Is It Safe to Omit the 37 °C Reading From Pretransfusion Red Blood Cell Antibody Detection Testing?

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The routine pretransfusion red blood cell antibody detection test (PADT), performed at the authors' institution, consists of a three-phase, saline-antiglobulin technique (immediate spin, 30-minute incubation at 37 °C, IgG indirect antihuman globulin test [IAT]), and each phase is examined for hemolysis and agglutination. To determine if it would be safe to omit reading the 37 °C phase of the PADT, a 6-year retrospective review of records (February 1986 to February 1992) was undertaken. Of approximately 280,000 sera tested for unexpected red cell alloantibodies, 1480 (.53%) were reactive at only 37 °C. Of 1480 sera tested to 1 in every 1875 sera tested, had they not been detected. The authors suggest that the increased risk of an acute or delayed hemolytic transfusion reaction would be too high to justify a procedural change. Based on these data, the authors continue to read the 37 °C phase of the PADT for hemolysis and agglutination. (Key words: Antibody detection testing; Pretransfusion testing; 37 °C incubation step reading) Am J Clin Pathol 1994;101:361-364.

METHODS AND MATERIALS

During the study period, the PADT consisted of a three-phase, saline-antiglobulin technique: a saline immediate spin test, a 30-minute incubation step at 37 °C (without the addition of a potentiator) and an IAT with anti-IgG. Group O R1R1 and Group O R2R2 reagent RBCs (Ortho Diagnostics, Redondo Beach, CA; Organon Teknika Corp, Durham, NC; Immucor, Norcross, GA; Gamma Biologicals, Houston, TX; and Dade, Miami, FL) were used; one of the reagent RBCs was centrifuged and examined for hemolysis and macroscopic agglutination. Incubation was for 30 minutes at 37 °C; at the conclusion, the mixture was again centrifuged and examined for hemolysis and macroscopic agglutination.

A pretransfusion antibody detection test (PADT) should detect antibodies that can cause hemolysis of red blood cells (RBC). Guidelines for performing a PADT have been established by the Food and Drug Administration, the College of American Pathologists, and the American Association of Blood Banks. In general, a PADT should consist of at least two phases: a 37 °C incubation step and an indirect antiglobulin test. Although a 37 °C incubation step is included as part of a PADT, there is no requirement that the 37 °C step be examined for a result. The PADT performed at our institution consists of a three-phase, saline-antiglobulin technique (immediate spin, 30-minute incubation at 37 °C, and IgG indirect antihuman globulin test [IAT]), and each phase is examined for hemolysis and agglutination. In response to workload pressures, however, we evaluated the safety of omitting the 37 °C phase reading from the PADT routine. We reasoned that if the 37 °C phase of the PADT routine could be omitted, that up to 93,000 technologist observations might be avoided annually. This, in turn, would simplify the PADT routine, reduce data recording, possibly reduce the number of antibody identification workups, and improve workflow. We did not consider omitting the immediate spin phase of the PADT, because the overwhelming majority of RBC units transfused at our institution are cross-matched by an immediate spin test. Furthermore, the immediate spin cross-match is usually not done until the RBC units are requested for transfusion. We were concerned that omitting the immediate spin phase from the PADT would result in an increase in the number of positive immediate spin cross-matches, which might delay blood availability for some patients while the cause of a positive cross-match was being determined.
glutination. The reagent red cells were then washed four times with 0.9% sodium chloride saline in preparation of the IgG-IAT phase. The supernatant was decanted, and anti-IgG (Gamma Biologicals, Immucor) was added; the mixture subsequently centrifuged and then read for agglutination. Each negative IAT was verified by using IgG-coated reagent RBCs (check cells). Antibodies in reactive sera were identified using standard methods. Medical records of patients with clinically significant antibodies whose serum samples reacted at only 37 °C were reviewed to determine pregnancy and transfusion history.

RESULTS

The results of approximately 280,000 antibody detection tests were reviewed retrospectively. Roughly 9500 sera (approximately 3.4%) demonstrated reactivity (agglutination, hemolysis, or rouleaux), of which 1480 (approximately 5%) showed reactivity at only the 37 °C reading (Table 1).

Of the 1480 sera that were reactive only at 37 °C, 86 (approximately 0.3%) of the total sera tested) contained alloantibodies, such as anti-D, anti-E, anti-K, anti-c, and anti-Fy(a), that had specificities known to cause hemolytic transfusion reactions or hemolytic disease of the newborn. These 86 sera were from 53 different patients, of whom 49 had one antibody identified in their serum samples, and 4 had two antibodies. At least 45 (85%) of the 53 patients had been pregnant or transfused (Table 2). In 49 of the 53 patients, the initial PADT performed was reactive only at 37 °C. The initial PADT for the remaining four patients was at first positive at the IAT phase of testing, but one or more follow-up serum samples reacted only at 37 °C. In 25 cases, there was only one reactive serum sample, and no follow-up serologic testing was performed. In 10 cases, however, repeat testing of the same serum sample showed reactivity in the IAT phase; in seven other cases, follow-up blood samples eventually showed reactivity in the IAT phase. Six of the 53 patients experienced a delayed serologic transfusion reaction (DSTR), as defined by Ness and colleagues; in all six cases, the first positive antibody detection test was reactive only at 37 °C, but two eventually became reactive at the IAT phase of testing. Five of these DSTRs were associated with anti-K and one was associated with anti-E. Two of the six DSTR cases showed evidence of immune hemolysis and were suspected of having had delayed hemolytic transfusion reactions (DHTR).

In addition to the 86 serum samples containing potentially significant antibodies, 154 sera showed reactivity only at the 37 °C reading because of innocuous causes (anti-I, other cold agglutinins, and rouleaux), and 1240 sera contained antibodies of no or questionable significance, such as anti-Le(a) (n = 907), anti-Le(b) (n = 38), anti-Le(a)+anti-Le(b) (n = 58), anti-Le(a)+cold agglutinins (n = 78), anti-P1 (n = 7), and others (n = 152).

DISCUSSION

The practice of transfusion medicine has changed greatly in the last decade, partly because of economic constraints. As a result, efforts have been made toward eliminating unnecessary tests and streamlining testing techniques. We therefore explored the possibility of eliminating the 37 °C-phase reading of the PADT from our routine, particularly because this step was not required for accreditation. We reasoned that if this reading could be omitted safely, that more than 93,000 technologist observations and test result recordings could be eliminated annually. In turn, this would reduce both the number of innocuous antibodies detected and the number of antibody identification workups performed. Fewer observations would decrease the number of manual clerical errors and future automation of pretransfusion antibody detection techniques would become more efficient with less observations. Furthermore, the PADT routine would be simplified, and work flow would be improved, thus possibly avoiding clerical and technical errors.

The immediate spin test is also not a required element of the PADT. However, we chose not to consider omitting the immediate spin phase from the PADT because we use an immediate spin cross-match for nonalloimmunized patients, and this cross-match test is not done until the blood is needed for transfusion. We were concerned that if the immediate spin phase of the PADT were omitted, we might miss detecting antibodies capable of causing a reactive immediate spin cross-match, which might result in a delay in blood availability while the cause of the positive immediate spin cross-match was being determined.

Based on our 6-year retrospective records review, had the 37 °C reading been omitted from our PADT routine, the detection of clinically insignificant antibodies would have been reduced approximately 25%. This reduction in antibody detection would have reduced the number of antibody identification
Within 4 mo (＞90% of cases) or because of technical errors (＜10% of cases).

To detect approximately one potentially significant antibody per 4700 sera tested if our testing workups, decreased workload, and improved work flow. In our view, however, this reduction in workload would cause an unreasonable increased risk of hemolytic transfusion reactions.

Even the most sensitive antibody screening techniques fail to detect some antibodies. 12-14 Currently, our PADT method fails to detect approximately one potentially significant antibody for every 4700 sera tested, either because of low test sensitivity (＞90% of cases) or because of technical errors (＜10% of cases). However, because approximately 1.4% of the sera tested at our institution contain significant alloantibodies, and because approximately 2.2% of these sera react at only the 37 °C reading, we estimate that if the 37 °C reading had been omitted, the cumulative risk of missing a potentially significant antibody would increase from approximately one in every 4700 sera tested to about one in every 1875 sera tested if our testing procedure were changed. During the study period, there were 132 patients who had a DSTR, six of whom (4.5% of all DSTRs diagnosed) had an emerging alloantibody that was initially detectable only at the 37 °C phase. In two of these six cases, evidence was found of possible immune red cell destruction, although definitive diagnoses of DHTR could not be made because of complicated clinical courses. Of the six patients with a DSTR or DHTR, a follow-up serum sample eventually showed reactivity in the IAT phase in only two. Had the reading of the 37 °C phase of the PADT not been done, the diagnosis of DSTR and possibly DHTR might have been delayed or missed altogether. We view this increase in risk to transfused patients as unacceptable.

Our results confirm and expand on those of other investigators who have also examined the importance of reading the 37 °C phase of PADT for hemolysis and agglutination. An albumin-antiglobulin method was evaluated by Reisner and colleagues,16 and a low-ionic strength salt-antiglobulin technique was studied by Judd and colleagues.17 Both groups of investigators found that clinically significant antibodies reacted only at 37 °C, and concluded that examining the 37 °C phase for a result was important when performing their respective methods of PADT. Neither group eliminated the 37 °C reading from their testing methods.

Of the 53 patients who had potentially significant antibodies detected only at the 37 °C reading, eight had no known history of pregnancy or transfusion; of these eight, five had anti-E. The lack of a known sensitization event raised the possibility that these five examples of anti-E could have occurred naturally. This is speculative, however, as a sensitization event might have occurred but not been adequately documented in the medical records. Furthermore, even if one or more of these anti-E had occurred naturally, it would not change our approach to transfusing these five patients, if transfusion became necessary. Examples of naturally occurring anti-E have caused shortened survival of transfused E-positive RBCs; thus, any one of these five patients who required RBC transfusions would receive donor RBC units that lacked the E-antigen.15

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The 37 °C reading appears to play an important role when performing the low-ionic strength salt, albumin, or saline methods of antibody detection. Furthermore, our data suggest that a prewarmed saline-antiglobulin technique would not be expected to detect more than approximately 98% of clinically significant antibodies, because the prewarmed saline-antiglobulin method is read only in the IAT, and no enhancement medium is used. Omission of the 37 °C reading might not be deleterious, however, when using other techniques, such as polyethylene glycol (PEG), which is routinely read in only the IAT phase.18 In our laboratory, the PEG method is used on a case-by-case basis. During the study period, PEG was used in five of the cases in which the routine PADT was reactive only at 37 °C. In four of these five patients, the serum samples were reactive when read in the IAT using PEG. However, in the one case where the PEG-IAT method did not detect the antibody, a delayed hemolytic transfusion reaction was suspected. Sera from this patient had previously reacted in the IAT phase using the saline-antiglobulin technique. Other methods that are routinely read only at the IAT phase include a gel test19 and a

### Table 2. History of Red Cell Exposure in Patients With Potentially Significant Antibodies That Reacted at Only 37 °C When Performing a Saline-Antiglobulin Technique

<table>
<thead>
<tr>
<th>RBC Exposure</th>
<th>E</th>
<th>K</th>
<th>C</th>
<th>c</th>
<th>D</th>
<th>M</th>
<th>Fya</th>
<th>K + C</th>
<th>E + D</th>
<th>E + c</th>
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<tr>
<td>Within 4 mo</td>
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<td>Pregnancy</td>
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<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
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<td></td>
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<tr>
<td>Over 4 mo</td>
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<td>1</td>
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<td></td>
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<tr>
<td>Patient subtotal</td>
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<td>14</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
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</table>

### Table 3. Serology of Patients Diagnosed With a Delayed Serologic Transfusion Reaction

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Initial Reactivity</th>
<th>Eventually IAT Reactive</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Anti-K</td>
<td>37 only</td>
<td>Yes</td>
<td>49</td>
<td>M</td>
<td>AML</td>
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<tr>
<td>Anti-K</td>
<td>37 only</td>
<td>Yes</td>
<td>43</td>
<td>F</td>
<td>Pancreatitis*</td>
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<tr>
<td>Anti-K</td>
<td>37 only</td>
<td>No</td>
<td>50</td>
<td>M</td>
<td>MVA</td>
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<tr>
<td>Anti-K</td>
<td>37 only</td>
<td>No</td>
<td>46</td>
<td>M</td>
<td>AIDS</td>
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<tr>
<td>Anti-E</td>
<td>37 only</td>
<td>No</td>
<td>33</td>
<td>M</td>
<td>AIDS</td>
</tr>
<tr>
<td>Anti-E</td>
<td>37 only</td>
<td>No</td>
<td>56</td>
<td>F</td>
<td>TTP*</td>
</tr>
</tbody>
</table>

AML = acute myelogenous leukemia; MVA = motor vehicle accident; TTP = thrombotic thrombocytopenic purpura; IAT = indirect antiglobulin test.

* Suspected delayed hemolytic transfusion reaction.

RBC = red blood cells.
microplate solid-phase technique. Continued investigation is required to evaluate the strengths and weaknesses of these new techniques. As discussed, one facet that needs evaluation is how effective these new techniques are at detecting antibodies that are reactive only at 37 °C.

In summary, we believe the data demonstrate that, depending on the PADT method used, the failure to read the 37 °C phase of the PADT could increase the risk of transfusion. Consequently, as long as a saline-antiglobulin PADT technique is used at our institution, the 37 °C phase of the PADT will be examined for hemolysis and agglutination.

REFERENCES