Platelet bacterial contamination and the use of a chemiluminescence-linked universal bacterial ribosomal RNA gene probe

M.E. BRECHER, J.J. HOGAN, G. BOOTHE, A. KERR, L. MCCLANNAN, M.R. JACOBS, R. YOMTOVIAN, V. CHONGOKOLWATANA, G. TEGTMEIER, S. HENDERSON, A. PINEDA, V. HALLING, M. KEMPER, K. KURAMATO, P.V. HOLLAND, AND M. LONGIARU

Background: Currently, the maximum outdate for platelets is 5 days, because of the increasing chance of bacterial growth over time. Various methods for rapid detection of bacterial contamination of blood components have been described, with mixed results and no general acceptance. A recently described, molecular biologic approach for the detection of bacterial contamination involves a chemiluminescence-linked universal DNA bacterial probe to a highly conserved bacterial region of ribosomal RNA (rRNA).

Study Design and Methods: A multicenter trial of a chemiluminescence-linked universal bacterial rRNA probe for the detection of bacterial contamination in platelet concentrates is described. At each of five sites, platelet concentrates (no older than 1 day from date of phlebotomy) were inoculated in triplicate with isolates of four bacterial species (*Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*) to a final concentration of 10 to 50 colony-forming units (CFUs) per mL and in triplicate to a final concentration of 1000 CFUs per mL. At one site, an additional 6 platelet concentrates were inoculated with sterile saline to serve as controls. Inoculated units were then subjected periodically to quantitative cultures and probe analyses. A total of 126 platelet concentrates were studied over a period of 7 days (120 inoculated with bacteria and 6 with sterile saline).

Results: This assay was, in some cases, able to detect *S. aureus* bacterial contamination in the range of 100 to 1000 CFUs per mL; the majority of samples (*B. cereus*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*) with contamination exceeding 10^4 CFUs per mL; and all samples with contamination of 2.1×10^5 CFUs per mL or greater. Increasing the sample size from the recommended 0.4 mL to 1.0 mL resulted in an unacceptable loss of specificity (83.3%).

Conclusion: The routine use of this assay would be expected to result in a decreased risk of septic platelet transfusion reactions and could lead to a lengthening of the current 5 day storage period for platelets. Further, the pooling of random-donor platelet concentrates before storage instead of immediately before transfusion may be possible if this rRNA probe is employed to detect bacteria in the pool. **TRANSFUSION** 1994;34:750–755.

Abbreviations: CFU(s) = colony-forming unit(s); PC(s) = platelet concentrate(s); RLU(s) = relative light unit(s); rRNA = ribosomal RNA.

CURRENTLY, THE maximum outdate for platelets is 5 days, because of the increasing chance of bacterial growth over time. Various methods for the rapid detection of bacterial contamination of blood components have been described, including Gram stain,[1,2] acridine orange staining,[3,4] endotoxin assays,[4,5] color change,[6] platelet swirling,[7,8] and blood gas analysis[9]; there have been mixed results and no general acceptance. Recently, molecular biologic techniques have been applied to the problem. Through the use of polymerase chain reaction, Feng et al.[11] detected 500 *Yersinia enterocolitica* organisms in 100 µL of whole blood (5×10^3 colony-forming units [CFUs]/mL). As Wagner et al.[12] pointed out, such a technique typically requires 3 hours for thermocycling and involves multiple processing steps including centrifugation, boiling, and protease treatment of samples. Such techniques are also subject to contamination problems leading to decreased specificity and, as described, were limited to one bacterial species. A more promising molecular bio-

From the University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; the Institute of Pathology, Case Western Reserve University School of Medicine and University Hospitals, Cleveland, Ohio; the Community Blood Center of Greater Kansas City, Kansas City, Missouri; the Mayo Clinic, Rochester, Minnesota; the Sacramento Medical Foundation, Sacramento, California; and Gen-Probe, San Diego, California.

Supported in part by Gen-Probe, Inc.

Received for publication February 4, 1994; revision received May 23, 1994, and accepted June 1, 1994.

logic approach for the detection of bacterial contamination involves a chemiluminescence-linked universal DNA bacterial probe to a highly conserved bacterial region of ribosomal RNA (rRNA). Brecher et al.[9] found that such a probe assay could, in some cases, detect *Staphylococcus epidermidis* in concentrations as low as 1×10^3 CFUs per mL in platelet concentrates (PCs) and that it reliably detected contamination with the same bacteria in all platelet units at a concentration of 1×10^4 CFUs per mL.

In a multicenter trial, we investigated the ability of a chemiluminescence-linked bacterial rRNA probe to detect bacterial contamination in PCs deliberately inoculated with known contaminants. Our goal was to evaluate the sensitivity and specificity of the chemiluminescence-linked rRNA probe with a variety of bacterial organisms at various concentrations.

Materials and Methods

Study design

Five clinical sites—the University of North Carolina at Chapel Hill (Site 1), the University Hospitals of Cleveland (Site 2), the Community Blood Center of Greater Kansas City (Site 3), the Sacramento Blood Center (Site 4), and the Mayo Clinic (Site 5)—participated in clinical trials to evaluate a chemiluminescence-linked universal DNA-rRNA bacterial probe. At each site, PCs (no older than 1 day from the date of phlebotomy) were inoculated in triplicate with one of four bacterial species (*Pseudomonas aeruginosa* #1915799, *Bacillus cereus* #1967467 and #1952142, *S. epidermidis* #2343536 and #1965845, and *Staphylococcus aureus* #2458334) to final concentrations of 10 to 50 and 1000 CFUs per mL. Thus, a total of 30 PCs were inoculated with each of the four organisms (15 at 10-50 CFU/mL and 15 at 1000 CFU/mL). At Site 1, an additional 6 PCs were inoculated with sterile saline to serve as controls. All bacterial isolates were originally obtained from actually contaminated PCs isolated at the Clinical Microbiology Laboratory University Hospitals of Cleveland. Each unit was then followed for up to 7 days after inoculation with periodic quantitative bacterial cultures and probe analyses.

To gain additional insight into the practicality of implementing such an assay in a busy transfusion service and to obtain another measure of specificity (but by routine laboratory technologists rather then research and development personnel), Site 1 implemented, on a trial basis, one strategy for testing. For a 6-week period the entire single-donor apheresis platelet inventory of Site 1 was sampled, probed, and cultured in the early hours of the morning. If the probe assay resulted in a value exceeding a predetermined threshold, the platelet unit was quarantined and the probe was repeated twice. Such repeat assays could be performed the same day or with the following day's batch run. Had a unit's repeat testing again exceeded the threshold ("best 2 of 3"-type testing), it would have been cultured and discarded. Those PCs that had probe assays below the testing threshold were made available for issue until the next batch testing was completed.

Blood collection

Each PC and single-donor apheresis platelet was obtained after standard processing of whole blood from healthy, volunteer (unpaid) blood donors.

Inoculation, sampling, and storage of units

On Day 0 of the study, sampling-site couplers were placed in the PCs and samples were obtained aseptically for baseline probe analysis and culture. We then inoculated individual units with sterile saline or with bacterial suspensions obtained from several colonies placed overnight (16-18 hours) on 5-percent sheep blood agar plates or in broth. The turbidity of the suspension was adjusted to match McFarland turbidity standards. A 1.0 McFarland standard is equivalent to approximately 3×10^8 CFUs per mL. Serial dilutions of these suspensions yielded concentrations from which small aliquots (0.2-1.5 mL) were inoculated into the PCs to obtain final concentrations of approximately 10 to 50 CFUs per mL or 1000 CFUs per mL. Thus, in total, 126 PCs were inoculated (120 with bacteria, 6 with sterile saline) and followed. We determined the actual inoculum dose from quantitative cultures of duplicate aliquots of the inoculum.

PCs were sampled aseptically in a laminar-flow hood on weekdays (Monday through Friday) until Day 7 after inoculation or until the relative light unit (RLU) signal from the probe exceeded 500,000 (Sites 2-5). Site 1 sampled daily for 7 days, irrespective of weekends or the magnitude of the RLU signal. Sample size was 1.5 to 2 mL per day. RNA preparation and the quantitative cultures were performed within 1 hour of sampling.

Sampling of the single-donor apheresis platelets was accomplished from a freshly made segment from the attached tubing of the bag of platelets obtained after thorough mixing of the bag's contents with the fluid within the tubing. This allowed the bag's contents to be tested without compromise of the shelf life of the PCs. Ideally, if it is anticipated that a unit will be tested over several days, long lengths of tubing should be left on these bags at the time of platelet production to facilitate testing.

Bacterial culture

We performed bacteriologic assays by serial 1-in-10 dilutions of 0.1 mL of platelets. We then plated 0.1 mL of sample (and dilutions thereof) on sheep blood agar and incubated it at 37°C for 48 hours. Colonies were then counted from plates with 25 to 300 colonies and the CFUs per mL were calculated. If the concentration exceeded 3×10^8 CFUs per mL, the concentration was recorded as $>3 \times 10^8$ CFUs per mL. Site 1 performed sufficient dilutions for accurate determination of the final plateau concentrations. The minimum sensitivity of the assay was 10 CFUs per mL.

Probe analysis

Probe analysis was performed as described previously.[9] In brief, a hybridization protection assay was performed employing chemiluminescence (acridinium ester)-labeled single-stranded DNA probes complementary to a highly conserved bacterial rRNA region. Bacteria are lysed and the rRNA released. Labeled DNA probe combines with the complementary rRNA to form a stable DNA:RNA hybrid, and unhybridized probe is selectively hydrolyzed. A chemiluminescent reaction is then initiated in the presence of base and hydrogen peroxide, and the amount of luminescence is quantitated in RLUs.

Directions and proprietary reagents employed in this assay were provided by Gen-Probe, Inc. The four steps of the assay (RNA preparation, hybridization, differential hydrolysis, and detection) are summarized in Fig. 1. Positive and negative controls typically had RLU values of more than 60,000 and less than 3000, respectively. Positive (RNA extracted from

RNA PREPARATION
0.4 mL of platelets, washed twice
0.05 mL of enzymatic lysis reagent added to pellet
37°C incubation x 15 minutes
↓
HYBRIDIZATION
0.05 mL of probe added
60°C incubation x 20 minutes
↓
DIFFERENTIAL HYDROLYSIS
0.3 mL of buffered alkaline selection reagent added
60°C incubation x 10 minutes
↓
DETECTION
Cool at room temperature x 5 minutes
0.4 mL of test mixture placed in luminometer
0.2 mL of 0.1 H_2O_2 in 0.001 *M* H_2NO_3
1-second delay
0.2 mL of 1 *M* NaOH
Luminescence measured x 2 seconds
Expressed in RLU

FIG. 1. Overview of the probe assay.

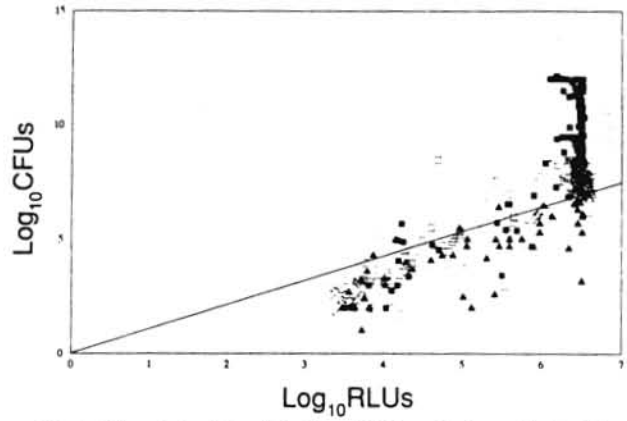

FIG. 2. The relationship of the $\log_{10}$ RLUs to the $\log_{10}$ CFUs. This relationship holds over approximately 10^3 to 10^6 RLU per mL (beyond this point, the probe is saturated). The line drawn is the line of identity, where RLU = CFU. The R^2 value for a regression over the range of 10^3 to 10^6 RLU per mL is 0.57. The similar clustering of points from each site about this line reflects the approximate reproducibility of the probe assay between sites. Site 1, ■, Site 2, ○; Site 3, △; Site 4, ▲; Site 5, □.

Escherichia coli) and negative (sterile-buffered detergent solution) controls were performed in duplicate with every run.

To assess the effect of sample volume, we probed 1.0-mL samples from Site 1 in parallel with the routine 0.4-mL samples of the PCs from Day 0 to Day 7.

Data analysis

We calculated sensitivity as the percentage of culture-positive PCs detected when a specified RLU threshold (15,000 or 30,000 RLU) was exceeded. These results were categorized by magnitude of bacterial contamination and bacterial species. We calculated specificity as the percentage of culture-negative samples with an RLU value below a specified threshold (15,000 or 30,000 RLU). These determinations included all values obtained on Day 0 prior to inoculation, daily sampling of the 6 units inoculated with sterile saline, and any culture-negative samples obtained from the inoculated units obtained on days before the units became culture-positive. For the single-donor apheresis transfusion service trial, a threshold of 15,000 RLU was employed.

Results

Bacterial growth

Bacterial growth occurred in all 120 PCs inoculated with bacteria (30 of each of 4 different organisms) during the 7-day observation period. The 6 PCs inoculated with sterile saline remained sterile throughout the study period. All 126 samples obtained on Day 0 prior to inoculation were culture-negative. For the 120 bacterially inoculated units, we were able to identify the day that 110 units became culture-positive (99 on Day 1, 8 on Day 2, 2 on Day 4, and 1 on Day 7); in 10 instances, the exact day of culture positivity could not be determined, because these units did not have a negative culture the day before they were first detected as culture-positive (as a result of the fact that some sites did not test on the weekends). No lag phase (days with little or no growth) was noted for *B. cereus* and *P. aeruginosa*, while 6 (20%) of 30 of *S. aureus*-inoculated units (5 with low inoculum, 1 with a high inoculum) showed no growth on Day 1. Overall, *S. epidermidis* grew notably slower than the other bacteria, with a lag time varying from 0 to 6 days. By Day 4 after inoculation, 10 (33%) of 30 *S. epidermidis*-inoculated PCs had not exceeded 1×10^5 CFUs per mL (all but 1 unit were culture-positive by Day 4), whereas all PCs inoculated with the other three isolates had exceeded, or (on the basis of extrapolation of their growth curves) should have exceeded, 1×10^5 CFUs per mL by Day 3 after inoculation.

Typical doubling times for the organisms were approximately 1 hour for *B. cereus*, 1.7 hours for *P. aeruginosa*, and 1.6 to 3.3 hours for *S. aureus*. *S. epidermidis* was the most variable, with doubling times ranging from 1 to 6 hours. Even within the same site, doubling times for *S. epidermidis* differed, depending on the units inoculated. At Site 1, doubling times were either 1 or 4 hours. This was a reproducible phenomenon. Growth curves based on data from Site 1 ultimately plateau at 10^{10} to 10^{12} CFUs per mL for each organism tested.

Probe analysis

The number of CFUs per mL approximated the RLU values of the probe analysis over the range of 10^3 to 10^6 (Fig. 2), after which no increase in RLU value was apparent. This was likely due to the saturation of available probe. The comparability between sites is illustrated in the overlapping values seen in Fig. 2. The sensitivity and specificity of the probe assay stratified by magnitude of CFUs per mL are summarized in Tables 1 and 2. At an RLU cutoff of 30,000, all contaminated units were detected at concentrations of 5×10^5 CFUs per mL or greater. At an RLU cutoff of 15,000, all contaminated units were detected at concentrations of 2.1×10^5 CFUs per mL or greater.

Day 7 probe testing of the 1.0-mL sample was invalid, because of positive negative controls (this was attributed to a small crack that was detected in the rubber light seal and/or to a possible static charge buildup); this run was not repeated. Specificity with the 0.4-mL samples was 100 percent for both the 15,000 and 30,000 RLU thresholds. The use of a 1.0-mL sample at Site 1 (Days 0-6) and a 30,000-RLU threshold approximated

Table 1. *Sensitivity of rRNA bacterial probe* by species and magnitude of concentration and overall specificity† (Days 0-7)*

Range (CFU/mL)	Sensitivity (%)									
	B. cereus		*P. aeruginosa*		*S. aureus*		*S. epidermidis*		Total	
10^2 - <10^3	0	-‡	0	(0/1)§	31	(4/13)	0	(0/14)	14	(4/28)
10^3 - <10^4	0	-	0	(0/5)	50	(2/4)	7	(2/29)	11	(4/38)
10^4 - <10^5	100	(2/2)‖	43	(3/7)	80	(8/10)	58	(7/12)	65	(20/31)
10^5 - <10^6	100	(3/3)	91	(10/11)¶	75	(3/4)**	100	(9/9)††	93	(25/27)¶
>10^6	100	(62/62)	100	(64/64)	100	(59/59)	100	(54/54)	100	(239/239)

* RLU cutoff = 30,000.
† 97.8 percent (177/181).
‡ Undefined values.
§ RLU positive/CFU positive.
‖ All cases detected at ≥5 × 10^4 CFU/mL.
¶ All cases detected at ≥5 × 10^5 CFU/mL.
** All cases detected at ≥2.5 × 10^5 CFU/mL.
†† All cases detected at ≥1 × 10^5 CFU/mL.

the use of a 0.4-mL sample and a 15,000 threshold. Both resulted in increased sensitivity overall compared to a 0.4-mL sample and a 30,000-RLU threshold, but with a modest decrease in specificity to 98.3 percent (59/60) with the larger sample and a 30,000 RLU threshold. However, the 1.0-mL sample detected one additional sample of *S. aureus* in the range of ≥10^4 to <10^5 CFUs per mL that was not detected with the 0.4-mL sample and a 15,000-RLU threshold; conversely, the 0.4-mL sample and a 15,000-RLU threshold detected one sample of *S. epidermidis* in the range of ≥10^3 to <10^4 CFUs per mL that was not detected with the 1.0-mL sample and the 30,000-RLU threshold. Specificity with the 1-mL samples decreased from 98.3 percent (59/60) with a threshold of 30,000 RLU to 83.3 percent (50/60) with a threshold of 15,000 RLU.

Transfusion Service Trial

In total, 347 single-donor apheresis platelets were tested on 509 occasions (220, 92, 33, and 2 units were tested on 1, 2, 3, or 4 occasions, respectively). No units were found to be culture-positive. Specificity during this exercise was 96.8 percent (336/347). No initially reactive unit repeated as reactive.

Discussion

This study confirms the previously reported sensitivities of the universal bacterial rRNA probe for *S. epidermidis* bacterial contamination of platelets (Table 2).[9] This assay (as in the previous study) was able in 11 (38%) of 29 cases to detect *S. epidermidis* bacterial contamination in the range of ≥10^3 to <10^4 CFUs per mL and all but 1 (92%) of 29 cases in the range of ≥10^4 to <10^5 CFUs per mL. For *S. aureus* contamination, 4 (31%) of 13 were detected with bacterial concentrations as low as the range of ≥10^2 to <10^3 CFUs per mL. Because of the rapidity of growth with *B. cereus* and *P. aeruginosa*, concentrations lower than 1 × 10^4 CFUs per mL were not detected or assayed. However, as with the two examples of Staphylococcal species, the majority of examples of *B. cereus* and *P. aeruginosa* were detected in the range of ≥10^4 to <10^5 CFUs per mL (100% and 71%, respectively). Of the 120 units, 1 would not have been detected by the probe assay because of low colony count (10^2 CFU/mL, Site 4, *S. epidermidis*). Overall, the probe assay reliably detected all contaminated samples with bacterial concentrations greater than 1 × 10^5 CFUs per mL with an overall specificity exceeding 92 percent. In a recent report by Chongokolwatana et al.,[13] the utility of the rRNA probe for detecting platelet bacterial contamination was compared with microscopy. The rRNA probe detected significantly more instances of contamination with levels of organisms in the range of 10^2 to 10^5 CFUs per mL than did the Gram and acridine orange stains. At contamination levels greater than 10^6 CFUs per mL, the detection methods were equivalent.

Comparisons of sample size were similar for a 0.4-mL sample with a threshold of 15,000 RLU and a 1.0-mL sample and a threshold of 30,000 RLU. Decreased specificity to 83.3 percent precluded the use of the 1.0-mL sample with a 15,000-RLU threshold. These results do not support the use of the larger, 1.0-mL sample size for probe analysis.

As currently configured (with one technologist and a batch size ≤30 samples), performance of a chemilumi-

Table 2. *Sensitivity of rRNA bacterial probe* by species and magnitude of concentration and overall specificity† (Days 0-7)*

Range (CFU/mL)	Sensitivity (%)									
	B. cereus		*P. aeruginosa*		*S. aureus*		*S. epidermidis*		Total	
≥10^2 - <10^3	0	-‡	0	(0/1)§	31	(4/13)	0	(0/14)	14	(4/28)
≥10^3 - <10^4	0	-	0	(0/5)	50	(2/4)	38	(11/29)	34	(13/38)
≥10^4 - <10^5	100	(2/2)‖	71	(5/7)	90	(9/10)	92	(11/12)	87	(27/31)
≥10^5 - <10^6	100	(3/3)	100	(11/11)¶	75	(3/4)**	100	(9/9)††	96	(26/27)¶
>10^6	100	(62/62)	100	(64/64)	100	(59/59)	100	(54/54)	100	(239/239)

* RLU cutoff = 15,000.
† 92.8 percent (168/181).
‡ Undefined values.
§ RLU positive/CFU positive.
‖ All cases detected at ≥5 × 10^4 CFU/mL.
¶ All cases detected at ≥2.1 × 10^5 CFU/mL.
** All cases detected at ≥1.8 × 10^5 CFU/mL.
†† All cases detected at ≥1.0 × 10^5 CFU/mL.

nescence-linked rRNA assay from the point of sampling to the final assay result requires from 2 to 3 hours. While no more difficult to perform than a manually performed enzyme-linked immunoassay, the rRNA assay remains relatively labor-intensive, with manual washes during the RNA preparation, manual pipetting of samples, and insertion of tubes for final signal detection in the luminometer. The use for the RNA preparation of automated cell washers similar to those currently in use in blood banks and transfusion services is one possible way to automate this procedure. Additional automation of sample handling and placement and reading within the luminometer are also possible. Such automation would greatly facilitate the implementation of widespread testing. It is anticipated that the cost of reagents and labor will be comparable to that of an enzyme-linked immunoassay.

Assessing the cost-to-benefit ratio for the implementation of any test for bacterial contamination of blood components is problematic. While it is known that approximately 1 in 1000 units of platelets are bacterially contaminated and that approximately 5 deaths per year attributed to that contamination are reported to the United States Food and Drug Administration, the actual morbidity is not known. It is suspected that in some cases bacterial infections attributed to catheter-related sepsis may actually be platelet transfusion-related.[14] Nevertheless, it could be argued that implementation of platelet bacteria testing might be more cost-effective in preventing transfusion-related deaths in the United States then the currently implemented testing for syphilis, alanine aminotransferase, and antibodies to hepatitis B core (antigen), human T-lymphotropic virus type I and II, and human immunodeficiency virus type 2. Unlike all other disease marker testing that currently is generally performed by collection facilities (or an affiliated laboratory), it is likely that larger institutions that maintain sizeable platelet inventories will require on-site testing. Thus, the cost will likely affect both transfusion services and collecting facilities.

We have described a once-a-day batch testing scheme that was implemented for a 6-week testing period at the University of North Carolina. A variation on this approach would be pooling of random-donor platelets before initial testing (ideally with a thermal weld connection device) and testing of the pools rather than each PC in the pool. Pooled stored platelets have been shown to have satisfactory in vitro properties after storage.[15,16] It would offer the advantages of decreased reagent costs and testing time. It would also allow for more rapid issuing of a therapeutic dose of platelets to a patient, as the platelets would already be pooled. Currently, such practice is not allowed by either the regulations of the US Food and Drug Administration or the standards of the American Association of Blood Banks, because of fears of increased risk of bacterial contamination and growth due to the pooling of platelets. If a reliable detection system for bacterial contamination, such as the described rRNA bacterial probe assay, were available, a case could be made for modifying such restrictions. However, any savings would be partially offset by the costs associated with the use of sterile connection devices, if employed.

Bacterial growth has previously been shown to be a function of the inoculating concentration.[17] However, Myhre et al.[17] also noted no individual variation in the bactericidal property of platelets. Our data suggest that for *S. epidermidis* there is variation in the antibacterial properties of platelets that may be organism-specific.

In conclusion, we have described a multicenter trial of a universal bacterial rRNA probe. This assay was in some cases able to detect *S. aureus* bacterial contamination as low 100 to 1000 CFUs per mL; the vast majority of all samples (*B. cereus*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*) with contamination exceeding 10^4 CFUs per mL; and all samples with contamination of 2×10^5 CFUs per mL or greater. The routine use of such an assay would be expected to result in a decreased risk of septic platelet transfusion reactions and could lead to a lengthening of the storage period of platelets and possibly allow the pooling of random-donor PCs at the time of preparation instead of just before issuance to a patient. Application of any method for the detection of bacterial contamination of platelets will be most cost-effective if the additional costs are offset by the ability to extend the shelf life of tested components to 7 days.

Acknowledgments

The authors thank Ruth Jacobson (Mayo Clinic), Saralee Bajaksouzian (Case Western Reserve University), Dale Rabe (Mayo Clinic), and Necia Williams (University of North Carolina) for technical assistance.

References

1. Barrett BB, Anderson JW, Anderson KC. Strategies for the avoidance of bacterial contamination of blood components. Transfusion 1993;33:228-33.
2. Yomtovian R, Lazarus HM, Goodnough LT, Hirschler NV, Morrissey AM, Jacobs MR. A prospective microbiologic surveillance program to detect and prevent transfusion of bacterially contaminated platelets. Transfusion 1993;33:902-9.
3. McCarthy LR, Senne JE. Evaluation of acridine orange stain for detection of microorganisms in blood cultures. J Clin Microbiol 1980;11:281-5.
4. Arduino MJ, Bland LA, Tipple MA, Aguero SM, Favero MS, Jarvis WR. Growth and endotoxin production of *Yersinia enterocolitica* and *Enterobacter agglomerans* in packed erythrocytes. J Clin Microbiol 1989;27:1483-5.
5. Kim DM, Brecher ME, Bland LA, et al. Prestorage removal of *Yersinia enterocolitica* from red cells with white cell-reduction filters. Transfusion 1992;32:658-62.
6. Kim DM, Brecher ME, Bland LA, Estes TJ, Carmen RA, Nelson EJ. Visual identification of bacterially contaminated red cells. Transfusion 1992;32:221-5.
7. Myhre BA, Demianew SH, Yosimori RN, Nelson EJ, Carmen RA. pH changes caused by bacterial growth in contaminated platelet concentrates. Ann Clin Lab Sci 1985;15:509-14.

8. Wenz B, Burns ER, Freundlich LF. Prevention of growth of *Yersinia enterocolitica* in blood by polyester fiber filtration. Transfusion 1992;32:663-6.
9. Brecher ME, Boothe G, Kerr A. The use of a chemiluminescence-linked universal bacterial ribosomal RNA gene probe and blood gas analysis for the rapid detection of bacterial contamination in white cell-reduced and nonreduced platelets. Transfusion 1993;33:450-7.
10. Kim DM, Estes TJ, Brecher ME, Carmen RA, Nelson EJ. WBC filtration, blood gas analysis and plasma hemoglobin in *Yersinia enterocolitica* contaminated red cells (abstract). Transfusion 1992;32(Suppl):41S.
11. Feng P, Keasler SP, Hill WE. Direct identification of *Yersinia enterocolitica* in blood by polymerase chain reaction amplification. Transfusion 1992;32:850-4.
12. Wagner SJ, Friedman LI, Dodd RY. Transfusion-associated sepsis. Clin Microbiol Rev 1994;7:290-302.
13. Chongokolwatana V, Morgan M, Feagin JC, Jacobs MR, Yomtovian R. Comparison of microscopy and a bacterial DNA probe for detecting bacterially contaminated platelets (abstract). Transfusion 1993;33:(Suppl)50S.
14. Krishnan LS, Brecher ME. Transfusion transmitted bacterial infection. Hematol Oncol Clin of North Am (in press).
15. Snyder EL, Stack G, Napychank P, Roberts S. Storage of pooled platelet concentrates: in vitro and in vivo analysis. Transfusion 1989;29:390-5.
16. Moroff G, Holme S, Dabay MH, et al. Storage of pools of six and eight platelet concentrates. Transfusion 1993;33:374-8.
17. Myhre BA, Walker LJ, White ML. Bacteriocidal properties of platelet concentrates. Transfusion 1974;14:116-23.

Mark E. Brecher, MD, Associate Director, Transfusion Medicine Service, and Associate Professor, Department of Pathology, University of North Carolina Hospitals, 101 Manning Drive, Chapel Hill, NC 27514. [Reprint requests]

James J. Hogan, PhD, Scientific Investigator, Hybritech, Inc., San Diego, CA.

Gregg A. Boothe, MS, MT (ASCP)SBB, Advanced Blood Bank Technologist, Hoxworth Blood Center, Cincinnati, OH.

Alan Kerr, BSc, MT(ASCP), Clinical Microbiology & Immunology, University of North Carolina Hospitals.

Laura S. McClannan, MSCLS, MT(ASCP)SBB, Component Supervisor, Transfusion Medicine Service, University of North Carolina Hospitals.

Michael R. Jacobs, MD, PhD, Professor of Pathology, Case Western Reserve University, Cleveland, OH.

Roslyn Yomtovian, MD, Assistant Professor of Pathology, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

V. Chongokolwatana, MD, Fellow, Blood Bank, Department of Pathology, Vanderbilt University, Nashville, TN.

Gary Tegtmeier, PhD, Director of Research, Community Blood Center of Greater Kansas City, Kansas City, MO.

Sharon E. Henderson, BS, MT(ASCP), Immunology/Virology Supervisor, Community Blood Center of Greater Kansas City.

Alvaro A. Pineda, MD, Professor, Mayo Medical School; Medical Director, Component Laboratory; and Consultant, Division of Transfusion Medicine, Mayo Clinic, Rochester, MN.

Valerie W. Halling, CLA, ASCP, Development Technologist, Component Laboratory, Division of Transfusion Medicine, Mayo Clinic, Rochester, MN.

Marti Kemper, MT(ASCP)BB, BS, Research Associate, Sacramento Medical Foundation, Sacramento, CA.

Ken Kuramato, MT, BS, Research Coordinator, Sacramento Medical Foundation.

Paul V. Holland, MD, Medical Director/CEO, Sacramento Medical Foundation.

Mathew Longiaru, PhD, Director, Gen-Probe, Inc., San Diego, CA.