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Intravenous Rh immune globulin prevents alloimmunization in D– granulocyte recipients but obscures the detection of an alloanti-K


Rh immune globulin (RhIG) has been used to prevent alloimmunization in D– recipients of apheresis platelet transfusions from D+ donors that may contain up to 5 mL of D+ red blood cells (RBCs). Granulocyte concentrates contain approximately 30 mL of RBCs and it has been necessary to give D– recipients granulocyte transfusions from D+ donors. Intravenous RhIG has not yet been demonstrated to be effective in preventing D alloimmunization with granulocyte transfusions. Four D– recipients received multiple D+ granulocyte transfusions from D+ donors and multiple injections of intravenous RhIG at a standard dose of 600 µg for each D+ transfusion. Two D– males with chronic granulomatous disease were given 32 and 13 daily granulocyte transfusions, 18 and 2 of which, respectively, were D+. After the first dose of intravenous RhIG, both patients exhibited circulating anti-D that was undetectable 3 to 4 years later. Two patients with severe aplastic anemia were given 5 and 14 granulocyte transfusions, 4 and 7 of which, respectively, were D+. Both patients died before the effectiveness of RhIG could be assessed. In one of these patients the indirect and direct antiglobulin tests became positive after the first dose of intravenous RhIG, which required that subsequent granulocyte transfusions from D+ donors be crossmatched by immediate spin (IS) testing only. A delayed hemolytic reaction attributed to alloanti-K occurred after granulocytes from a K+ donor were given to this patient. These results suggest that intravenous RhIG can be used to prevent alloimmunization to D in D– patients receiving large quantities of RBCs from D+ granulocyte transfusions. However, anti-D and other passive antibodies from RhIG prohibit the use of the antiglobulin crossmatch with antigen-positive granulocyte donor samples. It may be important to frequently collect new samples to screen for newly formed alloantibodies when IS crossmatches are used in place of the antiglobulin crossmatch. Immunohematology 2001; 17:37–41.

Key Words: granulocytes, granulocyte transfusions, intravenous immune globulin, Rh immune globulin, Rh alloimmunization, Rh D, transfusion reactions

The therapeutic use of granulocyte concentrates has increased as a result of the large quantity of cells that can be collected from donors mobilized with granulocyte colony-stimulating factor (G-CSF). Transfusion of granulocytes from D-incompatible donors is more problematic than transfusion of platelets from D-incompatible donors because of a higher contamination of red blood cells (RBCs) in granulocyte concentrates. Granulocyte concentrates contain an average of 27 mL of RBCs and may contain more than 40 mL of RBCs, compared with a maximum of 5 mL of RBCs permitted in apheresis platelet concentrates or 0.5 mL in platelet concentrates prepared from a whole blood donation. As donors for granulocyte collections must be willing to receive an injection of G-CSF, the pool of available granulocyte donors is of limited size. The number of D– granulocyte donors is limited further by the need to find donors who are ABO compatible with the recipient and, in some cases, who are seronegative for cytomegalovirus.

Preventing alloimmunization to the D antigen in women of childbearing age has been the goal of transfusion services and obstetricians for many years because anti-D has been implicated in fetal death and in severe hemolytic disease of the newborn. Preventing alloimmunization to the D antigen in other individuals would allow for the transfusion of D+ blood in an emergency, continued support with D+ granulocyte concentrates, and possibly prevent the concomitant formation of autoantibody. The timely administration of appropriately dosed intramuscular or intravenous Rh immune globulin (RhIG) can prevent alloimmunization of D– women during pregnancy. The incidence of alloimmunization to the D antigen in women receiving antepartum and postpartum prophylactic intramuscular RhIG was reduced to 0.1 percent. A similar incidence of D alloimmunization of 1 in 1122 (0.09%) in women
receiving antepartum and postpartum prophylactic intravenous RhIG was reported by Bowman et al. The transfusion of blood products that contain D+ RBCs also can cause alloimmunization in D– individuals. Timely and appropriate doses of intramuscular RhIG can prevent alloimmunization to the D antigen in nearly 100 percent of D– recipients of D+ RBCs. The use of intravenous RhIG has been approved by the Food and Drug Administration to treat children and adults with acute and chronic immune thrombocytopenia purpura and for suppression of D alloimmunization in pregnancy. The calculated dose of RhG for the WinRho SDFT™ product (WinRho SDFT™, Cangene Corporation, Winnipeg, Canada) can be administered by either the intramuscular or intravenous route. Dosage is based on 5 IU being equivalent to 1 µg and 18 µg is required for each mL of D+ RBCs. The product is available in 600 IU (120 µg) and 1500 IU (300 µg) vials. It has been shown to be effective in the prevention of D alloimmunization for recipients of D+ platelet products. There is evidence provided by Jouveneaux et al. that the intravenous route is more effective than the intramuscular route for protection against transfusion of D+ RBCs. The WinRho SDFT™ package insert recommends the administration of more intramuscular RhIG (120 IU or 24 µg per mL RBCs) than intravenous RhIG (90 IU or 18 µg per mL RBCs) for exposure to D+ RBCs. Recently, intravenous RhIG has been successfully used to prevent D alloimmunization in a D– child inadvertently transfused with D+ blood.

Materials and Methods

Study design

We evaluated the administration of intravenous RhIG to four D– pediatric patients transfused with granulocytes from D+ donors. Two of the children had chronic granulomatous disease (CGD) and two had severe aplastic anemia. Intravenous RhIG was chosen because repeated high doses were required, and the children being treated had a limited muscle mass and in some cases were thrombocytopenic. Our pharmacy routinely supplies intravenous RhIG.

RBC antibodies

Plasma from EDTA-anticoagulated whole blood was screened for atypical antibodies and, when indicated, antibody identification was performed using a low-ionic-strength solution (LISS) antiglobulin gel technique (Micro Typing Systems, Inc., Pompano Beach, FL).

The direct antiglobulin test (DAT) was performed by standard tube technique using patient RBCs from EDTA-anticoagulated whole blood and polyspecific, monospecific anti-IgG, and monospecific anti-C3d (BCA, West Chester, PA) antiglobulin reagents. Titration, adsorption studies, and antigen typings were performed using standard tube techniques. Eluates were prepared from RBCs using Elu-Kit™ II (Gamma Biologicals, Houston, TX). The patients were monitored for the presence of circulating anti-D using the antibody screen.

Mobilization and collection of granulocytes

Granulocyte concentrates were collected from donors only given dexamethasone, 8 mg PO, approximately 12 hours prior to collection; G-CSF (Filgrastim, Amgen, Thousand Oaks, CA) only, 5 µg/kg SQ, approximately 18 hours prior to the collection; or both dexamethasone and G-CSF. Granulocyte concentrates were collected using a CS3000 blood cell separator (Fenwal, Baxter Healthcare Corporation, Round Lake, IL). A granulocyte separation chamber was used and 7 liters of whole blood were processed per procedure, using 30 mL of 46.7% of trisodium citrate anticoagulant (tirCitrasol; Citra anticoagulants, Braintree, MA) in 500 mL of hydroxyethyl starch (Baxter). The interface offset was 33. The final volume of the concentrates was approximately 250 mL.

Administration of intravenous RhIG

For patients 1, 2, and 3, 3000 IU (600 µg) of WinRho SDFT™ were given intravenously for each D+ granulocyte concentrate transfused. RhIG was given within 48 hours following the incompatible transfusion. Because patient 4’s clinical condition was unstable, her antibody screen was monitored for circulating anti-D and she received a reduced dose of RhIG (600 µg for every 2 D+ granulocyte concentrate; Table 1).

Table 1. Number of granulocyte concentrates and doses of RhIG given to each patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>15</td>
<td>6</td>
<td>9</td>
<td>11</td>
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<tr>
<td>Diagnosis</td>
<td>CGD*</td>
<td>CGD†</td>
<td>SAA†</td>
<td>SAA</td>
</tr>
<tr>
<td>Blood type</td>
<td>B -</td>
<td>O -</td>
<td>B -</td>
<td>A -</td>
</tr>
<tr>
<td>Number of granulocyte concentrates given</td>
<td>32</td>
<td>13</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Number of D+ concentrates</td>
<td>18</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>RhIG doses (600 µg)</td>
<td>18</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* Chronic granulomatous disease  
† Severe aplastic anemia
Results

Patient 1, a 15-year-old male with CGD, received 32 granulocyte concentrates collected from dexamethasone-treated donors over 49 days. Eighteen of the granulocyte concentrates were from D+ donors. Circulating anti-D was detected after intravenous RhIG was administered, but the DAT was negative. Following the last intravenous RhIG infusion, his antibody screen was strongly reactive with D+ screening cells (3+). The antibody screen was repeated regularly for several weeks following the last infusion; 3 months after the last infusion, the antibody screen remained weakly reactive with D+ cells. The next antibody screen, which was performed 4 months later, was negative. More than 65 antibody screens were performed for anti-D over the next 4 years, all of which were negative (Table 2).

Patient 2, a 6-year-old male with CGD, was given 13 dexamethasone-mobilized granulocyte concentrates over 25 days, 2 of them from D+ donors. After intravenous RhIG was administered, anti-D was detected in his serum and the DAT was positive. Anti-D was eluted from his RBC sample, which contained D+ transfused RBCs. No additional granulocyte concentrates were transfused. His DAT remained positive for 2 weeks following the last granulocyte transfusion. The antibody screen was strongly reactive with D+ cells (2+) during the 2 weeks following the infusion of 6000 IU of intravenous RhIG but was only weakly reactive at 3 weeks. Titration studies with R,R, RBCs revealed an endpoint of w+ at 1, 4, and 4 for samples drawn on days 12, 15, and 19, respectively, of postinfusion intravenous RhIG. Four months after the infusion of RhIG, his antibody screen and DAT were negative, as were three assays performed during a 3-year follow-up period.

Patient 3, an 11-year-old male with severe aplastic anemia, was given five G-CSF plus dexamethasone-mobilized granulocyte concentrates over 9 days for a disseminated aspergillus infection of the lung and Enterobacter enteroccus bacteremia. Four of the granulocyte concentrates were from D+ donors. He was given four doses of 3000 IU (600 µg) of intravenous RhIG after receiving four granulocyte concentrates from D+ donors, which he tolerated without complications. Anti-D was detected 2, 6, and 9 days postinfusion of RhIG. A DAT was not performed. He died from the infections 10 days after the first granulocyte concentrate was given.

Patient 4, an 11-year-old group A girl with severe aplastic anemia, was treated with antithymocyte globulin and cyclosporin A. She developed an aspergillus infection of her sinuses despite treatment with amphotericin and was transfused with 14 G-CSF and dexamethasone-mobilized granulocyte concentrates, 7 of which were from D+ donors, over 23 days. She was given four infusions of 3000 IU (600 µg) of intravenous RhIG. Antibody screen results were negative prior to the administration of intravenous RhIG. Circulating anti-C and -D were detected after two doses of RhIG and infusion of two D+ granulocyte concentrates. Over the next 6 days, she received three D+ granulocyte transfusions and a third dose of RhIG. This lot of RhIG was shown to contain anti-D, -C, and -E at titration levels of >4096, 64, and 4, respectively, by indirect antiglobulin test (IAT). Subsequently, circulating anti-E as well as anti-D were detected in the patient’s plasma. She received multiple granulocyte and platelet transfusions from group O donors. The DAT was positive with both IgG (1+) and C3d (1+) antiglobulin. Anti-D and -A were eluted from her RBCs.

A fourth dose of RhIG and two more D+ granulocyte concentrates were given over the next 8 days. The last D+ granulocyte transfusion was associated with a pulmonary reaction due to a newly formed HLA antibody and the granulocyte transfusions were discontinued. The next day, the patient’s lactate dehydrogenase level (LDH) increased to 1257 U/L from 132 U/L the previous day. Her bilirubin level increased from 13.4 mg/dL to 17.0 mg/dL and her hematocrit fell from 24% to 19%. Repeat testing of her plasma detected a new anti-K in addition to passively acquired Rh antibodies. An aliquot of the RhIG lot reacted microscopically positive by tube LISS/IAT with

Table 2. Results of the antibody screen (AS) and the direct antiglobulin test (DAT) in granulocyte concentrate recipients before, during, and 4 months after the administration of RhIG

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before RhIG administration</td>
<td>AS: Negative</td>
<td>AS: Negative</td>
<td>AS: Negative</td>
<td>AS: Negative</td>
</tr>
<tr>
<td></td>
<td>DAT: Negative</td>
<td>DAT: Negative</td>
<td>DAT: Negative</td>
<td>DAT: Negative</td>
</tr>
<tr>
<td>During RhIG administration</td>
<td>AS: Anti-D</td>
<td>AS: Anti-D</td>
<td>AS: Anti-D</td>
<td>AS: Anti-D, -C, -E</td>
</tr>
<tr>
<td></td>
<td>DAT: Negative</td>
<td>DAT: Positive*</td>
<td>DAT: Anti-D</td>
<td>DAT: Positive†</td>
</tr>
<tr>
<td>After RhIG administration</td>
<td>AS: Negative</td>
<td>AS: Negative</td>
<td>AS: Not done</td>
<td>AS: Not done</td>
</tr>
<tr>
<td></td>
<td>DAT: Negative</td>
<td>DAT: Negative</td>
<td>DAT: Not done</td>
<td>DAT: Not done</td>
</tr>
</tbody>
</table>

*The DAT was reactive with anti-IgG; an eluate detected anti-D
†The DAT was reactive with anti-IgG and anti-C3d; an eluate detected anti-D and -A

Patient 2, a 6-year-old male with CGD, was given 13 dexamethasone-mobilized granulocyte concentrates over 25 days, 2 of them from D+ donors. After intravenous RhIG was administered, anti-D was detected in his serum and the DAT was positive. Anti-D was eluted from his RBC sample, which contained D+ transfused RBCs. No additional granulocyte concentrates were transfused. His DAT remained positive for 2 weeks following the last granulocyte transfusion. The antibody screen was strongly reactive with D+ cells (2+) during the 2 weeks following the infusion of 6000 IU of intravenous RhIG but was only weakly reactive at 3 weeks. Titration studies with R,R, RBCs revealed an endpoint of w+ at 1, 4, and 4 for samples drawn on days 12, 15, and 19, respectively, of postinfusion intravenous RhIG. Four months after the infusion of RhIG, his antibody screen and DAT were negative, as were three assays performed during a 3-year follow-up period.

Patient 3, an 11-year-old male with severe aplastic anemia, was given five G-CSF plus dexamethasone-mobilized granulocyte concentrates over 9 days for a disseminated aspergillus infection of the lung and Enterobacter enteroccus bacteremia. Four of the granulocyte concentrates were from D+ donors. He was given four doses of 3000 IU (600 µg) of intravenous RhIG after receiving four granulocyte concentrates from D+ donors, which he tolerated without complications. Anti-D was detected 2, 6, and 9 days postinfusion of RhIG. A DAT was not performed. He died from the infections 10 days after the first granulocyte concentrate was given.
one K:1,2 cell. The patient’s plasma reacted 2+ with the same cell by the gel antiglobulin test. The DAT remained positive with anti-IgG (1+) and anti-C3d (1+) and a weak panagglutinin was eluted from her RBCs along with anti-D and -K, which were demonstrable after removal of the autoantibody by adsorption.

Phenotyping of RBCs collected from Patient 4, 1 week before the onset of hemolysis, showed mixed-field reactivity with the A, E, and K antigen typings. During the 2 weeks before the hemolytic episode, three units of A– RBCs were transfused. Two units were C–, E+, K+, and one was C–, E–, K+. Two days after the onset of the hemolytic reaction, a titration of her serum against a K+ RBC revealed an endpoint of w+ at 32, which was indicative of a newly formed alloantibody. The presumed passively acquired anti-E and -D showed titration result endpoints of w+ at 2 and 8, whereas the anti-C was not demonstrable.

All further RBC transfusions were negative for the D, C, E, and K antigens. The DAT remained positive for the next 6 days. The aspergillus infection progressed and she died 15 days after the last granulocyte transfusion. At the time of death, her LDH had fallen to 514 U/L, and there were no other signs of hemolysis.

Discussion

Four D– recipients were given repeated granulocyte concentrates from D+ donors containing approximately 30 mL of RBCs and were treated with intravenous RhIG to prevent alloimmunization. Two patients with CGD were followed for more than 3 years after the transfusion of D+ granulocyte concentrates and neither produced anti-D. The effectiveness of RhIG could not be assessed in two other patients with severe aplastic anemia who died within weeks of their last granulocyte transfusion, but both patients tolerated the infusions of RhIG.

Although this was not a controlled study, the results suggest that intravenous RhIG is effective in preventing D alloimmunization in granulocyte transfusion recipients. CGD patients have defects in granulocyte respiratory burst but otherwise have normal immune systems. These results are similar to a previous study of 10 D– volunteers who received 2.5 mL of D+ RBCs and 120 µg doses of intravenous RhIG up to 48 hours after the RBCs and case reports on four D– women who were inadvertently transfused with one to three units of D+ RBCs and received 3000 to 7750 µg intravenous RhIG. The preventative dose of intravenous RhIG suggested by Mollison and colleagues is 10 to 15 µg per mL of RBCs. Hemoglobinuria was the only untoward effect listed for the administration of such large doses of intravenous RhIG in the presence of a significant amount of D+ RBCs.

The manufacturer of intravenous RhIG indicates that 18 µg will suppress the immunizing potential of 1 mL of D+ RBCs. Because a typical granulocyte concentrate contains approximately 30 mL of RBCs, initially we elected to give 600 µg of intravenous RhIG after each granulocyte transfusion. The number of RBCs in each granulocyte concentrate is variable, but when 600 µg of intravenous RhIG was given after each transfusion, circulating anti-D was readily detectable after one or two intravenous RhIG infusions, suggesting that the dose was adequate. In fact, Patient 4 received only four doses of intravenous RhIG to treat seven D+ granulocyte transfusions and passively infused anti-D, and -E were detected after the last D+ granulocyte transfusion. Although the optimum dose of intravenous RhIG for recipients of D+ granulocyte transfusions is not known, we recommend that initially 600 µg of intravenous RhIG be given for each D+ transfusion. However, if the antibody screen remains reactive with D+ cells after multiple doses of RhIG, it is likely that giving 600 µg for every two D+ granulocyte transfusions will be sufficient. The infusions of RhIG were well tolerated by the children in this study.

The antiglobulin crossmatch cannot be used for compatibility testing of granulocyte products with D+ RBCs in patients with passive anti-D. Circulating anti-D was detected in all four patients following the administration of intravenous RhIG. In addition, passively acquired anti-C and -E were detected in one patient. Because the antiglobulin crossmatch is of limited use, it is important to follow the recipient’s antibody screen closely and frequently analyze samples for the presence of newly developed alloantibodies. This is especially difficult when RhIG contains multiple antibody specificities. Passive transfer of anti-D, -C, -E, -Lea, and -Bg has been reported with RhIG prophylaxis following D+ platelet transfusions. Differentiating passively acquired antibodies from newly developed alloantibodies is not always possible. Therefore, it may be necessary to provide antigen-matched RBC units that are crossmatched by immediate spin to check for ABO compatibility between the donor and recipient. If possible, it may be prudent to perform extended RBC phenotyping on D– granulocyte transfusion recipients before transfusion of products containing RBCs. The phenotype information could be helpful in determining the antigens to which the recipient can produce alloantibodies.
In conclusion, intravenous RhIG prophylaxis was successful in two D– patients receiving granulocyte transfusions containing D+ RBCs. However, it is not known if these individuals were at risk for alloimmunization (i.e., immune responders) and further studies are needed. The use of intravenous RhIG was well tolerated and avoids the problems associated with multiple intramuscular injections. Approximately 15 percent of Caucasians are D–; therefore, the ability to transfuse D– patients with granulocyte concentrates from D+ donors increases the pool of donors available to these patients more than fivefold. This is particularly important because granulocyte concentrates must be collected from ABO compatible and, in some cases, CMV-negative donors. However, anti-D and other passive antibodies from RhIG prohibit the use of the antiglobulin crossmatch with antigen-positive granulocyte donors. Thus, for granulocyte concentrate recipients treated with RhIG to prevent D alloimmunization, it may be necessary to screen the recipient’s serum more frequently than every 3 days to prevent the transfusion of an incompatible product.

Acknowledgment

We thank Ellen Lazarus, MD, for reviewing the manuscript and for her suggestions.

References


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Rapid genotyping of the major alleles at the Duffy (FY) blood group locus using real-time fluorescence polymerase chain reaction

F. Araújo, C. Pereira, A. Alexio, I. Henriques, F. Monteiro, E. Meireles, P. Lacerda, and L.M. Cunha-Ribeiro

The Duffy blood group system has clinical importance due to involvement in transfusion reactions and hemolytic disease of the newborn. Recently, the molecular basis of the two alleles, FY*A and FY*B (125G>A), and the mutation situated in the promoter region of the FY gene (~35T>C), have been elucidated. In order to develop an accurate, easy, and rapid genotyping method, we describe a procedure using the LightCycler®. Samples from 53 Caucasian Portuguese blood donors and 7 black, healthy, European individuals were phenotyped with commercial antisera. DNA was extracted from blood samples and the relevant sequences were amplified with the same cycling conditions, using real-time polymerase chain reaction. The melting point of the FY*A allele was 63ºC and of the FY*B allele, 55ºC. The allele without mutation at the promoter region had a melting point at 64ºC and of the FY*B allele, 55ºC. The allele without mutation at the promoter region had a melting point at 64ºC and of the FY*B allele at 58ºC. The results in Caucasian individuals were similar to those found in European and American populations. When FY genotyping techniques are necessary, the methodology described is preferable to conventional methods as it is reliable, high speed, and uses small volumes, providing a highly competitive technology for use by a routine laboratory. Immunohematology 2001;17:42–44.

Key Words: Duffy, FY, real-time PCR, LightCycler

The Duffy protein, which carries the antigens of the Duffy blood group system, is of biological importance because it acts as a receptor for chemokines and as an invasion site for Plasmodium vivax. Its clinical significance is due to involvement in transfusion reactions and hemolytic disease of the newborn (HDN).

The molecular bases of the two alleles, FY*A and FY*B, were elucidated in 1995 as being due to a single G to A nucleotide substitution at position 125 of the FY gene (major transcript). In the same year, it was shown that a mutation situated in the promoter region of the FY gene (~35T>C) was responsible for a silent allele found in blacks. In these individuals, this mutation could falsely predict the presence of a normal FY*B allele when, in fact, it carried the silent FY allele. Other mutations in the FY gene were recently described in selected populations, which may have physiological and clinical implications.

The original FY genotyping methods were based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). More recently, using a duplex PCR approach with allele-specific primers (PCR-ASP), the three major alleles at the Duffy blood group locus could be detected using a simpler technique.

In order to develop a more accurate, easy, and rapid genotyping method, we describe a procedure using the LightCycler® (Roche Molecular Biochemicals, Mannheim, Germany). With this real-time fluorescence PCR instrument, adjacent hybridization probes monitor the appearance of a specific product using fluorescence resonance energy transfer technology. The fluorescence signals measured at each cycle were converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature against temperature. In this setting, PCR amplification (using hot air for heating) and detection occurred in a closed glass capillary in less than 35 minutes.

Material and Methods

Blood samples from 53 Caucasian Portuguese blood donors and 7 black, healthy, European individuals were drawn into EDTA.

Duffy phenotyping was performed with antisera from Gamma Biologicals (Houston, TX), Diagast (Immucor Medizinische Diagnostik GmbH, Rodermark, Germany), and DiaMed AG (Cressier sur Morat, Switzerland), according to the manufacturers’ instructions (the latter tests were performed on gel cards, and the other tests were performed in tubes).

DNA was extracted using QIAamp® (Hilden, Germany). The oligonucleotide primers and probes were synthesized by TIB MOLBIOL (Berlin, Germany).
The wild-type complementary detection probes 3’-labeled with fluorescein covered the mutation site (sensor) and the adjacent anchor probes were 5’-labeled with LC-Red640 dye (anchor; see Table 1).

Table 1. DNA sequences of the oligonucleotide primers and probes

<table>
<thead>
<tr>
<th>Primers and probes</th>
<th>Nucleotide sequence</th>
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<tbody>
<tr>
<td>125G&gt;A</td>
<td>Primer 1: position 859-879</td>
</tr>
<tr>
<td></td>
<td>5’-CAGCTGGGACTGGAAGATGTA-3’</td>
</tr>
<tr>
<td></td>
<td>Primer 2: position 1071-1053</td>
</tr>
<tr>
<td></td>
<td>5’-GGGGAAGAGGCTCTGAAA-3’</td>
</tr>
<tr>
<td></td>
<td>Anchor 1</td>
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<tr>
<td></td>
<td>TGGGAAGAATCTACACCCATAGGAAGAA</td>
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<td></td>
<td>Sensor 1</td>
</tr>
<tr>
<td></td>
<td>GTGGCCACCATAGTCTCAT</td>
</tr>
<tr>
<td>–33T&gt;C</td>
<td>Primer 3: position 379-398</td>
</tr>
<tr>
<td></td>
<td>5’-AGGGGCATAGGGATAAGGGACF-3’</td>
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<tr>
<td></td>
<td>Primer 4: position 666-646</td>
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<td>CTGGGCTTACACCCAGAGCACA</td>
</tr>
</tbody>
</table>

PCR reactions were performed in the LightCycler® glass capillaries, consisting of two mixes, one for each mutation: 2 µL of genomic DNA (100 ng), 10.8 µL H2O, 1.6 µL MgCl2 (3 mM), 1 µL of each primer 1 and 2 (10 µM), 0.8 µL of each probe 1 (5 µM), and 2 µL of DNA-Master Hybridisation Probes, for 125G>A; 2 µL of genomic DNA (100 ng), 11.6 µL H2O, 0.8µL MgCl2 (2 mM), 1 µL of each primer 3 and 4 (10 µM), 0.8 µL of each probe 2 (5 µM), and 2 µL of DNA-Master Hybridisation Probes, for –33T>C.

Cycling conditions were identical for both reactions, including initial denaturation, followed by 45 cycles with denaturation at 95°C for 0 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 28 seconds. Melting curves were generated by slowly heating the sample to 75°C.

Results

The melting point of FY*A was 63°C and of FY*B was 55°C, whereas the allele without mutation at the promoter region had a melting point of 64°C and the FY*B silent allele of 58°C. In different runs, the positions and distances of the melting peaks were identical and differed by less than 1°C for the same allele (Fig. 1). The genotypes obtained were confirmed by classical PCR-ASP using the protocol described by Olsson et al.4

The phenotype, genotype, and allele distributions are shown in Table 2. The results in Caucasian individuals were similar to those found in other European and American populations. However, in European blacks, we found that the Fy(a–b–) phenotype and the silent FY*B allele frequencies were lower than in other studies. This could be due to the small number of individuals tested or to the specific characteristics of this population.

![Fig. 1](image.png)

Table 2. Phenotype, genotype, and allele frequencies in the Caucasian Portuguese (53 samples) and the European blacks (7 samples)

<table>
<thead>
<tr>
<th>Phenotypes %</th>
<th>Genotypes %</th>
<th>Allele %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Fy(a+b-)</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Fy(a+b+)</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td>Fy(ab+b)</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Fy(a-b-)</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: FY = silent FY*B allele
Discussion

*FY* genotyping techniques are necessary when we need to resolve inconsistent, weak, or posttransfusion mixed-field reactions and positive direct antiglobulin tests, or HDN.

The classic nonhomogeneous gel-based methods are time-consuming, need PCR optimization, could cause concern with contamination, and could require digestion of PCR products. The technology described here is more reliable, quicker (the "hands-on" time for setting up the assays is shorter and no manual intervention is required after loading the instrument), and uses small volumes (lowering reagent costs), making it a highly competitive technology for a routine laboratory. Another advantage is the detection of other mutations that could occur under the probe by producing a mismatch with a typical melting curve.

References


Acute hemolytic transfusion reaction caused by anti-Co

R.B. COVIN, K.S. EVANS, R. OLSHOCK, AND H.W. THOMPSON

Coa is a high-frequency blood group antigen in the Colton blood group system expressed on red blood cells (RBCs) of approximately 99.8 percent of random persons. Anti-Coa has been reported to cause delayed hemolytic transfusion reactions, hemolytic disease of the newborn, and accelerated clearance of RBCs in vivo. Acute hemolytic transfusion reactions (AHTRs) have not previously been reported. A 58-year-old man was hospitalized for vascular surgery. Initial blood bank evaluation revealed anti-Fya. The patient received six units of RBCs during his initial hospitalization and developed anti-E. A subsequent sample was sent to the reference laboratory when all units of RBCs appeared incompatible. Additional studies, including alloabsorptions, revealed the presence of anti-E, anti-Fya, and an apparent warm autoantibody. One unit of least-incompatible RBCs was transfused during surgery. The patient had an increase in temperature. Hemoglobinuria and a decrease in hematocrit were also noted. Due to the clinical impression of an AHTR, the pre- and postreaction samples were re-evaluated in the reference laboratory and demonstrated the presence of anti-Coa in both. Based on clinical and laboratory evaluation this patient appears to have had an AHTR due to anti-Coa. This is the first known reported case of an AHTR caused by anti-Coa.

Immunohematology 2001;17:45–49.

Key Words: blood groups, transfusion reaction, hemolysis, anti-Coa, Colton A (Coa) antigen

Coa is a high-frequency blood group antigen expressed on red blood cells (RBCs) of 99.8 percent of Caucasians and may be more frequent in other ethnic-racial groups.1 The Colton protein has been identified as aquaporin 1, a red cell water-selective transport protein that regulates water homeostasis.2 The first examples of anti-Coa were described by Heisto et al. in 1967.3 Anti-Coa has been reported to cause delayed hemolytic transfusion reactions,4 hemolytic disease of the newborn,5 and accelerated clearance of RBCs in an in vivo survival study.6 Acute hemolytic transfusion reactions (AHTRs) due to anti-Coa have not previously been reported. The case presented here concerns a patient who developed an apparent AHTR due to anti-Coa. We believe that this case is the first reported case of an AHTR caused by anti-Coa.

Case Report

A 58-year-old group A, D+ man with longstanding venous insufficiency was admitted to the hospital on February 18, 2000 for left leg valve transplantation. His medical history was significant for a 38-year history of pain, edema, and discomfort in his left lower extremity due to venous insufficiency, ulcers, and thromboses. He had received multiple red cell transfusions during surgery for ulcer excisions followed by skin grafts, multiple nerve blocks, and a sympathectomy. He had a history of moderate-to-severe aortic stenosis and of gastrointestinal bleeding in 1993. At that time he was also noted to have anti-Fya. Medications on admission included oxycodone, amitriptyline, zolpidem, and warfarin. Admission hemoglobin was 11.9 g/dL. Initial blood bank evaluation confirmed anti-Fya. The patient’s RBC phenotype was reported as Fy (a+), Jk (a+), and S+. The patient underwent a left lower extremity venous valve transplant on February 18, 2000. A left deep venous thrombosis necessitating heparin therapy complicated the patient’s postoperative course.

Subsequently, the patient developed left thigh and popliteal hematomas that were evacuated on February 28, 2000. During this phase of the patient’s hospitalization, and prior to this second operation, the patient received 6 units of RBCs. An anti-E was detected for the first time. A type and crossmatch just prior to the patient’s second surgery revealed no compatible units and the specimen was sent to the reference laboratory. Additional studies including alloabsorptions revealed the presence of anti-E, -Fya, a cold autoantibody, and an apparent warm autoantibody. Further phenotyping revealed that the patients RBCs also were E– and K–. Eight units of least-incompatible RBCs were sent to the patient’s hospital. During the second surgery, the patient was transfused with one of the units and the other seven were returned. The transfused unit was E–, Fy (a–), K–, and Jk (a–). In the postanesthesia recovery unit, within 60 minutes from the start of the transfusion, the patient was noted to develop rigors, increased temperature, and hemoglobinuria. The hematocrit was also noted to have decreased. There was no clinical evidence of bleeding
to account for the decline in hematocrit. Due to the clinical impression of an AHTR, the pre- and immediate postreaction samples were re-evaluated in the reference laboratory. A panel of RBCs to test for the presence of antibodies to high-frequency antigens demonstrated anti-Co\(^a\). A posttransfusion sample revealed a new anti-Jk\(^a\). The patient’s RBCs were Co(a–) and Jk(a–).

Materials and Methods

Polyspecific anti-IgG, -C3d, murine monoclonal anti-IgG (Gamma Biologicals, Houston, TX) and murine monoclonal anti-C3b, C3d (Ortho-Clinical Diagnostics, Raritan, NJ) were used for the direct antiglobulin test (DAT). Testing was performed at immediate spin and after a 10-minute incubation.

The following monoclonal reagents were used for the initial reference laboratory RBC phenotyping: anti-A, -B, -D, -C, -c, -E, and -e (Ortho); the following indirect antiglobulin reagents were used: anti-K, -Fya, -S (Ortho) and -Jk\(a\) (Gamma). The patient’s ABO serum grouping was performed using A\(1\) and B cells (Ortho).

Initial panels used in the reference laboratory investigation included select cells from Panocell 16 (Lot # 09280, Immucor, Norcross, Georgia) and Panel One (Lot numbers 0222 and 0118, Gamma). Panels were read at immediate spin, followed by the addition of two drops of N-Hance (Gamma). Each tube was incubated at 37°C for 10 minutes, followed by centrifugation and reading. Each tube was washed x 4 with saline, two drops of anti-IgG (Gamma) were added, followed by centrifugation and reading. Negative antihuman globulin reactions were checked using Coombs Control Cells (Ortho).

A cold autoantibody, one of the suspected antibodies, interfered with the patient’s ABO serum grouping. The test was repeated and resolved using two drops of the patient’s serum plus A\(1\) and B cells, and O cord cells. A prewarmed panel was performed, using the same cells as the original select cell panel. The following were warmed separately at 37°C for 5 to 10 minutes: one drop of each panel cell to be tested, enough N-Hance to add two drops to each tube, and the patient’s serum. Two drops of prewarmed serum and N-Hance were added to each panel cell tube. All tubes were incubated at 37°C for 10 minutes. All tubes were washed x 4 with warm saline, followed by the addition of two drops of anti-IgG (Gamma) to each tube.

An acid eluate was performed using Elu-Kit II (Gamma). As a control, the last wash was tested against two selectogen cells (Ortho). Polyethylene glycol (PEG) adsorptions were performed to remove the apparent warm autoantibody from the serum. Raw PEG (Sigma Chemicals, St. Louis, MO) was obtained and prepared as a reagent for use in our laboratory using the following method: 20 grams of PEG dissolved into 100 mL of phosphate-buffered saline. Three different donor’s cells (Bonfils Blood Center, Denver, CO) lacking the following antigens were used to perform the adsorptions:

- Donor 1 (O, D+) lacking E, c, Fya, Jkb, and K antigens
- Donor 2 (O, D+) lacking C, E, Fya, Fyb, Jkb, K, and S antigens
- Donor 3 (O, D–) lacking E, Fya, Jk\(a\), K, and s antigens

The cells from the three donors were tightly packed, with as much donor plasma as possible removed. Equal volumes of packed adsorbing RBCs, PEG, and patient’s serum were incubated at 37°C for 15 minutes. Following centrifugation, the adsorbed serum was harvested and tested against a select cell panel from the initial panels used in the investigation.

Upon report of the suspected transfusion reaction, the patient’s pretransfusion serum was tested with the following cells: one Rhnull / Fy (a+), one Rhnull / Fy (a–), three E– / Fy (a–), and one Co (a–). The pretransfusion eluate was also tested against the Rhnull/Fy (a–) cell. The patient’s RBCs were further phenotyped for the following antigens: Vel, Kp\(b\), I, Co\(a\), Lu\(b\), Yt\(i\), PP1Pk, Ge, and LAN. A select cell panel was run using methods already described, testing at least one cell that was negative for each of the high-frequency antigens listed above. Seven more Co(a–), E–, Fy (a–) RBCs were tested with the patient’s serum. Six of these cells were Jk (a+). All rare cells and antisera were obtained from either the Serum, Cells, and Rare Fluids (SCARF) program or Bonfils Blood Center donors.

Results

Pretransfusion antibody study

As the patient’s type and crossmatch prior to his second surgery revealed no compatible units, the specimen was sent to the reference laboratory for further evaluation. This evaluation revealed an anti-E reactive at 37°C with N-Hance and anti-Fya plus -E at the antiglobulin test using anti-IgG. A cold autoantibody at immediate spin (IS) and an apparent warm autoantibody were also present. The serologic evaluation is shown in Figure 1. All negative antihuman globulin reactions showed agglutination with check cells.
An eluate showed a panagglutinin at IS. At the antiglobulin test, anti-E plus a panagglutinin was present and the adsorbed serum contained only anti-E plus -Fy\(^a\) (Fig. 1).

Table 1. Vital signs, laboratory, and blood bank evaluation pre- and post-AHTR\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
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<td>39.2</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>155/95</td>
<td>150/95</td>
</tr>
<tr>
<td>Pulse</td>
<td>115</td>
<td>105</td>
</tr>
<tr>
<td>Respiration</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Laboratory evaluation

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>22.3</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.1</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>—</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>—</td>
</tr>
</tbody>
</table>

Identification of anti-Co\(^a\)

Due to the AHTR, the reference laboratory re-evaluated the patient's pretransfusion sample in an attempt to identify an antibody to a high-frequency antigen. The results of this evaluation are shown in Figure 2. An antibody to Co\(^a\) by LISS IgG was identified. All negative anti-human globulin reactions showed

**AHTR DUE TO ANTI-CO\(^a\)**

Transfusion reaction clinical, laboratory, and serologic evaluation

Following transfusion of one of the units of RBCs, the patient developed signs and symptoms suggestive of an AHTR. The results of the hospital’s transfusion reaction evaluation are shown in Table 1. The sample was drawn within 3 hours from the time of the suspected AHTR. Most notable were the laboratory values and an increase in the strength of the DAT.

![Table 1. Vital signs, laboratory, and blood bank evaluation pre- and post-AHTR\(^a\)](image)

![Fig. 1. Pretransfusion evaluation performed by reference laboratory. Antibodies identified included anti-E, anti-Fy\(^a\), and “cold” and “warm” autoantibodies.](image)
Fig. 2. Re-evaluation performed by reference laboratory on the pretransfusion sample following the report of an AHTR. Anti-Co<sup>a</sup> was identified. Patient’s phenotype was Co (a–).

agglutination with check cells. Phenotyping of the patient’s RBCs revealed the patient was Co (a–).

In further testing, Rh<sub>null</sub> / Fy(a+) cells tested 2+ positive, whereas Rh<sub>null</sub> / Fy (a–) cells tested ± and antibodies to Vel, Kp<sup>b</sup>, I, Lu<sup>b</sup>, Yt<sup>e</sup>, PP<sub>p</sub>, P<sub>n</sub>, Ge, and LAN were ruled out. A posttransfusion sample, in addition to anti-Co<sup>a</sup>, -E, and -Fya, also revealed the presence of an anti-Jk<sup>a</sup>.

**Discussion**

An AHTR is defined as the rapid destruction of RBCs that occurs during or shortly after a blood transfusion (< 24 hours). The most common cause of AHTR is transfusion of ABO-incompatible RBCs with formation of antigen-antibody complexes that induce activation of the complement cascade and lead to intravascular hemolysis. Most often, these antibodies are IgM and are naturally acquired; however, antibodies may be IgG and can be acquired due to a previous pregnancy or blood transfusion. The clinical features of an AHTR are variable and depend on the quantity of incompatible RBCs transfused, the type of antibody and its antigen specificity, the thermal amplitude of the antibody, complement activation, and the clinical condition of the patient. The most frequent presentation is fever (greater than a 1°C rise in temperature) with or without chills. Other signs and symptoms include hemoglobinemia, hemoglobinuria, hypotension, chest pain, dyspnea, flushing, oliguria or anuria, and disseminated intravascular coagulation. Laboratory diagnosis usually includes a decline in hematocrit, hemoglobinemia, hemoglobinuria, and a positive DAT that may show a mixed-field appearance. An unconjugated hyperbilirubinemia, abnormal coagulation studies, and worsening renal function may also be identified. Our patient developed an increased temperature (although not greater than 1°C), a decrease in hematocrit, hemoglobinuria, and increased strength of the DAT consistent with the clinical impression of an AHTR. Re-evaluation of the patient’s serum samples identified an antibody to the high-frequency antigen Co<sup>a</sup> and a new anti-Jk<sup>a</sup>. Phenotyping of the patients RBCs revealed the patient to be Co (a–). Because Co (a–), Jk (a+) cells did not show agglutination during repeat testing of the pretransfusion sample, we feel that it is highly likely that the transfusion reaction was caused by anti-Co<sup>a</sup>. In addition, the reaction was most consistent with a preformed antibody and the anti-Jk<sup>a</sup> was identified only after the transfusion reaction. The only Jk (a+) units that were transfused were given 1 month prior to the reaction and this length of time is not consistent with a delayed transfusion reaction. Our patient did not receive any further transfusions during his hospital stay so we cannot document an increase in hematocrit and a lack of reaction to a transfusion of Co(a–) RBCs.

The differential diagnosis for an AHTR includes thermal destruction of RBCs by heating and cooling devices, concomitant administration of drugs or
nonisotonic fluids, bacterial contamination, or a hemolytic condition within the recipient (e.g., autoimmune hemolytic anemia). In our patient, no other causes for the AHTR were identified.

This case describes what we believe to be the first reported case of an AHTR caused by anti-Co\textsuperscript{a}. Previous reports have implicated anti-Co\textsuperscript{a} in delayed hemolytic transfusion reactions\textsuperscript{4} and hemolytic disease of the newborn.\textsuperscript{5} In addition, accelerated clearance of Co(a+) RBCs has been identified in patients with anti-Co\textsuperscript{a} during in vivo survival studies.\textsuperscript{3,6} In their initial report, Heisto et al.\textsuperscript{3} described a shortened T\textsubscript{50} when Co(a+) RBCs were injected into a patient with anti-Co\textsuperscript{a}. These findings were later confirmed by Kurtz et al.\textsuperscript{6} who found a T\textsubscript{50} of only 5 minutes in a 56-year-old G3P2 woman with a history of four previous transfusions. She was identified as having anti-Co\textsuperscript{a} and rapidly cleared \textsuperscript{51}Cr-labeled Co(a+) RBCs within 24 hours. The authors suggested that Co(a-b+) RBCs be used for transfusion in patients with anti-Co\textsuperscript{a}. Although anti-Co\textsuperscript{a} is most often IgG, some of these antibodies have been shown to bind complement and examples of IgM antibodies have been reported.\textsuperscript{6} Even though Co\textsuperscript{a} has not previously been reported to cause an AHTR, Lee and Bennett\textsuperscript{8} reported a case of a 74-year-old man who developed an AHTR due to anti-Co\textsuperscript{b}.

In our case, the anti-Co\textsuperscript{a} was initially diagnosed as a warm autoantibody (WAA) due to its reactivity with all test cells. The differentiation between a WAA and a high-frequency antigen can be difficult. Cash et al.\textsuperscript{9} reported similar difficulty with a 60-year-old female who was initially suspected to have a warm autoantibody. After transfusion of two units of RBCs, she developed a delayed hemolytic transfusion reaction. The antibody was subsequently identified as anti-At\textsuperscript{a}.

We believe the clinical presentation and laboratory evaluation is consistent with an AHTR due to anti-Co\textsuperscript{a}.

References
8. Lee EL, Bennett C. Anti-Co\textsuperscript{a} causing acute hemolytic transfusion reaction. Transfusion 1982; 22:159–60.

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Tube and column agglutination technology for autocontrol testing

J. E. COURTNEY, J. L. VINCENT, AND A. J. INDRIKOVS

The incidence of positive autocontrol test results with column agglutination technology is a concern. This study investigates the incidence and significance of positive autocontrols in the ID Micro Typing System™ (gel) and the Gamma ReACT™ (ReACT). The study encompassed a total of 1021 randomly selected samples from patients and 95 samples from donors collected during 1 month. The autocontrol testing was carried out according to the manufacturer's instructions for the column agglutination tests. The tube method was carried out using low-ionic-strength solution (LISS). The direct antiglobulin test (DAT) was performed using the tube method, and further investigated with elution studies if warranted. Seventy-nine patient's samples (7.74%) had a positive autocontrol: the gel test, 72 (91.13%); ReACT, 21 (26.58%); and the tube method, 27 (34.18%). Of the 79 positive autocontrols, 44 samples had a negative DAT. Of the samples with positive DAT results, only one possessed a clinically significant antibody, anti-D. Moreover, the same sample also tested positive in all three methods. Column agglutination techniques have increased sensitivity for a positive autocontrol beyond the conventional tube method. However, ReACT and gel tests differ significantly in their frequency of positives. Investigation of the significance of a positive autocontrol in column agglutination technology when the conventional tube method is also positive is suggested.

Materials and Methods

During March 2000, 1021 samples from patients were randomly selected. As a control, 95 specimens were collected from donor units. The patients' samples were collected in EDTA and segments from donor units were collected in CPDA-1. All samples were tested within 48 hours of collection and were centrifuged for 5 minutes prior to testing.

Three test systems were used to investigate autocontrols: the ID Micro Typing System™ (gel; Ortho-Clinical Diagnostics, Raritan, NJ), the Gamma ReACT™ (ReACT; Gamma Biologicals, Houston, TX), and the conventional tube method. Manufacturers' instructions for autocontrol testing were used in the gel and ReACT test systems. The conventional tube method was performed with low-ionic-strength solution (LISS; ImmuAdd™, Immucor, Norcross, GA) as the potentiator. A 15-minute incubation at 37°C preceded conversion to the indirect antiglobulin test.

Any positive autocontrol in any one of the test systems employed was further investigated by performing a DAT using the conventional tube method. For DAT-positive samples, a transfusion history was reviewed. If the patient had been transfused within the past 3 months, elutions were performed.

Key Words: autocontrol testing, ID Micro Typing System™, ReACT™
time, antibody screening and DAT studies were negative. DATs due to medication were not considered in this study. This study was carried out by one individual to minimize interpretive errors.

Results

Overall, 79 (7.7%) of the patient’s samples exhibited a positive autocontrol. There were no positive autocontrols found in the donor segments. Figure 1 illustrates the strength of reaction observed for the positive autocontrols. A positive result for the tube method usually was observed on the microscopic level, whereas the other test systems gave a 1+ or stronger reaction. Table 1 compares gel and ReACT autocontrol reactivity to tube reactivity. Fifty-two tests positive in gel and/or ReACT were negative by the tube test.

![Figure 1](percent-positive-autocontrols.png)

**Fig. 1** Percent of positive autocontrols found with gel, ReACT, and tube methods (m+ = microscopically positive).

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>Tube 0</th>
<th>Tube m+</th>
<th>Tube 1+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel 0, ReACT 0</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel 0, ReACT 1+</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel 0, ReACT 2+</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel 1+, ReACT 0</td>
<td>38</td>
<td>7</td>
<td></td>
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<tr>
<td>Gel 2+, ReACT 0</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gel 1+, ReACT 1+</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gel 2+, ReACT 1+</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gel 3+, ReACT 1+</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>26</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Comparison of gel and ReACT autocontrol reactivity to tube reactivity

- Tube 0 = negative by tube test
- Tube m+ = microscopically positive by tube test
- Tube 1+ = macroscopically positive by tube test

A DAT was performed on all samples exhibiting a positive autocontrol in any of the three test systems employed. Forty-four samples (55.7%) were negative with the polyspecific antiglobulin reagent and 35 samples (44.3%) were positive. Table 2 illustrates the results of the DAT done on samples demonstrating one or more positive autocontrols.

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>Poly m+, IgG 0, Complement 0</th>
<th>Poly m+, IgG m+, Complement 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel 0, ReACT 1+, Tube 0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Gel 0, ReACT 2+, Tube 0</td>
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<tr>
<td>Gel 0, ReACT 0, Tube m+</td>
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<td>Gel 3+, ReACT 1+, Tube m+</td>
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<tr>
<td>Gel 3+, ReACT 1+, Tube 1+</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Direct antiglobulin test results done on samples with a positive autocontrol

- Poly 0 = negative with the polyspecific reagent
- Poly m+ = positive microscopically

The 35 samples with a positive DAT were further evaluated using elution studies. Thirteen samples were excluded because the patients had not been transfused within the past 3 months. Of the 22 remaining samples, an elution could not be performed on 7 samples due to inadequate volume for the procedure. Of the remaining 15 samples meeting criteria, anti-D was identified in only one sample. In this sample, all autocontrol tests were positive (gel 3+, ReACT 1+, and tube 1+).

The gel test gave a 1+ or stronger reaction in 43 samples when both ReACT and tube were negative (Table 1). ReACT gave a positive result in four samples when both gel and tube were negative. This confirms an observation of increased incidence of positive autocontrols observed in the gel system when compared to the tube method.\(^1\,^2\) In the case where the tube was microscopically positive (26 samples), both gel and ReACT gave a negative result in 3 samples, whereas ReACT was negative in an additional 12 samples. In the single case when the tube reacted 1+, both column agglutination systems were positive.

All positive controls were investigated by performing a DAT (Table 2). The majority of DAT-negative reactions were observed when only the gel was positive (33 samples). The four samples that were positive with ReACT alone in the autocontrol testing also had a negative DAT. When either the gel or ReACT system alone attributed to a positive autocontrol, the tube DAT
was negative in the majority of the cases (37 out of 47 samples). Our data suggests a direct correlation between the ReACT and the tube method when the DAT is negative. The tube was negative in 12 samples and ReACT in 22 samples. This data suggests that when the DAT is positive, the gel system will render a positive control.

Discussion

There was an incidence of 7.7% (79) positive autocontrols in this study. The gel detected 72 positive controls (91.1%), ReACT detected 21 (26.6%), and the tube method detected 27 (34.2%). The incidence of a positive autocontrol for the gel test alone in this study was 7.1 percent, as compared to the 0.7 percent reported in a comparative study. Possible explanations for this deviation may be attributed to the amount and type of samples tested. In our study, 1021 EDTA samples were investigated, whereas the other study used 13,280 serum samples.

The column agglutination technology has an increased sensitivity. However, there is apparently a higher detection of positive autocontrols using the gel system. There is better correlation between the tube method and the ReACT system than between tube and gel methods. The deviation between gel and ReACT systems can be best attributed to the dynamics. The ReACT system employs an active matrix, selective for IgG; the gel system does not. This matrix may contribute to a different level of specificity along with increased sensitivity. This sensitivity differs from the conventional tube method and may explain the deviation when the DAT is positive and the tube demonstrates a microscopic autocontrol. However, this sensitivity must ensure the detection of all clinically significant antibodies, and further investigation with the ReACT system is required to determine if this is true.

In our study, all tests were positive in the one incidence when a clinically significant antibody was identified. In addition, a direct relationship between a positive DAT and a positive autocontrol in the gel system also was observed.

Column agglutination technology has found its niche in blood banks where it often has replaced tube method in routine screening. However, until sensitivity is refined to ensure only the detection of clinically significant antibodies, the conventional tube method will not become obsolete.

References


J.E. Courtney, MT (ASCP) SBB, University of Texas Medical Branch, Galveston, TX; J.L. Vincent, MS, SBB (ASCP), corresponding author, Educational Coordinator, Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0743; and A.J. Indrikovs, MD, MBA, Director of Blood Bank, University of Texas Medical Branch, Galveston, TX.

Attention: State Blood Bank Meeting Organizers: If you are planning a state meeting and would like copies of Immunohematology for distribution, please contact Mary McGinniss, Managing Editor, 3 months in advance, by phone or fax at (301) 299-7443.

Phone, Fax, and Internet Information: If you have any questions concerning Immunohematology, Journal of Blood Group Serology and Education, or the Immunohematology Methods and Procedures manual, contact us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org.
Comparison of three low-ionic diluents for dilution and storage of reagent A₁ and B cells for testing in gel technology

E.A. STEINER AND L. DAKE

Currently, ABO serum grouping performed by gel technology employs a red cell diluent containing EDTA (MTS Diluent 2 Plus™) that does not permit extended storage of the red cell suspensions. A diluent currently used for suspension and long-term storage of reagent red cells for antibody detection and identification (Ortho 0.8% Red Cell Diluent™) was evaluated for use with A₁ and B cells. Because this diluent does not contain EDTA, testing was limited to EDTA samples. As a comparison, a Micro Typing Systems (MTS) diluent not containing EDTA (MTS Diluent 2™) was also tested. MTS-suspended red cells were maintained for 24 hours and compared with Ortho-Clinical Diagnostics 0.8% suspended red cells maintained for 7 days. ABO serum grouping was performed on 144 EDTA plasma samples using all three cell suspensions. Acceptable results were noted in all aspects of testing. Immunohematology 2001;17: 53–56.

Key Words: gel technology, ABO serum group, blood typing in gel, MTS Diluent 2 Plus

This study compares the reactivity of red blood cells (RBCs) used in ABO serum grouping tests prepared and stored for 1 day in MicroTyping Systems (MTS, Pompano Beach, FL) Diluent 2 Plus™ with those prepared and stored for 7 days in Ortho-Clinical Diagnostics (Raritan, NJ) 0.8% Red Cell Diluent™. RBCs used in ABO serum grouping tests performed in ID-MTS™ gel technology must be prepared in a specific diluent. Manufacturer’s guidelines indicate that these cells be diluted to an 0.8% concentration in MTS Diluent 2 Plus™, a hypotonic, low-ionic saline containing EDTA.1 It is recommended by the manufacturer that these RBCs be used on the day of dilution. Ortho-Clinical Diagnostics markets Ortho 0.8% Red Cell Diluent™, a low-ionic saline for use in diluting cells for gel testing.² The purine nucleoside and antibiotics present in the Ortho 0.8% diluent create a low-ionic environment that maintains antigen stability under extended storage conditions. RBCs manufactured in this diluent for use in antibody detection and antibody identification have been granted a Food and Drug Administration license that includes a 35-day dating period, under the controlled conditions of manufacturing. Extended storage time, 7 days vs. 1 day, would permit a more efficient use of these reagents and personnel preparation time. A storage time longer than 7 days was not chosen for RBCs diluted in Ortho 0.8% Red Cell Diluent™ because of the potential for microbial contamination.

MTS Diluent 2 Plus™ contains EDTA so that fresh serum samples containing antibody cannot activate complement and cause hemolysis, rather than initiate agglutination. Because Ortho 0.8% Red Cell Diluent™ does not contain EDTA, this study was confined to the testing of EDTA plasma samples.

The second phase of this study compares the reactivity of ABO serum grouping RBCs prepared in MTS Diluent 2™, which also does not contain EDTA,³ with those prepared in MTS Diluent 2 Plus™.

Methods

Samples

The 144 random EDTA samples were obtained from a hospital clinical hematology laboratory. They were collected at various times throughout Day 0, stored at room temperature throughout the rest of that day, and then stored refrigerated until this study was initiated on Day 2. At that time, samples were centrifuged and the plasma transferred to clean labeled test tubes. Samples were stored at 2°C to 8°C between testing events and were brought to room temperature prior to testing.
Red blood cells

Ortho Affirmagen™ A and B RBCs, in-date and of a single lot number, were used for all testing. OCD guidelines were used to prepare the 0.8% cell suspensions in the three diluents. RBCs, both before and after 0.8% preparation, were stored at 2° to 8°C and brought to room temperature prior to use. The aliquots of cells were prepared as follows:

- Suspension A = Affirmagen cells suspended in Ortho 0.8% Red Cell Diluent™
- Suspension B = Affirmagen cells suspended in MTS Diluent 2 Plus™
- Suspension C = Affirmagen cells suspended in MTS Diluent 2™

Evacuated, 7mL Vacutainer™ tubes (red tops) were used for the cell suspensions because of their sterility and the convenience of prefixed labels and stoppers.

Gel cards

MTS Buffered Gel Cards™, in-date and of a single lot number, were tested as described in the manufacturer’s package insert, other than when substituting Ortho Diluent or MTS Diluent 2™ for MTS Diluent 2 Plus™.

ABO serum grouping test

Tests were set up by pipetting 50µL of 0.8% RBCs into the gel column, followed by 50µL of EDTA plasma and centrifugation. Although there was no intent or need to incubate these tests prior to centrifugation, samples were tested in large batches. Because of this, the time interval between dispensing of RBCs and plasma addition ranged from 10 to 15 minutes and the time between addition of plasma and initiation of centrifugation ranged from 5 to 10 minutes.

RBCs in Suspension A (Ortho 0.8% Red Cell Diluent™) were tested on the day of dilution, the following day (22 to 26 hours after dilution, henceforth referred to as “24 hours”), and 7 days after preparation. RBCs in Suspension B (MTS Diluent 2 Plus™) were tested on the day of dilution and at 24 hours and RBCs in Suspension C (MTS Diluent 2™) were tested only at 24 hours. For nine samples, there was insufficient plasma to test with Suspension C or Suspension A at 24 hours. For an additional 16 specimens, there was not enough plasma to test Suspension C. All other readings were performed and confirmed as described.

Additionally, quantity of plasma permitting, repeat testing was performed whenever there was non-agreement of results, positive vs. negative; the graded reactivity differed by one or more gradations; or unusual findings were noted.

Results

All gel cards were read in an MTS Reader M™, a viewing device specifically approved for the reading of gel card reactions. Following the automated reading, all reactions were further evaluated for—

- Operator agreement with the MTS Reader’s suggested analysis,
- Evidence of any unusual findings, and
- Intermediate reactivity between the +, ++, ++++, and ++++ scale employed by the Reader software. The Reader gradations of strength of reactivity are comparable to the 1+, 2+, 3+, and 4+ defined by Marsh for hemagglutination readings in test tubes. For example, one 3+ reaction might be noted as 3+w because it was closer to a 2+, whereas another 3+ reaction was noted as 3+s because it was closer to a 4+ reading.

All unusual findings could be attributed to fibrin or particulate matter trapping a few RBCs in the top of the gel. This “top line” phenomenon was eliminated when tests were repeated with recentrifuged samples.

Using the intermediate grading scale, samples were evaluated for relative strength and result concordance. Translation of intermediate reactions into final graded strength is described in Table 1.

Table 1. Translation of intermediate readings into final graded strength

<table>
<thead>
<tr>
<th>Intermediate readings*</th>
<th>Final grading†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+s and/or 4+‡</td>
<td>4+</td>
</tr>
<tr>
<td>2+, 3+, 3+ and/or 3+</td>
<td>3+</td>
</tr>
<tr>
<td>1+, 2+, 2+ and/or 2+</td>
<td>2+</td>
</tr>
<tr>
<td>1+ and/or 1+</td>
<td>1+</td>
</tr>
</tbody>
</table>

* = Readings derived from subjective evaluation of MTS Reader-generated reaction gradings
† = Reaction grading used for comparison of different tests
‡ = Graded reaction strengths as defined in the text

With these guidelines in place, readings were reviewed for discordant findings, defined as non-agreement of positive vs. negative results as well as a difference of one or more in the level of graded strength of positive results. Readings for Suspension B, tested fresh and after 24 hours, were compared with those of Suspension A at the same intervals to establish a baseline (Table 2). There were no discordant results, although insufficient sample volume prevented the testing of nine samples at 24 hours with Suspension A. In order to determine if results were affected by the
absence of EDTA in the RBC diluent, readings of Suspension B at 24 hours were compared with those of Suspension C at the same interval of storage. Sample volume permitted testing of 119 plasma samples (Table 3). There were no discordant results.

**Discussion**

All samples demonstrated agreement of results. Repeat testing was done in smaller batches than initial testing in order to resolve any significant differences that may have resulted from the inadvertent delays and variables associated with large batch testing. Repeat testing using recentrifuged samples eliminated the problems noted with top lines in the gel column of some otherwise negative tests. Other than two samples whose volume did not permit retesting, there were no significant variations in the final graded strength of any reactive samples. The following conclusions can be made:

- RBCs diluted in MTS Diluent 2 Plus™ gave comparable reactivity when tested fresh and at 24 hours.
- RBCs diluted in Ortho 0.8% Red Cell Diluent™ gave comparable reactivity when tested fresh and at 7 days.
- There was equivalent reactivity between RBCs diluted in MTS Diluent 2 Plus™ and those diluted in Ortho 0.8% Red Cell Diluent™, both freshly prepared and at the end of their respective storage periods.
- When testing EDTA plasma samples, RBCs diluted in MTS Diluent 2™ reacted the same as RBCs diluted in MTS Diluent 2 Plus™.

Fresh serum samples, with the ability to activate complement, would not be expected to demonstrate these same results. These conclusions are based only on the testing of EDTA plasma samples.

**Table 2. ABO serum grouping results for red cells freshly prepared and after 24 hours of storage in MTS Diluent 2 Plus™ and in OCD 0.8% Red Cell Diluent™**

<table>
<thead>
<tr>
<th>Graded strength</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>60&lt;sup&gt;†&lt;/sup&gt;(3)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>55(2)</td>
</tr>
<tr>
<td>++</td>
<td>67(5)</td>
<td>67(2)</td>
</tr>
<tr>
<td>+</td>
<td>3(1)</td>
<td>25(4)</td>
</tr>
<tr>
<td>2+</td>
<td>5</td>
<td>10(1)</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N&lt;sup&gt;§&lt;/sup&gt;</td>
<td>135(9)</td>
<td>135(9)</td>
</tr>
</tbody>
</table>

<sup>*</sup> = Degrees of reactivity as defined in the text  
<sup>†</sup> = Number of samples with this result for all four readings  
<sup>‡</sup> = Number of samples with this result for three readings; insufficient sample to test Suspension A at 24-hours  
<sup>§</sup> = Total number of samples tested

**Table 3. ABO serum grouping results for red cells stored for 24-hours in MTS Diluent 2 Plus™ and in MTS Diluent 2™**

<table>
<thead>
<tr>
<th>Graded strength</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>52&lt;sup&gt;†&lt;/sup&gt;</td>
<td>26</td>
</tr>
<tr>
<td>++</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>2+</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>119</td>
<td>119</td>
</tr>
</tbody>
</table>

<sup>*</sup> = Degrees of reactivity as defined in the text  
<sup>†</sup> = Number of samples with this result for both readings  
<sup>‡</sup> = Total number of samples tested

**Table 4. ABO serum grouping results for red cells stored for 24 hours in MTS Diluent 2 Plus™ and in Ortho 0.8% Red Cell Diluent™ for 7 days**

<table>
<thead>
<tr>
<th>Graded strength</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>63&lt;sup&gt;†&lt;/sup&gt;</td>
<td>35</td>
</tr>
<tr>
<td>++</td>
<td>70&lt;sup&gt;†&lt;/sup&gt;</td>
<td>69</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>2+</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>142</td>
<td>144</td>
</tr>
</tbody>
</table>

<sup>*</sup> = Degrees of reactivity as defined in the text  
<sup>†</sup> = Number of samples with this result for both readings  
<sup>‡</sup> = Two samples that were initially 4+ in MTS Diluent and 3+ in Ortho Diluent lacked sufficient volume for confirmation testing; see text for further discussion  
<sup>‡</sup> = Total number of samples tested
The findings of this study suggest that commercially prepared RBCs (Affirmagen) can be diluted with Ortho 0.8% Red Cell Diluent™ and used for ABO serum grouping over a period of 7 days.

Acknowledgments
We thank the staff at the Hematology Laboratory, University of Michigan Hospitals, Ann Arbor, MI, for supplying the samples used for this study.

References

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Attention: Presidents of State Blood Bank Associations—In order to increase the number of subscribers to Immunohematology, we are soliciting membership lists of your organizations. Upon receipt of such a list, each person will receive a complimentary copy of Immunohematology, and, if desired, a personal letter from the association president. For further information, contact: Mary H. McGinniss, Managing Editor, by phone or fax at (301) 299–7443.

Free Classified Ads and Announcements: Immunohematology will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) without charge. Deadlines for receipt of these items and the projected mailing weeks of the journal are as follows:

**Deadlines**

1st week in January for the March issue  
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1st week in October for the December issue

Fax these items to Mary H. McGinniss, Managing Editor, (301) 299–7443.
BOOK REVIEW


This monograph is devoted to the management of deviations and sentinel events that occur in a quality system with the intent of reducing or eliminating those deviations. It complements the American Association of Blood Banks (AABB) Quality Assurance Essentials, a system providing a means for error and accident identification, root cause analysis, and corrections and corrective actions.

Authors contributing to this monograph share their expertise in the areas of process improvement, identification of sentinel events, classifying errors and accidents, using audits to detect errors, analyzing process data, implementing corrective actions, and efficacy evaluations.

Of particular value is Chapter 3 by Boyd Fogle of the Food and Drug Administration (FDA), Centers for Biological Evaluation and Research. Mr. Fogle explains the agency’s interpretation of the Food, Drug and Cosmetic Act, as extended by the US Code of Federal Regulations, Title 21, Parts 7 (recalls), 211 (pharmaceutical products), 606 (blood and blood products), and 820 (quality systems regulations). This chapter clearly defines the FDA’s policy regarding process or product deviations. It also clearly differentiates those deviations requiring a recall action from those that the agency considers nonrecall events. Other chapters discuss deviations occurring in the blood center or hospital-based transfusion service arenas and provide recommendations on handling such events.

Chapter 2, which focuses on errors occurring in hospitals, rather than in blood centers, defines sentinel events—those leading to serious patient hazard morbidity and mortality—and the systems required by the FDA and Joint Commission on the Accreditation of Healthcare Organizations (JCAHO) to identify, evaluate, and correct them.

Chapter 6 provides a brief, yet concise, introduction to the quality tools used to analyze numeric and nonnumeric data derived from quality system operations. This chapter will be of particular help to new quality assurance professionals who are not familiar with the various analysis methods (such as pareto charts, histograms, and statistical process control maps) but who wish to gain a better working knowledge of the differences and applications of each. The chapter also provides a listing of sources for quality software.

The final chapter of the monograph discusses the measures needed to prevent recurrence of errors over the short and long term. A useful set of case studies completes the book. These studies represent actual cases reported to the FDA and were presented at a 1998 AABB workshop on error and accident reporting. They are structured to test the reader’s understanding of what constitutes an error or accident, and whether the event should be reported to the FDA.

The shortcoming of this monograph is that error and accident reports were replaced in November 2000 by Biological Product Deviation Reports, as defined in 21 CFR Part 606. In spite of this change in reporting policy, this monograph remains a considerable resource of information for quality personnel seeking information regarding the identification, documentation, reporting, and resolution of deviations and sentinel events that indicate failures within established quality system operations.

Susan Rolih, MS, MT(ASCP)SBB
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Letter to the Editors

Re: Use of red blood cell units containing alloantibodies

I have a question concerning the paper titled “Large-scale use of red blood cell units containing alloantibodies” published in Vol. 16, No. 3, 2000. The question is: Did the study utilize CPDA-1 red blood cell (RBC) units, which were required to have a final hematocrit of 80 percent or less, or were they using the newer additive units, which have even more of the original plasma removed? I would think that with the greater percentage of plasma removed from the “additive” units, any problems or concerns associated with alloantibodies in RBC units would be considerably less than those experienced when CPDA-1 units are used. For this reason I would like to know what type of RBC units the authors used in the study. At this time my facility is receiving additive units and we have no hesitation in receiving antibody positive units. Maybe we should negotiate a price break as well. Thank you.

John C. Staley, CLS/MT
Transfusion Service Supervisor
McKay-Dee Hospital Center
3939 Harrison Blvd.
Ogden, UT 84409

The above letter was sent to M.R. Combs, et al. The authors offer the following reply.

Mr. Staley brings an important point that we neglected to discuss in our paper. Twenty-eight (11%) of the 259 antibody-positive units we received during the study period were CPDA-1 units. The remaining units contained additives. Four (27%) of the 15 units transfused to patients who developed detectable passive antibody were CPDA-1 units. Three patients received one CPDA-1 unit and one additive unit containing alloantibodies.1

We agree that passive antibody may be detected more often with the transfusion of CPDA-1 units, which contain more plasma than additive units. We had referenced an abstract by Nobiletti et al. that confirms that antibody is often not detected in the supernates of additive units. They tested 169 units drawn from donors with alloantibodies and found that 78 (46%) had no detectable antibody in the supernate.2 Indeed, our rate of detection of passive antibody may have been lower if only additive units had been transfused.

We thank Mr. Staley for bringing this to our attention and hope this added information will encourage more institutions to accept red blood cell units containing alloantibodies. Although CPDA-1 units from donors with alloantibodies may contain detectable alloantibody more often than additive units, both types of blood products can be safely transfused.

2. Nobiletti J, Badon S, Cable R, et al. Unexpected red cell antibodies are not detected in 46% of additive red cells from antibody positive donors. Transfusion 1998;38(Suppl):87S.

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Donald H. Bennett, BS, MT(ASCP)
Marilyn J. Telen, MD
Duke University Medical Center
DUMC 2615
Durham, NC 27710

SBB Student Paper

In many of our issues of Immunohematology, the readers have seen appeals for papers. Over the years we have encouraged SBB students to submit papers written in support of projects that are part of the SBB program. The students of today are the investigators and authors of tomorrow and we are proud to support these individuals.

The editors are pleased to present in this issue the article “Tube and Column Agglutination Technology for Autocontrol Testing” by J.E. Courtney, J.L. Vincent, and A.J. Indrikovs. Ms. Courtney performed this study as part of her SBB studies at the Blood Bank at the University of Texas Medical Branch, Galveston, TX, under the guidance of Ms. Vincent, Educational Coordinator, and Dr. Indrikovs, Director of the Blood Bank. We congratulate Ms. Courtney for a good study and a fine paper. We hope this will encourage other SBB students to submit data from studies for publication.

Delores Mallory
Editor-in-Chief
Mary McGinniss
Managing Editor
ANNOUNCEMENTS

International Society of Blood Transfusion. The 11th Regional Western Pacific Congress of the International Society of Blood Transfusion will be held in Shanghai, China, from November 10–13, 2001 at the Exhibition and Conference Hall located in the Shanghai Rainbow Hotel. The program will offer plenary lectures, scientific symposiums, and poster sessions on the latest advances in blood banking and transfusion medicine. There also will be planned social activities to promote understanding and camaraderie among the different nationalities and cultural backgrounds.

Important Dates
- Early registration deadline: July 31, 2001
- Hotel registration deadline: August 30, 2001; phone (81–62) 62755388; fax (86–21) 62753736; e-mail: sales@rainbowhotel.net
- Preregistration deadline: October 31, 2001

For further information, Contact: Congress Secretary, Prof. ZHU Youg Ming, Shanghi Blood Center, #1191, Hong Qiao Road, Shanghai 200051, China; phone: (81–62) 62780789; fax: (86–21) 62958414; e-mail: isbt2001@sbc.org.cn

Monoclonal antibodies available. The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive direct antiglobulin test. Anti-Rh:17 is a directly agglutinating monoclonal antibody. Anti-Fy², anti-K, anti-Js², and anti-Kp² are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. Contact: Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

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  - detection of heparin-induced antibodies (PF4 ELISA)
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• Monoclonal antibody immobilization of platelet antigens (MAIPA)

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or call:
Maryann Keashen-Schnell
(215) 451-4041 office
(215) 451-4205 laboratory

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(215) 451-4362

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**Immunohematology**

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**Instructions for Authors**

**SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS**

Before submitting a manuscript, consult current issues of *Immunoheematology* for style. Type the manuscript on white bond paper (8.5" x 11") and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 6 under Preparation
8. Figures—see 7 under Preparation

**Preparation**

1. **Title page**
   A. Full title of manuscript with only first letter of first word capitalized
   B. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES
   C. Running title of ≤ 40 characters, including spaces
   D. 3 to 10 key words
2. **Abstract**
   A. 1 paragraph, no longer than 200 words
   B. Purpose, methods, findings, and conclusions of study
   C. Abstracts not required for reviews
3. **Text (serial pages)**
   Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.
   A. **Introduction**
      Purpose and rationale for study, including pertinent background references.
   B. **Case Report (if study calls for one)**
      Clinical and/or hematologic data and background serology.
   C. **Materials and Methods**
      Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patients' names or hospital numbers.
   D. **Results**
      Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
   E. **Discussion**
      Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.
4. **Acknowledgments**
   Acknowledge those who have made substantial contributions to the study, including secretarial assistance.
5. **References**
   A. In text, use superscript, arabic numbers.
   B. Number references consecutively in the order they occur in the text.
   C. Use inclusive pages of cited references, e.g., 1431–7.
   D. Refer to current issues of *Immunoheematology* for style.
6. **Tables**
   A. Number consecutively, head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and use no punctuation at the end.
   B. Use short headings for each column, and capitalize first letter of first word.
   C. Place explanations in footnotes (sequence: *, ‡, §, ‡, ††).
7. **Figures**
   A. Figures can be submitted either drawn or photographed (5" X 7" glossy).
   B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, put title of paper and figure number on back of each glossy submitted.
   C. When plotting points on a figure, use the following symbols when possible: ○ ● △ ▲ ■ □
8. **Author information**
   A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and complete address (including zip code) for all authors. List country when applicable.

**SCIENTIFIC ARTICLES AND CASE REPORTS SUBMITTED AS LETTERS TO THE EDITOR**

**Preparation**

1. **Heading—To the Editor:**
2. **Under heading—title with first letter capitalized**
3. **Text—write in letter format (paragraphs).**
4. **Author(s)—type flush right; for first author: name, degree, institution, address (including city, state, and zip code); for other authors: name, degree, institution, city, and state.**
5. **References—limited to ten.**
6. **One table and/or figure allowed.**

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