Use of the RQI Test for Bacterial Screening of Whole Blood Platelets

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Abstract

We compare our experience using a new rapid qualitative immunoassay (RQI) test (platelet Pan Genera Detection, Verax, Worcester, MA) for bacterial screening of whole blood platelet (WBP) pools with our previous WBP bacterial screen, pH testing.

All WBP pools were RQI tested at the time of issue. All RQI+ pools were cultured in an automated culture system, with subsequent bacterial identification if the culture was positive.

During approximately 5.5 months, 7,733 WBP pools were RQI tested. There were 14 positive RQI tests; 12 WBP pools were sterile when cultured and considered false-positive RQI tests. One pool was positive for coagulase-negative Staphylococcus, while another was positive for group B Streptococcus. The specificity and positive predictive value of the RQI test were 99.85% and 14.3%, respectively. The specificity and positive predictive value of the RQI test were higher than pH testing, leading to less waste of sterile WBP pools.

Bacterial contamination of platelets is of great clinical concern, affecting approximately 0.007% to 0.07% of whole blood platelets (WBPs) (reviewed in detail by Blajchman et al3). The AABB (formerly known as the American Association of Blood Banks) has mandated that steps be implemented to detect and limit the growth of bacteria in platelet units.4 To comply with this regulation, several techniques have been advanced for detecting contaminated platelets, including measurement of pH, Gram stain,5 platelet swirling,6,7 2 different automated culture systems,8-11 chemiluminescent DNA probes for bacterial ribosomal RNA genes,12 epifluorescence techniques for specific bacteria,13 fluorescent probes for bacterial DNA,14 and laser-based solid phase cytometry for leukoreduced platelets.15 The US Food and Drug Administration (FDA) has approved the Acrodose system (Pall Medical, East Hills, NY), a novel device whereby WBPs can be pooled, leukoreduced, and sampled for culture on day 2 of storage and then maintained for up to 5 days at room temperature. The use of a diversion pouch that retains a small quantity of the first blood phlebotomized from the donor, including the skin plug, has also been demonstrated to reduce the incidence of platelet contamination by skin flora.16-18 Each of these tests have trade-offs in ease of performance, rapidity of reporting a result, and sensitivity and specificity for detecting bacterially contaminated platelets (reviewed by Müller et al19). Time-of-issue tests, such as pH testing, have the advantage of providing real-time information about the platelet unit just before it is transfused, although the sensitivity and specificity of these tests tend to be lower than culture-based tests, which can detect very small bacterial loads, often fewer than 10 colony-forming units (CFU)/mL.8,10
The Centralized Transfusion Service (CTS), Pittsburgh, PA, provides transfusion medicine support to 16 area hospitals and uses predominantly WBPs. Transfusing a large number of WBPs required the use of a rapid and simple time-of-issue test for bacterial contamination, namely pH testing. Unfortunately the low specificity and positive predictive value (PPV) of this test resulted in a 1% wastage of sterile platelets. We thus sought another time-of-issue test that would accommodate the large volume of WBPs transfused within our network of hospitals, would have a lower detection threshold for bacteria than pH testing, and would have a higher specificity and PPV than pH testing, thereby reducing the wastage of WBPs.

The rapid qualitative immunoassay (RQI) test (platelet Pan Genera Detection, Verax, Worcester, MA) for bacterial contamination of platelets has received 501(k) clearance from the FDA for use as an adjunct in detecting bacteria in leukoreduced apheresis platelets that have also been tested by an FDA-approved bacterial detection system specific for this type of platelet product. The device is a small cartridge featuring separate lanes that detect gram-positive or gram-negative bacteria in the platelet product by targeting conserved bacterial cell membrane constituents, lipoteichoic acids or lipopolysaccharides, respectively. According to the manufacturer, the RQI test can detect bacteria as low as $10^3$ to $10^5$ CFU/mL, which is several magnitudes lower than the detection threshold of pH testing, $10^7$ to $10^8$ CFU/mL. The RQI test is rapid and easy to perform; thus, it is suitable for time-of-issue platelet testing. We report our initial experience of screening WBP pools for bacteria using the RQI test.

Materials and Methods

The database of the CTS was reviewed to establish the number of WBP pools issued after the implementation of the RQI test on October 13, 2008, through March 31, 2009. Approximately 90% of the WBPs were obtained from 1 local FDA-licensed blood center. A diversion pouch was used in the phlebotomy of all donors from this blood center, although it is unknown if one was used by the blood centers from which approximately 10% of our WBPs were imported. The standard pool size was 5 units of WBPs, although periodically, in times of shortages, the pool sizes were reduced in accordance with the day’s platelet inventory. The WBP units were pooled according to standard transfusion service protocols when an order for platelet transfusion was received and then leukoreduced, if indicated.

Standard growth detection following inoculation of products with lyophilized organisms was not appropriate for the validation of the RQI test because in the lyophilization process, some bacterial antigen sites are altered or destroyed. The ZeptoMetrix Mixed Titer Bacteria Panel for Platelets (ZeptoMetrix, Buffalo, NY) was used to validate the RQI test. This panel included 8 species of bacteria and 2 sterile control samples that were thawed before use. The lowest concentration of live bacteria in the panel was $4 \times 10^2$ CFU/mL (ZeptoMetrix, oral communication, customer service representative, April 2009). Two nonleukoreduced and 2 leukoreduced platelet pools were used to resuspend each panel sample. The RQI test gave a positive result with the samples containing bacteria and a negative result with the sterile samples. In addition, 6 randomly selected WBP pools with a pH of 7.0 or more, our previous criterion for issuing WBPs, were RQI tested, and negative results were obtained. These 6 WBP pools were cultured using the eBDS automated culture system (Pall Medical) and were found to be sterile.

After validation, the RQI test was performed on all WBP pools at the time of issue per the manufacturer’s instructions; briefly, after thorough mixing, 500 μL of the WBP pool was aspirated from the WBP unit, and the aliquot was cultured in an aerobic bottle using an automated culture system (BacT/ALERT, bioMérieux, Durham, NC) for 5 days. Any units that were culture-positive during the 5-day incubation were sent to an outside laboratory for bacterial identification and Gram stain. Simultaneously, all of the available cocomponents from the WBP units in the pool were quarantined and the RBCs cultured on the BacT/ALERT. If RBCs were not available, the plasma cocomponents were cultured. If the culture of the WBP pool and the individual RBC cocomponents were sterile after the 5-day incubation, the RBCs were returned to the blood bank’s inventory and could be issued for transfusion, and the RQI test result was considered to be a false-positive.

**Table 1**

<table>
<thead>
<tr>
<th>Bacteria Present in the ZeptoMetrix Mixed Titer Bacteria Panel for Platelets Used to Validate the Rapid Qualitative Immunoassay Test</th>
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<tbody>
<tr>
<td>Bacillus thuringiensis</td>
</tr>
<tr>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
</tr>
</tbody>
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true-positive test was defined as one in which the RQI test was positive and bacteria were isolated from any of the cultured material. If a WBP unit involved in a positive RQI test was imported, the blood supplier was notified. All tests were performed in accordance with the manufacturer’s instructions.

The total number of WBP pools subjected to the RQI test during the study period, the results of this testing, and the results of any Gram stain and bacterial identification were recorded. Statistical differences between categorical variables were assessed by using a 2-tailed Fisher exact test (GraphPad Software, San Diego, CA). The study protocol was approved by the University of Pittsburgh Institutional Review Board.

Results

The results of screening WBP pools using the RQI test are given in Table 2, along with the previously reported results of screening individual WBPs using the pH test. There were a total of 43 individual WBP units involved in the 12 pools that produced a false-positive RQI test. All false-positives occurred in the gram-positive lane. The average age of these WBPs was 4.2 days (median, 5 days; range, 2-5 days). In 1 of these 12 cases, an initially positive RQI test was found to be negative when the test was repeated and the WBP pool was sterile when cultured; thus, this was considered a false-positive RQI test result.

Bacteria were identified in culture from 2 of 14 RQI-positive pools. The pool in which coagulase-negative Staphylococcus was identified consisted of 5 U of WBPs. All of these WBPs were 5 days old, and 4 of the RBC cocomponents were shown to be sterile after testing with the BacT/ALERT. Coagulase-negative Staphylococcus was identified in the remaining RBC cocomponent, implicating the donor as the source of the bacteria rather than a laboratory contaminant. The donor was a 47-year-old man who passed all of the donor screening questions and transmissible disease tests on the day of his donation and, on questioning after the contamination was found, confirmed that he had no infectious ailments on the day of his donation and that he was not under the care of a physician at that time. The pool in which group B Streptococcus was identified also consisted of 5 WBPs that were all 5 days old. All of the RBC cocomponents were sterile when cultured in the BacT/ALERT; nevertheless, this was thought to be a true-positive RQI test result because group B Streptococcus is not likely to be an environmental contaminant, nor does it grow well under RBC storage conditions.

No septic transfusion reactions to platelets were reported to the blood bank during the study period.

Discussion

There are several factors to consider when deciding which time-of-issue test to use for detecting bacterial contamination of WBP products: The test must be rapid and simple so that it can be performed nearly 60 to 80 times per day in a large transfusion service. It must have sufficient sensitivity to prevent issuing a contaminated product, yet its specificity must also be high enough to avoid wasting a large number of sterile platelets. Unfortunately, there is no FDA-approved time-of-issue test for the bacterial screening of WBPs, and so from March 2004 until mid October 2008, we used pH testing to comply with the AABB mandate for bacterial detection. As we reported earlier (Table 2), 405 (1.1%) of 37,060 of the WBPs screened had a pH less than 7.0 and, thus, were not issued, while bacteria were isolated from only 4 of those 405 WBP units.20 Thus, 401 sterile WBPs were wasted, and the PPV and specificity of the pH test were 1% and 98.93%, respectively.

In the current study, 14 of 7,733 WBP pools (corresponding to 53 individual WBPs) tested positive using the RQI test. The PPV of the RQI test was significantly higher than that of the pH test (14.3% vs 1%, respectively; \( P = .014 \)), and the clinical impact of the higher specificity of the RQI test was apparent. Had 1.1% of the 7,733 WBP pools failed the RQI test, a total of 85 WBP pools (corresponding to approximately 255-425 individual WBP units based on a pool size of 3-5 WBP units) would have been discarded.

<table>
<thead>
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<th>Table 2</th>
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<tr>
<td>Results of Screening WBPs With the RQI Test</td>
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<table>
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<tr>
<th>Platelet Screening Test</th>
<th>No. of WBP Products Screened</th>
<th>No. (%) of Positive Tests</th>
<th>No. of Positive Tests From Which Bacteria Were Identified in Culture</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RQI</td>
<td>7,733</td>
<td>14 (0.2)</td>
<td>2</td>
<td>99.85</td>
<td>14.3</td>
<td>.014</td>
</tr>
<tr>
<td>pH †</td>
<td>37,060</td>
<td>405 (1.1)</td>
<td>4</td>
<td>98.93</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

PPV, positive predictive value; RQI, rapid qualitative immunoassay; WBP, whole blood platelet.

* See text for details.
† \( P \) value compares the PPV of the RQI and pH tests.
‡ Data from Yazer and Triulzi.20
Although neither the pH test nor the RQI test are approved by the FDA for standalone time-of-issue bacterial screening of WBPs, the RQI test has a distinct advantage over the pH test insofar as the RQI test directly detects bacterial cell membrane components instead of relying on a surrogate marker for the presence of bacteria (ie, decreased pH). Because WBCs and the platelets themselves are metabolically active during storage, decreased pH is not specific for bacterial contamination; evidence for the metabolic activity of WBCs during storage comes from the observation that prestorage leukoreduced platelets had a lower rate of pH testing failures compared with nonleukoreduced platelets, and, in our hands, the leukoreduced WBP units with a pH less than 7 had, on average, double the WBC content of those with a pH of 7 or more. Thus, while WBCs contribute to the decreased specificity of pH screening for bacterially contaminated platelets, they are unlikely to cause a false-negative reaction in the RQI test.

In addition to not being approved by the FDA for the bacterial screening of WBPs, there are several other drawbacks of the RQI test. While the cost of performing a pH test on each individual WBP unit was negligible, each RQI test costs approximately $40 per pool to perform. In our hands the test takes approximately 30 to 35 minutes to perform from the time the WBP pool sample is loaded into the cartridge until the platelets have entirely traversed the gram-positive and gram-negative test lanes. Data from the manufacturer indicate that the majority of positive reactions will be noted within the first 12 to 15 minutes; thus, our policy for issuing platelets under urgent clinical situations is to release the WBP pools after this shorter incubation as long as the test remains negative. The test continues to incubate for the full 30 to 35 minutes after the WBP pools have been issued and, in the unlikely event that it should turn positive, an attempt would be made to recover the WBP pools before they are transfused. If even a 12- to 15-minute delay is not clinically acceptable, WBPs would be issued immediately after the inoculation of the RQI test cartridge. Unfortunately, we do not know how long it took for either of our bacterially contaminated WBP pools to demonstrate a positive reaction in the RQI test.

This study has several limitations. Because the WBP pools that passed the RQI test and were issued for transfusion were not cultured, sensitivity could not be evaluated. The manufacturer claims that the test can detect bacteria as low as 10^3 to 10^4 CFU/mL, and in our validation studies, the RQI test gave a positive reading when WBPs containing on the order of 10^2 CFU/mL were tested. It would have been interesting to have performed pH testing on the 14 units that failed the RQI test to see if they would also have been detected by this method. Furthermore, because we did not culture the WBP pools that passed the RQI test, it is possible that bacteria might have been identified from some of these pools had a more sensitive test, such as culture, been used. In this study, and in our previous study of pH screening, we assumed that all pools that passed the RQI test or the pH test were truly negative for bacteria. Thus, our specificity rates for the RQI and pH tests might have been affected by this assumption.

In this study, the rate of bacterially contaminated WBPs was approximately 1:19,000 (0.005%) based on a pool size of 5 WBPs. The rate of bacterial contamination in this study was in the range of our previous study of pH screening at 1:37,060 to 1:9,265, despite the fact that diversion pouches were not routinely used at our center when the data for the pH study were collected. Nevertheless, the rate of 1:19,000 is on the lower end of that reported in the literature, and there are several possible explanations: The RQI test has a higher detection threshold for bacteria (ie, it is less sensitive) compared with culture-based tests, which possibly led to the lower overall rate of contamination when compared with tests with a lower detection threshold (ie, more sensitive tests). The RQI test is performed with only 500 μL of the WBP unit compared with the 4- to 8-mL aliquot that is commonly inoculated into automated culture systems; thus, a contaminated WBP pool with a low bacterial load might avoid detection owing to the small amount of fluid used for the RQI test. Last, it is common to use almost all of the WBPs in our inventory during the day and then replenish the stock overnight. Thus, given such a brisk turnover of WBPs, it is possible that we are transfusing fresher WBPs, which are less likely to be bacterially contaminated, although data on the average age of the transfused WBPs at the CTS are not available.

This is the first large-scale report detailing the off-label use of the RQI test for bacterial screening of WBP pools. This test is more specific and has a higher PPV for detecting bacteria, which resulted in fewer wasted sterile WBP units. Although the bacterial detection threshold of the RQI test is expected to be superior to pH testing, this remains to be proven under actual transfusion service conditions.

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References


