stain and stained with acridine orange (0.1 g/L in 0.5M acetate buffer) for 2 min. The smear is read on a suitable fluorescent microscope with a 40X dry or 100X oil objective. The sensitivity of the acridine orange stain is approximately 10^4 CFU/mL.[45]

Interpretation of Gram and acridine orange stains involve consideration of staining characteristics and cell size, shape, and arrangement. Sample preparation, reagents, and staining procedures may influence these characteristics. Therefore, use of quality control slides and competency training of the personnel reading the stains is essential. It is advisable that these staining methods be done in a clinical microbiology laboratory. In institutions where a microbiology laboratory is not available, the Gram stain can be performed by blood bank or transfusion service personnel that have been adequately trained to perform and interpret Gram stains. In addition, these laboratories should be participating in a Gram stain proficiency testing program.

pH and glucose analysis: Testing can be done separately using a handheld device or pH paper, or in combination on a multi-reagent dipstick. Although, handheld pH meters provide a more accurate result than do dipsticks, both methods are easy and rapid to perform and, for that reason, they have been adopted for many blood banks and transfusion services for testing RDP units.[46-49]

The sensitivity of pH and glucose testing, using one or both methods, is approximately10^6 to 10^7 CFU/mL. Because of this limited sensitivity, the AABB recommends that facilities use these methods on individual units rather than pooled units. A recent study showed that as platelets age, the rate of pH failures increases, and only 4 of 405 random platelet units that failed pH were culture positive.[49] A lower rate of pH failure has been demonstrated in random units that have undergone prestorage leukoreduction compared to non-leukoreduced, random platelets.[50]

IMPLEMENTATION OF DETECTION METHODOLOGY

A summary of the AABB fall 2004 survey of members on practices related to the new guidelines on detection and prevention of bacterial contamination in platelet units was presented at the US-FDA Blood Products Advisory Committee Meeting in January of 2005.[51] The survey results showed that the majority (88%) of blood centers were using a culture method – usually BacT/ALERT- to screen for bacterial contamination of SDP units. Most centers (85%) using BacT/ALERT did only aerobic cultures and held the cultures for 5 to 7days. Four out of the 34 blood centers surveyed used glucose or pH by dipstick or meters. A few transfusion services cultured platelets by plate culture or performed Gram stains. Among hospital blood banks, most cultured platelets if their supplier did not, or if the platelets were collected in-house. Half of the blood banks performing cultures used anaerobic and aerobic cultures, while the remainder used only aerobic culture.[51]

It is clear from the results of the survey that many transfusion services are using less sensitive techniques – glucose, pH or Gram stain as compared to methods cleared by the US-FDA - for quality control of platelets, particularly RDP units. The advantage of using these methods is that they are easy to implement in any hospital blood bank or transfusion service and they can be performed at or near the time of issue. However, there have been reports of false negatives with fatal consequences with these methods,[10] and investigators have strongly advised against the use of surrogate markers of bacterial metabolism, such as pH and glucose concentration, for detecting bacterial contamination of platelets due to the analytic insensitivity of these methods.[52]

Some of the practical aspects relating to implementation of the US-FDA-cleared detection methods are discussed below. In addition, the BacT/ALERT and the eBDS systems have also been evaluated for screening of individual and of pooled RDP units, and have been found to be reliable for monitoring bacterial contamination in these products.[53, 54] Table 2 summarizes the published contamination rates found by routine testing in a clinical setting using different detection methods. The rates of bacterial contamination varied considerably between and within methods, with methods such as plate culture, eBDS and BacT/ALERT generally reflecting realistic true positivity rates (0.02-0.05% for SDP and RDP units, and 0.1-0.25% for RDP pools), while Gram stain, pH determination and glucose determination resulted in low true positive rates while in the case of pH and glucose determination, very high false positive rates were also found. In many instances especially when the BacT/ALERT method was positive, the platelet product had already been transfused and was no longer available for retesting, and these cases are regarded as unconfirmed positives.

Table 2: Results of various methods used for routine testing of platelet products to detect bacterial contamination. Realistic true positive rates based on 1:1,000 to 1:3,000 units being contaminated are 0.03-0.1% for SDP and RDP units, and 0.17-0.5 for RDP pools of 5 units. Data was obtained or derived from the references indicated.

Method	Platelet product (total tested)	Overall positive rate (%)	True positive rate (%)	False positive rate (%)	Unconfirmed positive rate* (%)	False negative rate (%)	Reference #
Gram stain at issue	RDP pools of 5 units (N=7716) SDP (N=8761)	0.12 0	0.078 0	0.039 0	0 0	0.16 0.046	11
pH at issue	Individual RDP (N=37067)	1.09	0.01	1.08	0	NR	49
	Individual RDP (N=6590) SDP (N=671)	1.97 1.93	0 0	1.97 1.93	0 0	0.03 0.30	11
pH + glucose at issue	Individual RDP (N=3093)	0.96	0.06	0.9	0	0 (N=203)	47
BacT/ALERT at 12-24 h	SDP (N=350658)	0.06	0.02	0.03	0.01	0.0008	55
	RDP pools and SDP (N=4000)	1.2	0.13	0.9	0.02	NR	57
	Individual RDP units (N=10141) RDP pools of 5 units (N=2063)	1.4 3.4	0.02 1.9	0.75 1.5	0.64 0	NR NR	54
	RDP pools (N=28104)	0.72	0.65	0.06	0	NR	24
	RDP pools of 4 units and SDP (N=36896)	0.24	0.03	0.04	0.12	0.18 (N=1061)	20
eBDS at 24 h	Individual RDP units (N=12062) RDP pools of 5-6 units(N=2201)	0.04 3.6	0.02 0.04	0.008 3.5	0.008 0	NR NR	53
Plate culture at issue	RDP pools of 5 units (N=12,961) SDP (N=15,493)	0.26 0.077	0.24 0.045	0.023 0.032	0 0	0.0077 0	11

SDP: Single donor platelets; RDP: Random donor platelets; NR: Not reported
* Unconfirmed positives are those where the platelet product had been used at the time the bacterial detection system became positive

Implementation of BacT/ALERT: Laboratory and clinical studies have demonstrated the efficacy of this system in identifying many instances of bacterial contamination of platelets.[19-22] The American Red Cross implemented routine quality control testing for bacterial contamination in SDP products with BacT/Alert in all 36 regional blood centers in March 2004.[55] Platelet samples (4 ml) were obtained at least 24 h after collection and cultured in BacT/ALERT aerobic bottle only until the end of the product shelf life or until a positive reaction occurred.[55] Products were released for use if negative after incubation for at least 12 h, and, if not used, removed from supply when culture became positive. Reports and investigations of potential septic reactions to SDP platelets were also reviewed. In the first 10 months of bacterial testing, 226 of 350,658 collections tested initially positive. Sixty-eight (30%) were confirmed as true positives. Of the 354 apheresis platelet products derived from all 226 initial test-positive cases, 38 (10.7%) had been transfused by the time of the initial positive result, although none of these were confirmed positive. During this period, three septic episodes probably related to transfusion of bacterially contaminated platelets, which had negative screening, were documented, with *S. lugdunensis* implicated in one and other coagulase-negative staphylococci in the other two instances. In Norway, from May 1999 to May 2004, RDP pools were monitored for bacterial growth using BacT/ALERT during an average period of 6.5 days. The investigators found that from 88 platelet pools that were initially positive, only 12 (13.6%) were confirmed as true positives.[20] The main limitations related to culturing, either using traditional culture or using an automated system, is the time required to obtain results. In order to increase the sensitivity of culture, sample for testing should be taken 24 hours after collection, inoculated into culture media and incubated for at least five days before reporting it as negative. Although most of the bacterial organisms implicated in bacterial contamination of platelets are detected after 24 hours of incuba-

tion (48 hours after collection), slow growing organisms cannot be detected for several days. As documented in the survey described above, in most instances, because of the logistics of sample handling, BacT/ALERT testing is performed at the collection center rather than the transfusion service. Documentation of training of the personnel performing the testing is required by accrediting organizations. While inter-laboratory proficiency testing is currently not required due to lack of available defined methodologies, it is anticipated that this will soon be required.[56]

The survey did not evaluate whether the blood centers were performing Gram stains or confirmation cultures from positive BacT/ALERT bottles on site or whether the bottles were sent to an external microbiology laboratory. Because it is known that false positive bacterial contamination results may occur with this system,[57] if the bottles are sent out for confirmation results could be delayed, which may lead to an increase in wastage of products and unnecessary treatment of patients who received these units.

Another limitation of culturing using an automated system is the specimen volume required for testing, usually 4 ml per bottle, which for a SDP unit represents only 1.5% of the unit volume if using aerobic bottles only, or 3% if using both aerobic and anaerobic bottles, but if testing is done on a single RDP unit, the volume required for testing could represent 10% to 40% of the unit volume. This limitation makes routine culturing of individual random units impractical.

Implementation of Pall eBDS*:* The features of this culture-based US-FDA cleared test are similar to those of the BacT/ALERT. This system also requires that a large sample (3 ml) be obtained at least 24 hours after collection and requires an incubation period of 24 hours. Published laboratory studies have shown that the Pall eBDS system is able to detect 1 to 15 CFU/ml of aerobic bacteria in a contaminated unit.[58] The initial version of this system (BDS) gave some invalid or negative readings with organisms that rapidly consume O_2 during their metabolism, particularly *Klebsiella pneumoniae*, but the newer enhanced version (eBDS) has overcome this problem.[15, 58, 59]

There are several potential advantages of the eBDS system compared to automated blood culture systems: (1) it is easier to utilize in a laboratory environment not generally familiar with bacterial testing instrumentation; (2) the sampling and inoculation occurs in a closed system, reducing the opportunity for false positive culture results; and, (3) unlike the BacT/ALERT system in which continuous monitoring of the culture bottles is performed throughout the shelf-life of the platelet unit, the eBDS system is read at a single, discreet time point, eliminating the possibility that a positive result will be generated after the platelet unit has been transfused. It is believed that many of the positive results generated after the platelet unit has been transfused are either false positives or are not clinically significant, while adding a significant burden of work. A potential disadvantage of the eBDS compared to the BacT/ALERT is that only aerobic organisms are detected. And with a smaller sample volume than the BacT/ALERT, it is possibly slightly less sensitive. With both systems, since sampling is done relatively early in the storage period when the bacteria have had limited time for growth, it is possible that bacterial contamination will be missed entirely. While the number of such "breakthrough" cases is uncertain they have occurred, and some have been clinically significant.[29]

Implementation of the Scansystem: Our group and other investigators have evaluated this system and found that this method is able to detect most typical bacterial platelet contaminants in leukocyte-reduced SDP units 30 h after contamination, with a sensitivity equal to that of BactT/ALERT.[60, 61] The Scansystem can detect both live and dead bacteria, so that the presence of bacterial species such as *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, which are frequently inhibited or killed in the presence of human plasma,[62, 63] may still be detected, provided some bacterial growth occurred.

Advantages of the Scansystem method are that platelets, from 30 to 72 h after collection, can rapidly be tested for the presence of bacteria, with a total test time of approximately ninety minutes.[60, 64] Thus the Scansystem allows testing of platelets closer to time of use, and allows platelet units to be used as early as 32 h after collection, compared to typically 48 h for the BactT/ALERT and eBDS methods. Moreover, the Scansystem method is able to detect both live and dead bacteria, which may prevent transfusion of endotoxin-containing products. Disadvantages of the Scansystem are the labor-intensive nature of the system, the need to verify visually the presence or absence of bacteria for each positive test, and the need to test platelet units within 72 hours of collection as platelet aggregation – a requisite of the testing methodology as noted above – is less efficient after 3 days of storage.

Implementation of non US-FDA cleared culture methods: Traditional culture using plated or broth media as well as commercially available automated blood culture systems other than BacT/ALERT can be used for platelet bacterial contamination testing but requires strict validation to ensure that the performance of the technique is as expected.[11, 65, 66] The validation plan should be developed by each institution or laboratory before routine testing can be in place. AABB Association Bulletins, published to supplement the required AABB Standard, provide guidance on acceptable validation methods.[31, 67] Published studies have documented these systems to reliably detect bacterial contamination of platelets.[65, 66] In many cases, the burden of the internal validation pro-

cess is placed on the microbiology laboratory[68] and the cost of testing is passed on to the hospital. The legal risks of using a non-US-FDA-cleared detection system for quality control of platelets for which US-FDA cleared tests are available also need to be considered.

It is important to note that in emergency situations, as promulgated by the FDA any standard procedure may be bypassed if the clinical need is paramount. (see 21 Code of Federal Regulations 606.121(h) and www.fda.gov/ora/inspect_ref/igs/blood.html)

CHANGES IN REGULATIONS FOR PLATELET STORAGE

As discussed above, in 1984 the US-FDA extended the platelet storage from five to seven days based on the functional quality of the platelets. However, in response to an increasing number of reports of bacterial contamination episodes, the US-FDA reduced the storage time back to five days in 1986. Nevertheless, to comply with the new AABB and CAP recommendation for screening platelet products for bacterial contamination, the average time between collection and issuing the product has been increased and most SDP products are released three days after collection, making a difficult task for blood centers to satisfy the need for platelet products. In 2005, the US-FDA approved platelet bags made by Gambro BCT and Baxter for 7-day storage of SDP platelets provided that a US-FDA-cleared detection method is used,[15] and the US-FDA-approved protocol, the PASSPORT Study (www.passportstudy.com) is followed. The extended storage time would improve detection and confirmation of positive results before units have been used, and could limit the wastage of units.

SDP VERSUS RDP UNITS

Although several reports have demonstrated a reduced incidence of transfusion-associated platelet bacterial contamination with the use of SDP platelets compared with pooled RDP units,[11, 25, 36] the difficulty of maintaining a SDP platelet inventory has forced transfusion services to continue using pooled RDP units. However, if RPD units are screened for bacterial contamination with a sensitive method at the time of issue, the risk of transfusing a bacterially contaminated unit should be similar to that of SDP units. The obstacle in reaching that goal is that the US-FDA-approved methods for detection of bacterial contamination have been approved for quality control of SDP and, in some cases, RDP units, but not until recently (see below) for RDP pools, making approved systems prohibitively expensive to use on each RDP unit used to prepare a pool. Most hospital blood banks are therefore using methods such as pH or glucose determination, which have low sensitivity, but are rapid and easy to perform at the time of issue. The main reason that transfusion services and blood banks need to do the testing of RDP units is because the pooling of the units is done just before releasing the product for transfusion. This problem could be overcome by the allowance of early pooling along with screening for bacterial contamination with one of the methods already cleared by the US-FDA for SDP units.

Investigators have assessed the effectiveness of storing RDP platelets as a pool compared to SDP platelets and found that there is no detrimental effect of pooling RDP platelets as relates to platelet quality for products stored for up to 7 days.[69, 70] In February 2006, the US-FDA approved a platelet storage bag, the Pall Acrodose PL system, for the pooling of 4 to 6 ABO identical, leukocyte-reduced, RDP units shortly after collection and subsequent storage for up to 5 days after blood collection when coupled with a device cleared by US-FDA for detection of bacterial contamination in pooled, leukoreduced RDP units.[71]

Although some investigators have stated that the sensitivity of culture testing of pools may be insufficient to detect all contaminated products, and that manipulation of pools can also increase false positive results,[54] others have found pool testing to be reliable.[72-74] In addition, the use of both aerobic and anaerobic BacT/ALERT bottles may enhance the detection of contaminated units. This finding seems to be related to the volume of sample cultured rather than the isolation of strict anaerobic organisms.[74]

DETECTION METHODS UNDER DEVELOPMENT

Several detection methods suitable for use at the time of issue of platelet products are currently being developed, and include the following:

Detection of bacterial 16S ribosomal RNA with oligonucleotide probes. A chemiluminescence-linked universal bacterial rRNA probe for the detection of bacterial contamination in 0.4 mL aliquots of platelet concentrates detected the majority of samples containing 10^4 CFU/mL of *Bacillus cereus, P. aeruginosa, S. aureus* and *S. epidermidis*, and all samples with 2.1×10^5 CFU/mL or greater of these species.[75] Further development of this method is currently not being pursued.

Detection of conserved regions of bacterial 23S rRNA and a heat shock protein by real-time, reverse transcriptase PCR assay. Two real-time reverse transcriptase PCR (RT-PCR) assays performed in a LightCycler instrument were developed and compared regarding specificity and sensitivity by the use of different templates to detect the majority of the clinically important bacterial species in platelets.[76] Primers and probes specific for the conserved regions of the eubacterial 23S rRNA

gene or the *groEL* gene (encoding the 60-kDa heat shock protein Hsp60) were designed. With r*Tth* DNA polymerase in a one-enzyme system, 500 CFU/mL of *Escherichia coli* or *Staphylococcus epidermidis* were detected. With a two-enzyme system consisting of Moloney murine leukemia virus RT and *Taq* DNA polymerase, 16 CFU/mL were detected. With *groEL* mRNA as the target of RT-PCR under optimized conditions, 125 CFU/mL of *E. coli* were detected. False-positive results caused by reagent contamination or a cross-reaction with human nucleic acids were found with the 23S rRNA, but not with the *groEL* gene detection systems.

BacSTAT Antibody-based lateral flow device for detection of bacteria. This system, being developed by GenPrime Inc., Spokane, WA, (www.genprime.com) is a broad-spectrum, antibody-based lateral flow device for detection of bacteria in SDP or RDP units, and uses a unique lateral-flow scanning system and a reader with pass/fail software.[77] The antibody targets used in this system are unique surface antigens. Specimen processing is simple, and total test time is less than 20 minutes. The expected sensitivity of this method is 10^3 to 10^4 CFU/mL.

Rapid Bioluminescent Bacterial Detection System using Luciferase. This method, which detects bacterial ATP by generation of light in the presence of bioluminescent luciferase firefly extract, is being developed by SUBC Inc., Rochester, Minnesota.[78] The method consists of removing platelets and cellular debris from 2-3 mL platelet concentrate samples, isolation and immobilization of any bacteria present in an isolation chamber, rinsing the isolation chamber with buffer, adding a lysing solution in combination with localized heat to rapidly lyse bacterial membranes and release bacterial ATP, adding bioluminescent firefly extract, and detecting generated light with a photon counter. The burst of light is then converted into a bacterial inoculum in CFU/mL. The sensitivity of this method is claimed to be 10^2 to 10^3 CFU/mL. A fully automated, microprocessor-driven instrument with an internal reagent cartridge, GloBac, has been developed for this application, and processing time is 4 min per specimen.

Bacterial lipotechoic acid and lipopolysaccharide detection by lateral flow immunoprecipitation. This test is being developed as the Verax Biomedical Platelet PGD Test by Verax Biomedical Inc., Worcester, MA (www.veraxbiomedical.com).[79] This system consists of test kits with a double-sided, lateral flow test cartridge and a central specimen well. Testing is performed by adding 8 drops of a platelet lysis reagent to 0.5 mL of a platelet sample, and centrifuging for 5 min to sediment any bacteria present. The supernatant is decanted off and 8 drops of a resuspension agent and 4 drops of a bacterial lysis reagent are then added, and the fluid is then added to the central well of the lateral flow test cartridge. The test cartridge contains antibodies to bacterial lipotechoic acid on one side and lipopolysaccharide on the other side to detect Gram-positive and –negative bacteria, respectively. A positive test is shown by the development of a pink precipitation line on one side. The system is designed for use with leukoreduced or non-leukoreduced RDPs, pooled RDPs and SDPs, with testing taking 30 min, with 2 to 3 minutes attended labor per test. The sensitivity of this method is claimed to be 10^3 to 10^4 CFU/mL.

Bacteriological Biosensor using engineered spores as fluorogenic nanodetectors. This system, being developed by BCR Diagnostics, Jamestown, Rhode Island (www.bcrbiotech.com), is able to detect and count low bacterial levels quantitatively in less than 20 minutes with a linear detection response over a range of one to 10,000 bacteria per sample.[80] The instrumentation cost is low and the system is suitable for automated high-throughput operations. A 0.5 mL platelet sample is processed to separate bacteria, and the resulting suspension is mixed with engineered bacterial spores. The mixture is then combined with a germinogenic substrate and then filtered through a filter mounted on a biochip – the filter contains 80,000 micro-colanders, which are 20 µm in diameter and contain engineered biosensor bacterial spores. The presence of a bacterial aminopeptidase, present in all bacteria, in the platelet specimen triggers the biosensors on the spores, resulting in production of fluorescent light, which is detected by a camera. The expected sensitivity of this method is 10^3 CFU/mL.

Bacterial peptidoglycan chromogenic immunoassay. This method is being developed as BacTx by Immunetics, Inc., Boston, MA (www.immunetics.com) and consists of a test kit containing all needed reagents and a chromogenic reader.[81] The system has a turnaround time of less than one hour, a 1 mL sample volume, and premeasured vial reagent dispensers. The system detects bacterial peptidoglycan in Gram-positive and Gram-negative bacteria when a peptidoglycan binding protein triggers enzymatic conversion of a chromogenic substrate to a visible product with measurable absorbance. A 1 mL platelet sample is centrifuged for 10 min, the sediment incubated for 30 min with reagents, and absorbance is read on a reader. Assay sensitivity is claimed to be 10^3 to 10^4 CFU/mL.

CONCLUSIONS

Bacterial contamination of platelet units is the most prevalent infectious risk of blood products in the United States. In the last 3 years, a unified effort from professional and regulatory agencies has been committed to limiting bacterial contamination of platelet products. Strategies beginning with donor selection, collection guidelines, use of detection methods after collection and at time of issue have been developed in order to deal with this problem. Because of the multi-factorial etio-

logy of bacterial contamination and the heterogeneous growth and metabolic characteristics of the organisms involved, different strategies should be used in combination. Now that FDA-cleared methods and guidelines are available to meet the requirement that all platelet units be tested for bacterial contamination, it is hoped that data will continue to be collected and new strategies will be developed to reduce the occurrence and the fatalities associated with bacterial contamination of platelets. Several promising methods for at-issue testing are being developed and will hopefully obtain regulatory approval soon.

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