CONTENTS

149

Original Report
Transfusion of D+ red blood cells to D– individuals in trauma situations
A. Tchakarov, R. Hobbs, and Y. Bai

153

Serologic Method Review
Allogeneic red blood cell adsorption for removal of warm autoantibody
C. Barron

156

Case Report
Anti-Ge2: further evidence for lack of clinical significance
D. Karunasiri, F. Lowder, N. Ostrzega, and D. Goldfinger

158

Serologic Method Review
Polyethylene glycol antiglobulin test (PEG-AGT)
L. Weldy

161

Report
Validation of a blood group genotyping method based on high-resolution melting curve analysis
T. Gong, Y. Hong, N. Wang, X. Fu, and C. Zhou

166

Announcements

170

Advertisements

174

Instructions for Authors
Oscar-Claude Monet achieved greatness both as a virtuoso colorist and master of atmosphere and light and as a primary architect of the Impressionism Movement, which transformed French painting and 19th century art. Towards the end of the 1860s, Monet settled into domestic routines with his future wife, Camille Doncieux, and seldom traveled far from Bougival. His brief sojourns to Louveciennes in fall and winter to visit his friend and co-visionary, Camille Pissarro, led to a number of landscapes set locally. Road at Louveciennes, Melting Snow, Sunset, painted in 1870, shares its theme of deliquescence with Gong et al.’s article, which appears in this issue.

David Moolten, MD
Transfusion of D+ red blood cells to D− individuals in trauma situations

A. Tchakarov, R. Hobbs, and Y. Bai

To conserve D− red blood cells (RBCs), our facility developed a policy for transfusion of D+ units to D− patients, particularly in trauma situations. To our knowledge, this is the first study looking at D-mismatched RBC transfusion in trauma patients. We developed guidelines for the transfusion of D-mismatched RBCs. Patients were followed by antibody screening and direct antiglobulin testing. Twenty-six patients were identified, and 57.7 percent of the cases were the result of trauma. Follow-up ranged from 7 to 455 days. The trauma cohort had a follow-up of 7 to 102 days. Overall, patients were transfused with 1 to 36 units of D-mismatched RBCs. Three patients produced alloanti-D, resulting in a 20 percent rate of antibody formation in trauma patients. We found an overall antibody formation rate of 11.5 percent compared with 21.4 to 30.4 percent in previous studies. Approximately 58 percent of our study population was composed of trauma patients. Within that cohort, the formation of anti-D was 20 percent, similar to the rate seen in previous studies looking at primarily non-trauma populations. Immunohematology 2014;30:149–152.

Key Words: blood management, RBC transfusion, transfusion practices (adult)

The Rhesus (Rh) blood group system is second only to the ABO blood group system in importance for the transfusion of compatible blood components. This system consists of greater than 50 antigens, with D being recognized as one of the most highly immunogenic of the Rh antigens. Anti-D can lead to hemolytic transfusion reactions or hemolytic disease of the fetus and newborn in patients who have been alloimmunized. It has been found that a single exposure to as little as 0.5 mL of D+ red blood cells (RBCs) can lead to antibody formation in up to 80 percent of D− healthy volunteers and that multiple exposures of 0.5 to 1 mL can lead to antibody formation in up to 93 percent of healthy volunteers. Because of these findings, it has been widely accepted that D− individuals, particularly female children and women of childbearing age, should be transfused with D− RBCs and platelets to avoid potential complications of alloantibody formation.

In the United States, approximately 15 percent of the Caucasian and 5 percent of the African-American population are D−, resulting in a limited supply of D− RBCs available for transfusion. Studies looking at hospitalized D− patients receiving D+ RBCs observed an alloimmunization rate of nearly 22 percent. Additional studies performed on transplant and oncology patients have shown 10 to 15 percent alloimmunization in these populations. A study looking at the rate of anti-D formation in AIDS patients transfused with D-mismatched RBCs found that none of the patients formed anti-D.

We practice in a hospital with a high-volume, level one trauma center and a cardiovascular institute and frequently have found that we face a potential shortage of D− RBC units. To manage this shortage, we developed a policy for the transfusion of D+ RBCs to D− patients. We retrospectively studied the effect of this change by evaluating alloimmunization to D, particularly in the trauma patient population. Our findings show a decreased rate of alloimmunization in trauma patients when compared with healthy volunteers. This finding is similar to previous studies looking at hospitalized non-trauma patients.

Materials and Methods

To reserve D− RBC units for female children and women of childbearing age, our blood bank developed guidelines for the transfusion of D+ RBCs to D− patients (Table 1). When it was determined that the stock of D− RBCs was below critical value (35 units), patients were screened to identify possible D− individuals who could be transfused with D+ RBCs. Acceptable patients included (1) all males in addition to females older than 55 years or those with documented hysterectomy, (2) patients with no history of anti-D, and (3) patients with a current antibody screening test and direct antiglobulin test must be negative.

Table 1. Guidelines for the transfusion of D-mismatched RBCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs = red blood cells.</td>
<td></td>
</tr>
</tbody>
</table>

To justify the transfusion of D-mismatched RBCs during times of supply shortage, the following parameters must be met:

- There should be fewer than 35 units of D− RBCs in stock.
- The patient should be male or, if female, be older than 55 years of age or have documented hysterectomy in the electronic medical record.
- The patient must have no history of anti-D.
- Routine antibody screening test and direct antiglobulin test must be negative.
negative antibody screen and negative direct antiglobulin test (DAT).

A three-cell antibody screening test was performed using a gel method (Surgiscreen, Ortho Clinical Diagnostics, Raritan, NJ), and DAT was performed with polyclonal anti-human globulin containing antibodies to immunoglobulin G (IgG) and C3d (Biocline Anti-IgG, -C3d polyclonal, Ortho Clinical Diagnostics). After identification of an appropriate patient, the treating clinician was notified of the need to switch the patient to D-mismatched RBCs because of low supply. Clinicians were not given the option to opt out of the switch to D-mismatched RBCs.

The RBC units were apheresis and whole blood units prepared from volunteer donors by the Gulf Coast Regional Blood Center (Houston, TX) using standard preparation methods and leukocyte reduction. All RBCs were stored in regulated conditions and transfused within 42 days. None of the RBCs were irradiated or washed.

After patients were transfused with D-mismatched RBCs, per blood bank guidelines, they underwent gel antibody screening and DAT every 3 days for the remainder of the hospitalization. In addition, if a study patient was discharged and later readmitted, a three-cell antibody screen and DAT were performed on readmission. If patients exhibited a positive antibody screen test with anti-D identified, or a positive DAT with anti-D in the eluate, they were immediately switched to D– RBCs. Once RBC stock was replenished to greater than the critical 35 units, patients were transfused with D– units regardless of their antibody status.

Patient charts and blood bank records were retrospectively reviewed to determine relevant patient history, number of mismatched units transfused, antibodies formed, length of time required for antibody formation, and possible transfusion reactions. Patients who had a follow-up of fewer than 5 days were rejected from the study. This study had approval from the Institutional Review Board of Memorial Hermann Hospital and The University of Texas Medical School at Houston.

### Results

From January 2012 to November 2013, we identified 38 D– patients who were transfused with D+ RBCs because of a shortage of D– RBC units. Twelve patients were rejected as a result of follow-up of fewer than 5 days; the remaining 26 patients were transfused with 179 D-incompatible units and were included in our study population.

Within our study population, there were 22 males (84.6%) and 4 females (15.4%) ranging in age from 14 to 87 years (mean, 49 ± 23.1 years). Study patient blood types were group O, D– (n = 25), and group A, D– (n = 1).

Trauma without significant past medical history accounted for 15 (57.7%) of the cases. Three cases were identified in which the patients were expected to be immunocompromised: lymphoma, metastatic prostate cancer, and liver transplant. The diagnosis of the remaining cases included three aortic valve replacements, one coronary artery bypass graft, one aortic aneurysm repair, one meningioma resection, one ischemic bowel, and one pancreatitis. See Table 2 for relevant patient demographics.

<table>
<thead>
<tr>
<th>Diagnosis (n, %)</th>
<th>All patients receiving D-mismatched RBCs (n = 26)</th>
<th>Patients producing anti-D (n = 3)</th>
<th>Patients not producing anti-D (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trauma</td>
<td>17 (65.4)</td>
<td>3 (100)</td>
<td>14 (60.9)</td>
</tr>
<tr>
<td>Cardiovascular surgery</td>
<td>5 (19.2)</td>
<td>5 (11.1)</td>
<td>10 (43.5)</td>
</tr>
<tr>
<td>Organ transplant</td>
<td>1 (3.8)</td>
<td>1 (33.3)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>1 (3.8)</td>
<td>1 (33.3)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Leukemia/lymphoma</td>
<td>1 (3.8)</td>
<td>1 (33.3)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (11.5)</td>
<td>3 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; SD = standard deviation.

Follow-up ranged from 7 to 455 days (mean, 67.6 ± 104 days). The number of D-mismatched units transfused among all the patients ranged from 1 to 36 units (mean, 6.88 ± 7.9 units; median, 4 units). Among patients who did not form anti-D, the number of transfused D-mismatched units ranged from 1 to 23 units (mean, 5.7 ± 5.5 units). Patients who produced anti-D were transfused with 6 to 36 units of D-mismatched RBCs (mean, 16.3 ± 17 units; median, 7 units).

Sixteen (61.5%) of the study patients were undergoing massive transfusion protocol during transfusion of D-incompatible RBCs. The majority of these cases (n = 14) were trauma cases, with the remaining cases being an aortic valve replacement and an abdominal aortic aneurysm. Twenty-three (88.4%) patients were given additional D– RBCs during their hospitalization, ranging in number from 2 to 33 units (mean, 10.1 ± 7.14 units). None of the patients (n = 3) who were given only D+ blood formed anti-D. The number of D– units given to patients who did not form antibodies (n = 20) ranged from 2 to 19 units (mean, 8.4 ± 4.9 units). Patients who formed anti-D
were given a range of 13 to 33 units (mean, 22 ± 10.2 units) of additional D– RBCs. Table 3 summarizes the transfusion differences between patients who formed anti-D and patients who did not.

Table 3. Transfusion characteristics of study patients

<table>
<thead>
<tr>
<th>Antibody producers and nonproducers</th>
<th>Patients producing anti-D</th>
<th>Patients not producing anti-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of follow-up (days)</td>
<td>67.6 ± 104</td>
<td>47.6 ± 47.2</td>
</tr>
<tr>
<td>D+ RBCs transfused (units)</td>
<td>6.88 ± 7.9</td>
<td>16.3 ± 17</td>
</tr>
<tr>
<td>D– RBCs transfused (units)</td>
<td>10.1 ± 7.14</td>
<td>22 ± 10.2</td>
</tr>
<tr>
<td>Number of distinct D+ transfusion episodes</td>
<td>1.9 ± 1.4</td>
<td>3.3 ± 2.5</td>
</tr>
</tbody>
</table>

Table 3. Transfusion characteristics of all D– patients receiving D+ RBCs

<table>
<thead>
<tr>
<th>Length of follow-up (days)</th>
<th>67.6 ± 104</th>
<th>47.6 ± 47.2</th>
<th>70.2 ± 110.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+ RBCs transfused (units)</td>
<td>6.88 ± 7.9</td>
<td>16.3 ± 17</td>
<td>5.7 ± 5.5</td>
</tr>
<tr>
<td>D– RBCs transfused (units)</td>
<td>10.1 ± 7.14</td>
<td>22 ± 10.2</td>
<td>8.4 ± 4.9</td>
</tr>
<tr>
<td>Number of distinct D+ transfusion episodes</td>
<td>1.9 ± 1.4</td>
<td>3.3 ± 2.5</td>
<td>1.65 ± 1.15</td>
</tr>
</tbody>
</table>

Results are mean ± 1 standard deviation. RBCs = red blood cells.

When looking at the cohort of trauma patients transfused with D-mismatched RBCs (n = 15), there was a follow-up of 7 to 102 days (mean, 27.2 ± 25.5 days). Overall, the trauma patients were transfused with 1 to 36 units (mean, 7.2 ± 8.6 units; median, 6 units) of D-mismatched RBCs. Those trauma patients who were transfused with D– RBCs in addition to D+ RBCs (n = 14) received 2 to 33 units (mean, 12.6 ± 7.7 units; median, 10 units). Table 3 summarizes the transfusion characteristics of the trauma cohort.

Three patients (11.5%) produced anti-D after the transfusion of D+ RBCs. Of the patients who formed anti-D (n = 3), all were male and all were trauma patients undergoing massive transfusion protocol, resulting in a 20 percent rate of antibody formation in trauma patients. The ages of the patients developing anti-D were 20, 21, and 54 years. The first patient was transfused with 6 units of D+ RBCs in a single transfusion episode, along with 20 units of D– RBCs during his hospitalization and exhibited anti-D 12 days after the transfusion. The second patient was transfused with 36 units of D+ RBCs during six separate episodes as well as 13 units of D– RBCs and exhibited anti-D, anti-E, and nonspecific IgG antibodies 24 days after the first transfusion. The third patient was transfused with 7 units of D+ RBCs during three separate episodes along with 33 units of D– RBCs and exhibited anti-D and -E 21 days after the first transfusion episode. Table 4 summarizes the characteristics of the patients who formed anti-D. In addition to forming anti-D, 3 patients (13%) exhibited a weakly positive DAT with negative eluate. One patient produced anti-Jk and another patient, anti-Lu. There was a single patient who formed a warm autoantibody 6 months after the initial transfusion of D-mismatched RBCs.

Table 4. Demographics of patients forming anti-D

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>D+ RBCs transfused (units)</th>
<th>D– RBCs transfused (units)</th>
<th>Number of distinct D+ transfusion episodes</th>
<th>Time until antibody formation (days)</th>
<th>Additional antibodies formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>20</td>
<td>Male</td>
<td>Motor vehicle collision</td>
<td>6</td>
<td>20</td>
<td>1</td>
<td>12</td>
<td>None</td>
</tr>
<tr>
<td>Patient 2</td>
<td>21</td>
<td>Male</td>
<td>Gunshot wound</td>
<td>36</td>
<td>13</td>
<td>6</td>
<td>24</td>
<td>Anti-E and nonspecific IgG</td>
</tr>
<tr>
<td>Patient 3</td>
<td>54</td>
<td>Male</td>
<td>Gunshot wound</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>21</td>
<td>Anti-E</td>
</tr>
</tbody>
</table>

Discussion

Previous studies looking at the transfusion of D-mismatched RBCs to the general patient population found a rate of anti-D formation ranging from 21.4 to 30.4 percent.4,5,11 The upper limit of the formation rate (30.4%) was calculated using a statistical model to account for patients who would exhibit antibodies after the screening period had ended.11 Our study found an antibody formation rate of 11.5 percent in our study population, even though our patients were transfused more D-mismatched units on average (median, 10 units) than in previous studies (median, 1.1–6 units).4,5,11 One possible reason for this may have been a short period of follow-up leading to decreased detection of anti-D.

A significant proportion of our study population, 57.7 percent (n = 15), was trauma patients. Within that cohort of patients, the formation of anti-D was 20 percent (n = 3). Theoretically, this cohort of patients was normal and healthy immediately before the event requiring the transfusion of RBCs. However, after the transfusion of D-mismatched RBCs, this patient population had an anti-D formation rate similar
to that seen in previous studies of hospitalized patients. One possible reason for this is that because the patients were actively bleeding, there was a decreased period during which the D-mismatched RBCs were present in the circulation. However, trauma patients were transfused with D-mismatched RBCs for a mean of 1.9 days, indicating that many patients had transfusions of D-mismatched RBCs after their bleeding had slowed significantly or stopped. Another likely reason for the low rate of antibody formation in trauma patients is that both trauma and transfusion can have immunomodulatory effects and lead to a level of immunosuppression. To the best of our knowledge, this is the first study to look at the rate of D alloimmunization in trauma patients transfused with D-mismatched RBCs.

During the study, we managed to conserve 179 units of D– RBCs after transfusion of 26 patients. Many of the patients in our study were switched back to D– RBCs after the supply returned to adequate levels. Gonzalez-Porras et al. noted that 88 percent of patients who produced anti-D did so within the first four transfusions of D-mismatched RBCs. Accordingly, had we kept patients on D-mismatched RBCs after the first transfusion, we could have saved a total of 261 D– RBC units. This savings would be vital during times of D– RBC supply shortage, and by following a more aggressive schedule of transfusion of D-mismatched RBCs to eligible D– patients, the supply can be preserved for patients who vitally need D– units, including girls and women of childbearing potential and patients with existing anti-D.

References


Amanda Tchakarov, MD (corresponding author), Pathology Resident, University of Texas Medical School at Houston, Department of Pathology and Laboratory Medicine; Rhonda Hobbs, BS, Blood Bank Supervisor, Memorial Hermann Hospital, Texas Medical Center, 6411 Fannin, Houston, TX 77030; and Yu Bai, MD, PhD, Assistant Professor, University of Texas Medical School at Houston, Department of Pathology and Laboratory Medicine, 6431 Fannin Street, MSB 2.262, Houston, TX 77030.
Allogeneic red blood cell adsorption for removal of warm autoantibody

C. Barron

Adsorption studies are usually required to confirm or rule out the presence of underlying alloantibodies in samples containing warm autoantibody. Allogeneic adsorptions are necessary if the patient has been recently transfused. Most commonly, allogeneic adsorptions are performed using a trio of phenotyped reagent red blood cells to rule out clinically significant alloantibodies to common antigens. The adsorbing cells may be used untreated or treated with enzymes or with ZZAP before adsorption. Adsorption may also be performed using enhancement such as low-ionic strength saline or polyethylene glycol added to the mixture. Multiple adsorptions may be necessary to remove strongly reactive autoantibodies. Allogeneic adsorptions will not detect alloantibodies to high-prevalence antigens. *Immunohematology* 2014;30:153–155.

Key Words: AIHA, adsorption, autoantibody, alloantibody, allogeneic RBCs

The serum from patients with warm autoimmune hemolytic anemia (WAIAH) typically demonstrates broad reactivity with all reagent red blood cells (RBCs). This reactivity usually necessitates the use of incompatible blood for transfusion. When blood is incompatible as a result of an RBC autoantibody, transfused RBCs will survive at a rate similar to that of the patient’s own RBCs. However, autoantibody reactivity may mask the reactions of underlying clinically significant alloantibodies to common antigens, which can cause rapid RBC destruction. The rate of alloimmunization in patients with warm autoantibodies varies among investigators. Reported alloimmunization rates range from 17.5 to 38 percent, with an average rate of 32 percent. Issitt et al. found that 47 percent of alloantibody specificities identified after adsorption were actually partially adsorbed autoantibodies with mimicking specificities. Mimicking autoantibody specificities were found most often when autologous adsorption was used to remove autoantibody reactivity. Identification of alloantibody specificities underlying a warm autoantibody is critical for a safe and effective transfusion.

Methods used to determine the presence of an alloantibody underlying an autoantibody include serum dilution, autologous adsorption, and allogeneic adsorption. The dilution screening method uses a 1:5 dilution of serum in phosphate-buffered saline. The diluted serum is tested to determine whether the autoantibody has been removed and whether alloantibody is present. Although this may be useful as a screening tool, this method has been shown to be unreliable in consistently detecting alloantibody underlying autoantibody. Autologous adsorption, which uses the patient’s own RBCs to remove the autoantibody, is a preferred method. Using autologous RBCs ensures that only autoantibodies are removed from the serum, leaving intact any alloantibody the patient may have produced, including antibodies to high-prevalence antigens. However, autologous adsorptions may only be performed when the patient has not been recently transfused or pregnant.

Reagents/Supplies

<table>
<thead>
<tr>
<th>Treatment Method</th>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain</td>
<td>• 1% cysteine-activated papain</td>
<td>• Pipettes</td>
</tr>
<tr>
<td>Ficin</td>
<td>• 1% ficin</td>
<td>• 37°C water bath</td>
</tr>
<tr>
<td>ZZAP</td>
<td>• 1% cysteine-activated papain or 1% ficin</td>
<td>• Large-bore test tubes</td>
</tr>
<tr>
<td></td>
<td>• 0.2 M DTT</td>
<td>• Calibrated centrifuge</td>
</tr>
<tr>
<td></td>
<td>• Commercial LISS enhancement reagent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 20% PEG or commercial PEG enhancement</td>
<td></td>
</tr>
</tbody>
</table>

DTT = dithiothreitol; RBCs = red blood cells; LISS = low-ionic-strength saline; PEG = polyethylene glycol.

Procedural Steps

**Preparation of RBCs for adsorption**
• Select reagent RBCs for use as adsorbing cells.
• Treat the adsorbing cells with the desired enzyme or ZZAP.

**Adsorption**
• Add 1 to 2 volumes of treated adsorbing cells to 1 volume of patient serum or plasma and 1 volume of enhancement reagent (if applicable).
• Incubate for 30–60 minutes at 37°C without enhancement, or 15 minutes at 37°C with LISS or PEG enhancement.
• Harvest the adsorbed serum or plasma and test for the presence of underlying alloantibodies.

RBCs = red blood cells.
commonly defined as having no transfusions or pregnancy in the prior 3 months. Alloantibody may be removed from the test system if autologous adsorptions are performed using a sample from a recently transfused patient. Laine et al.\textsuperscript{10} showed that even small amounts of transfused RBCs are capable of removing all alloantibody reactivity. Allogeneic RBC adsorption is the method of choice for removal of warm autoantibody reactivity in patients with recent transfusions. Note that the term "serum" is used to denote the patient sample throughout this article; however, either serum or plasma may be used for testing.

**Principle**

Allogeneic adsorptions are used to remove broadly reactive autoantibody from a patient’s serum to allow for detection of underlying clinically significant alloantibodies to common antigens. The adsorbing cells will remove autoantibody along with any additional antibody specificities to antigens that are present on the RBC. When performing allogeneic adsorptions, the goal is to eliminate or confirm the presence of those alloantibodies directed at the common antigens D, C, E, c, e, K, S, s, Fy\textsuperscript{a}, Fy\textsuperscript{b}, Jk\textsuperscript{a}, and Jk\textsuperscript{b}.

Traditional allogeneic adsorptions use a set of three adsorbing cells with known phenotypes: R\textsubscript{1}, R\textsubscript{2}, and rr. At least one of the adsorbing cells should be negative for K and Jk\textsuperscript{a} or Jk\textsuperscript{b}. In addition, at least one of the reagent cells should also lack S or s and Fy\textsuperscript{a} or Fy\textsuperscript{b} unless the cells are pretreated to denature the antigens. A separate aliquot of patient serum is incubated with each adsorbing cell. Once all autoantibody reactivity is removed, the three adsorbed serums are tested to eliminate or prove the presence of any underlying alloantibody. Each aliquot of adsorbed serum contains alloantibody corresponding to those antigens for which the adsorbing cell was negative.

If a patient’s RBC phenotype is known, it may be possible to adsorb using a single phenotypically similar reagent RBC. For instance, if a patient is only negative for E, K, S, and Fy\textsuperscript{a}, one reagent RBC negative for those antigens can be used for adsorption. After adsorption, the adsorbed serum can be tested to confirm or eliminate the presence of anti-E, -K, -S, and/or -Fy\textsuperscript{a}.

Various methods are used for performing the adsorption. Treatment of the adsorbing cells using enzymes or ZZAP may enhance the uptake of the autoantibody.\textsuperscript{11} These cell treatments will also destroy some antigens, which may be advantageous when selecting cells for adsorption and testing the adsorbed serum. Rarely, autoantibodies may demonstrate specificity corresponding to an antigen that is destroyed by enzymes (e.g., anti-Ge\textsuperscript{2}) or ZZAP (e.g., anti-LW\textsuperscript{6} or Kell system antibodies). In these cases, adsorption with untreated reagent RBCs is necessary.

The addition of enhancement media to the adsorbing mixture has also become commonplace and can be used in place of RBC pretreatment. The addition of low-ionic-strength saline (LISS) or polyethylene glycol (PEG) reduces the processing time and may decrease the number of adsorptions required to remove the autoantibody.\textsuperscript{9,11,12} However, some investigators caution that some underlying alloantibodies may not be detected when PEG adsorptions are performed.\textsuperscript{13,14}

**Indications**

Allogeneic adsorptions should be used when a patient has been recently transfused or when autologous cells are of limited supply. Adsorption eliminates the reactivity of the warm autoantibody, allowing for the identification of alloantibodies in the adsorbed serum.

**Procedure**

Select cells for adsorption. If the patient’s RBC phenotype is known, select a phenotypically similar reagent RBC that lacks the same common RBC antigens as the patient’s RBCs. If the patient’s RBC phenotype is unknown, select a trio of reagent RBCs: R\textsubscript{1}, R\textsubscript{2}, and rr. Additionally, at least one cell of the set should be negative for K and Jk\textsuperscript{a} or Jk\textsuperscript{b} so that all common alloantibodies can be detected using the adsorbed serum. If adsorptions will be performed using untreated reagent RBCs, at least one cell should be negative for S or s and Fy\textsuperscript{a} or Fy\textsuperscript{b} as well.\textsuperscript{15–18}

If desired, treat the adsorbing cells with enzymes (ficin or papain) or ZZAP (ficin or papain and 0.2 M DTT). This pretreatment will typically enhance the adsorbing process.

Following pretreatment, wash the adsorbing cells, centrifuge, and remove all excess saline before use. Perform the adsorption by mixing one to two volumes of allogeneic RBCs with one volume of patient serum at 37°C for 30 to 60 minutes.

Another option is to perform the adsorption by mixing one volume of patient serum and one volume of LISS or PEG\textsuperscript{19} with one to two volumes of allogeneic reagent RBCs at 37°C for 15 minutes.\textsuperscript{15–18} After the adsorption, centrifuge the mixture and harvest the adsorbed serum. Test the adsorbed serum to determine whether adsorption is complete. This may be accomplished
by testing the adsorbed serum against the adsorbing cells or by testing the adsorbed serum against a reagent RBC phenotypically similar to the adsorbing cell. If necessary, repeat the adsorption using a new aliquot of adsorbing cells.

Once adsorption is complete, test the adsorbed serum against reagent RBCs to rule out the presence of underlying alloantibodies. For instance, if the adsorbing cell is R, R, S, K– Jk(a–), then the following alloantibodies can be ruled out in that aliquot of adsorbed serum; anti-E, -c, -S, -K, and -Jk×.

Note: Test the adsorbed serum from the LISS or PEG enhancements using four drops of the serum/enhancement reagent mixture.

**Limitations**

Allogeneic adsorption will not rule out the presence of alloantibodies to high-prevalence antigens. The adsorbing cells are presumed to be positive for all high-prevalence antigens; therefore, an alloantibody to a high-prevalence antigen would be adsorbed along with the autoantibody.

When a patient’s serum contains a very potent warm autoantibody, multiple adsorptions are needed to remove the broad reactivity. Generally, for each grade of reactivity, at least one adsorption will be necessary (a serum sample with an autoantibody that reacts 2+ will require a minimum of two adsorptions). Multiple adsorptions have the potential to dilute the serum. Increasing the ratio of adsorbing cells to serum may enhance the removal of the autoantibody.

The use of PEG adsorption may cause weakening or loss of antibody reactivity in some samples. The use of six drops of the serum/enhancement reagent mixture tests may minimize this effect.

**Quality Control**

To determine whether the adsorbing cells are adequately treated with enzymes, test the cells with Glycine max var. soja lectin. Enzyme-treated RBCs should demonstrate positive reactivity with G. max var. soja and untreated RBCs should be nonreactive.

**References**


Christina Barron, MT(ASCP)SBB, Manager, Immunohematology Reference Laboratory, American Red Cross, Missouri-Illinois Region, 4050 Lindell, St. Louis, MO 63108.
Anti-Ge2: further evidence for lack of clinical significance

D. Karunasiri, F. Lowder, N. Ostrzega, and D. Goldfinger

Anti-Ge2 may be immune or naturally occurring, and it reacts with an antigen on glycophorin D. Ge2 is encoded by a gene, \textit{GYPC}, which is located on the long arm of chromosome 2. Anti-Ge2 is usually an immunoglobulin G (IgG) antibody. In the available literature, we have not been able to find any reported cases of proven acute hemolytic transfusion reactions caused by anti-Ge2. We present the case of a 67-year-old man with metastatic pancreatic carcinoma who had symptomatic anemia and a hemoglobin concentration of 6.3 g/dL. During pretransfusion testing, anti-Ge2 was identified in his serum. Only a single unit of compatible, Ge:–2 frozen red blood cells (RBCs) could be provided by the blood supplier. A second unit of crossmatched, least-incompatible, leukocyte-reduced RBCs, presumably Ge:2, was also transfused. The transfusion was completed without incident, and the patient's hemoglobin concentration rose appropriately. Posttransfusion values for haptoglobin, lactate dehydrogenase, and urine hemoglobin were within normal limits. A monocyte monolayer assay performed on this anti-Ge2 supports the data that antibodies of this specificity do not cause hemolysis. The clinical and laboratory data obtained in our patient clearly indicated that no hemolysis of transfused RBCs occurred during and for 24 hours after transfusion. We believe that this report adds to a limited experience with anti-Ge2 and provides further evidence for concluding that, in all likelihood, this is not a clinically important RBC antibody. The risk of transfusing apparently “incompatible” (Ge:2) RBCs seems remote and should allow for timely administration of RBCs when treating patients with serious anemia. \textit{Immunohematology 2014;30:156–157.}

The Gerbich blood group system is composed of 11 antigens that are expressed on glycophorin C (GPC) and glycophorin D (GPD).\textsuperscript{1} Both are encoded by a single gene, \textit{GYPC}, which is located on the long arm of chromosome 2. Gerbich antigens are inherited as autosomal-dominant traits. Of the 11 antigens in the Gerbich system, 6 of them are high-prevalence antigens, including Ge:2. Anti-Ge2 may be immune or naturally occurring, and it reacts with an antigen on GPD. Anti-Ge2 is usually an immunoglobulin G (IgG) antibody.

In the available literature, we have not been able to find any reported cases of proven acute hemolytic transfusion reactions caused by anti-Ge2. Yet the presence of this antibody in patient plasma is cause for concern when transfusing incompatible Ge:2 red blood cells (RBCs). We found two reports of successful transfusion of Ge:2 RBCs in patients with strongly reactive anti-Ge2.\textsuperscript{2,3} We now report another case of successful transfusion of Ge:2 RBCs in a patient with demonstrable anti-Ge2.

\textbf{Case Report}

A 67-year-old man with metastatic pancreatic carcinoma and biliary obstruction was admitted to the Olive View UCLA Medical Center with symptomatic anemia and a hemoglobin concentration of 6.3 g/dL. His blood group was O Rh (D) positive. During pretransfusion testing, an antibody of undetermined specificity was detected (1+ to 2+) by the polyethylene glycol indirect antiglobulin test. A blood sample was sent to a reference laboratory for further testing, and anti-Ge2 was identified. Only a single unit of compatible, Ge:–2 frozen RBCs could be provided by the blood supplier.

We transfused the compatible unit of RBCs without incident. A second unit of crossmatched, least-incompatible, leukocyte-reduced RBCs, presumably Ge:2, was then transfused. We advised the clinician to transfuse this unit slowly and monitor the patient closely for any adverse effects. The transfusion was completed without incident, and the patient's hemoglobin concentration rose appropriately. Table 1 shows the values for laboratory tests performed before and 1 day after transfusion. Values for haptoglobin, lactate dehydrogenase, and urine hemoglobin were within normal limits. Serum bilirubin was elevated before transfusion and could not be used as a measure of hemolysis.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Date} & \textbf{Hemoglobin concentration} & \textbf{Haptoglobin (normal range 36–195 mg/dL)} & \textbf{Lactate dehydrogenase (normal range 98–192 U/L)} & \textbf{Urine hemoglobin} \\
\hline
Before transfusion & 6.3 g/dL & Not available & Not available & Not available \\
1 day after transfusion & 9.2 g/dL & 255 mg/dL & 179 U/L & Negative \\
\hline
\end{tabular}
\caption{Patient's laboratory test results}
\end{table}

A monocyte monolayer assay was performed by the American Red Cross Reference Laboratory as previously described.\textsuperscript{4} Reactivity greater than 5 percent can be associated with overt transfusion reactions in patients who receive
incompatible RBCs. Our patient demonstrated less than 1 percent reactivity, indicating that this anti-Ge2 would not be expected to cause acute hemolysis of transfused Ge:2 RBCs.

**Discussion**

Antibodies to high-prevalence antigens pose an especially difficult problem regarding patient management. The delays encountered in obtaining compatible, antigen-negative units of RBCs can place patients who require timely transfusion for severe anemia in real jeopardy. Therefore, knowledge of whether particular antibody specificity is clinically significant can be of huge importance in guiding patient care. However, the rarity of these cases makes obtaining adequate data for evidence-based decisions a challenge. The clinical and laboratory data obtained in our patient clearly indicate that no hemolysis of transfused RBCs occurred during and for 24 hours after transfusion.

We believe that this report adds to a limited experience with anti-Ge2 and provides the basis for concluding that, in all likelihood, this is not a clinically important RBC antibody. The risk of a patient experiencing an adverse reaction after transfusion of apparently “incompatible” Ge:2 RBCs seems remote, and this information should allow for more timely administration of RBCs when treating patients with serious anemia.

**Acknowledgments**

The authors thank blood bank staff at Los Angeles County–Olive View/UCLA Medical Center and the American Red Cross reference laboratory for diagnostic testing and supplying blood.

**References**


Deepthi Karunasiri, MD, MBBS (corresponding author), Blood Bank Director, Co-Director, Clinical Laboratory, Los Angeles County–Olive View/UCLA Medical Center, Department of Pathology, dkarunasiri@dhs.lacounty.gov; Frederick Lowder, CLS, MT(ASCP) SBB, Blood Bank Supervisor, Los Angeles County–Olive View/UCLA Medical Center, Department of Pathology; Nora Ostrzega, MD, Clinical Professor of Pathology, David Geffen School of Medicine at UCLA, Chair, Department of Pathology, Los Angeles County–Olive View/UCLA Medical Center, 14445 Olive View Drive, Sylmar, CA 91342; Dennis Goldfinger, MD, Professor, Division of Transfusion Medicine, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Consultant, Department of Pathology and Laboratory Medicine, Los Angeles County–Olive View/UCLA Medical Center, 757 Westwood Plaza, Suite B403, Los Angeles, CA 90095.
Polyethylene glycol antiglobulin test (PEG-AGT)

L. Weldy

Polyethylene glycol (PEG) was described in 1987 as a new technique for immunohematology testing. The original paper described its use in detection and identification of weakly reactive antibodies. PEG is used as an additive to enhance reactivity and to reduce incubation time when testing for unexpected antibodies. PEG can be used as an alternative to low-ionic-strength saline and whenever weak reactions are encountered. *Immunohematology* 2014;30:158–160.

**Key Words:** polyethylene glycol, PEG-AGT, reactivity enhancement, weak antibody detection

**Principle**

Polyethylene glycol (PEG) is a water-soluble linear polymer that has been demonstrated to enhance sensitivity in the detection of unexpected blood group antibodies although the mechanism by which PEG enhances antigen–antibody interactions is not fully understood. It is used in industry for lubrication and in immunology for detection of immune complexes and immunoglobulin precipitation. It was the immunoglobulin precipitation experience that led to PEG being considered for immunohematology testing, since this phenomenon/property might cause antigen and antibody molecules to be in closer proximity to each other. PEG may act to decrease water molecules around the red blood cell (RBC) membrane or decrease the net negative charge of the RBC. Its effect is to allow for improved antibody sensitization and agglutination.

**Background**

In the original studies of Nance and Garratty, the use of anti-immunoglobulin G (IgG)—and not polyspecific antiglobulin reagent—was noted to be important to yield fewer false-positive reactions. In the testing of 25 patient samples with weak (microscopic) to 2+ antibody reactivity in LISS (low-ionic-strength saline), 16 (64%) demonstrated strongest reactivity in PEG; 7 (28%) had the same reactivity in PEG, Polybrene (hexadimethrine bromide), or LISS; and 2 (8%) were weaker in PEG. In titration studies with 11 of the strongly reactive antibodies, 6 (54%) gave higher agglutination scores (>10 score difference) with PEG as compared with LISS.

The same authors tested 24 samples from patients who had experienced a hemolytic transfusion reaction (HTR) but without detectable antibody in their plasma and 10 samples from patients suspected of having direct antiglobulin test–negative autoimmune hemolytic anemia (DAT–AIHA). In 2 of 24 HTR patients, antibody was detected with PEG as well as with other sensitive methods: anti-Jk by Polybrene and anti-e by Polybrene and a 4 percent ficin capillary technique. Of the 10 DAT–AIHA samples that were positive by another sensitive method (enzyme-linked antiglobulin test), only 2 were positive by the direct PEG test. Thus, although the direct PEG test did detect more than the routine tube DAT, it was not as sensitive as the enzyme-linked antiglobulin test.

**Reagents/Supplies**

- **Reagents**
  - PEG (commercially or in-house prepared)
  - Anti-IgG
  - IgG-coated RBCs
  - Isotonic saline or PBS (pH 6.5–7.5)
- **Supplies**
  - Centrifuge
  - Test tubes
  - Pipettes
  - 37°C incubator or water bath
  - Timer
  - Optical aid (optional)

PEG = polyethylene glycol; IgG = immunoglobulin G; RBCs = red blood cells; PBS = phosphate-buffered saline.

**Procedural Steps**

**Process**

- Mix test serum with RBCs.
- Add PEG according to manufacturer’s or procedural directions.
- Incubate the tubes at 37°C for 15–30 minutes.
- DO NOT CENTRIFUGE. Wash the RBCs 3–4 times with saline.
- Add anti-IgG.

RBCs = red blood cells; PEG = polyethylene glycol; IgG = immunoglobulin G.
**Indications**

PEG is generally used as an enhancement medium to aid in the identification of potentially clinically significant antibodies. It also can enhance clinically insignificant antibodies (i.e., cold autoantibodies), and it can be used to minimize detection of clinically insignificant antibodies, especially those that demonstrate high-titer, low-avidity–like reactivity.

**Procedure**

For testing samples, label a test tube for each RBC sample to be tested. Wash the RBCs once with saline and prepare a 3–4 percent RBC suspension. Add 2 drops (0.1 mL) of test serum, plasma, or eluate to each tube followed by the addition of 1 drop of the appropriate RBC suspension. Then add 2 drops of PEG, or as according to manufacturer’s direction if a commercial source of PEG is used, and mix well. In-house prepared PEG may require 4 drops. It is important to add the PEG last, since it has a tendency to precipitate serum globulins; it is not intended as a cell-suspending medium.

Incubate the tubes at 37°C ± 1°C for 15 to 30 minutes. Do not centrifuge the test mixture after the incubation phase because centrifugation can lead to aggregates that are difficult to disperse and interpret. Immediately wash the RBCs three to four times with saline and completely decant the final supernatant. Add 2 drops of anti-IgG, mix well, and centrifuge for the time indicated in a calibrated centrifuge. Be sure to use anti-IgG and not polyspecific anti-human globulin to avoid unwanted positive reactions caused by C3-binding autoantibodies. Resuspend the RBCs, examine for hemolysis and agglutination macroscopically, and record results. Microscopic examination is not recommended when using PEG, since RBCs tend to be aggregated to some extent, and experience in this technique is required for microscopic interpretation.

Add IgG-coated RBCs to all negative tests, mix, and centrifuge in a calibrated centrifuge. Read macroscopically for agglutination. If negative results are obtained, the test must be repeated.

**Limitations**

As in all serologic procedures, such factors as contaminated materials; improper incubation time, temperature, or centrifugation; and deviation from manufacturer’s directions may produce false results.

If using PEG for crossmatching, an immediate spin phase before adding the reagent is recommended to avoid missing some IgM examples of anti-A and anti-B. IgM antibodies may not be detected by the PEG test procedure. In addition, the use of anti-IgG may result in failure to detect complement-dependent antibodies.

PEG has a tendency to precipitate serum globulins. Thus, it is important to ensure that the RBCs are thoroughly resuspended in each change of saline during the washing phases. If precipitated globulin remains enmeshed in the RBC button, it may neutralize the anti-IgG. This result may be overcome by increasing the number of washes or by manual washing. Alternatively, it may be necessary to use another method with similar sensitivity.

The addition of PEG to samples with high levels of globulin, e.g., those from patients with multiple myeloma, may cause a gel to form. This step will preclude the use of the PEG test procedure for these samples.

Precipitation of fibrinogen may be observed when testing plasma samples. In such cases, it may be necessary to perform additional washes of the RBCs.

**Quality Control**

To control the efficacy of the product in enhancing reactivity, a sample containing a weak IgG antibody may be selected and tested at regular intervals.

An autologous control (patient serum or plasma plus own RBCs) should be included for antibody identification tests at least the first time the media are used with the patient’s sample. This step will help to inform the tester of the presence of an autoantibody that is enhanced by PEG.

**Comparative Studies**

There are comparative studies using PEG and other media that may be of interest. The first, “Analysis of the routine use of polyethylene glycol (PEG) as an enhancement medium,” by Barrett et al. compares PEG with LISS. The authors report that PEG detected 35 clinically significant antibodies and 10 clinically insignificant antibodies, whereas LISS detected only 15 clinically significant antibodies and 33 insignificant antibodies. Thus, the sensitivity and specificity were better with PEG than with LISS. The second publication is “Comparison of tube and gel techniques for antibody identification” by Novaretti et al. In this study, duplicate antibody detection with the European-sourced IgG gel method and laboratory-developed PEG on 10,123 samples yielded a total of 628
reactive samples by gel or PEG, of which 430 were reactive by both methods. These authors reported that 196 samples with potentially clinically significant antibodies (by specificity) were reactive only by gel and 2 samples only by PEG. Most of the PEG-negative, gel-positive samples contained Rh, K, and Jk<sup>a</sup> antibodies. There were 25 anti-D, 33 anti-C, 76 anti-E, 13 anti-c, 6 anti-e, 18 anti-K, and 7 anti-Jk<sup>a</sup> antibodies that were not detected by PEG but were detected by the gel method. Of interest is that in the anti-E group, of 144 total anti-E, 76 (53%) were not detected by PEG. PEG continues to have its highest utility in increased sensitivity for antibody identification and has been used to improve specificity of more sensitive but less specific automated testing such as solid phase.

References


Larry Weldy, MT(ASCP)SBB, Supervisor, IRL, American Red Cross, Lewis and Clark Region, 6616 South 900 East, Salt Lake City, UT 84121.
Validation of a blood group genotyping method based on high-resolution melting curve analysis

T. Gong, Y. Hong, N. Wang, X. Fu, and C. Zhou

The detection of polymorphism is the basis of blood group genotyping and phenotype prediction. Genotyping may be useful to determine blood groups when serologic results are unclear. The development and application of different methods for blood group genotyping may be needed as a substitute for blood group typing. The purpose of this study is to establish an approach for blood group genotyping based on a melting curve analysis of real-time polymerase chain reaction (PCR). Using DNA extracted from whole blood, we developed and validated a DNA typing method for detecting **DO***01/*DO*02, **DI***01/*DI*02, **LU***01/*LU*02, and **GYPB***03/*GYPB*04 alleles using a melting curve analysis. All assays were confirmed with a commercial reagent containing sequence-specific primers (PCR-SSP), and a cohort of the samples was confirmed with sequencing. Results for all blood groups were within the range of specificity and assay variability. Genotypes of 300 blood donors were fully consistent with PCR-SSP data. The obtained genotype distribution is in complete concordance with existing data for the Chinese population. There are several advantages for this approach of blood group genotyping: lower contamination rates with PCR products in the laboratory, ease of performance, automation potential, and rapid cycling time. *Immunohematology* 2014;30:161–165.

**Key Words:** blood group genotyping, real-time PCR, melting curve analysis

Currently, typing for blood group antigens is performed by hemagglutination. As the gold standard for blood group phenotyping, hemagglutination assays may be inaccurate in some cases, such as for multiply transfused patients and patients with autoantibodies. In addition, the necessary reagents are costly and often limited in availability. Molecular typing techniques are emerging as a supplement to standard serology. Genotyping is often focused on a specific single-nucleotide polymorphism (SNP) of a blood group gene; as a result, the assay may not detect additional rare polymorphisms that may be responsible for the formation of a different antigen. In addition, the method of polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) for blood group genotyping may lead to contamination from the laboratory during the process of opening the reaction tube for electrophoresis, especially when screening a large number of samples. The purpose of this study was to explore a new method of blood group genotyping as a substitute for blood group typing. We developed a DNA method for genotyping **DO***01/*DO*02, **DI***01/*DI*02, **LU***01/*LU*02, and **GYPB***03/*GYPB*04 alleles using a melting curve analysis. Assay results were within the range of specificity and assay variability.

**Materials and Methods**

**Samples and Primers**

A total of 300 EDTA blood samples were obtained from volunteer blood donors at Chengdu Blood Center, Sichuan, China. Genomic DNA was extracted using the commercially available TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) from 500-μL blood samples and prepared for subsequent PCR amplification. The special primers for **DO***01/DO*02, **DI***01/DI*02, **LU***01/LU*02, and **GYPB***03/GYPB*04 were designed with Primer Premier 5.0 (Premier Biosoft, Palo Alto, CA). The forward primers for the alternative pairs of each blood group gene are the same, whereas the reverse primers are different by only one or two bases at the 3’ terminal because of the specific SNP of the blood group genes. Human β-actin primer served as the internal control for **DO***01/DO*02, **DI***01/DI*02, and **LU***01/LU*02, and human growth hormone (HGH) primer was the internal control for **GYPB***03/GYPB*04. Sequences of primers are shown in Table 1.

**Melting Curve Analysis**

PCR was carried out in a 10-μL reaction volume consisting of 10 ng purified genomic DNA, 10 pmol of each of the primers, and 1 U Taq DNA polymerase (Takara, Dalian, China). PCR was performed with 1 cycle at 94°C for 2 minutes, followed by 30 cycles of 94°C for 30 seconds, 62°C for 15 seconds, and 72°C for 15 seconds. After the amplification phase, a melting curve analysis for **DO***01/*DO*02, **DI***01/DI*02, **LU***01/LU*02, and **GYPB***03/GYPB*04 was performed. Each allele was amplified separately; for example, **DO***01* was interrogated in one tube, and **DO***02* in another tube.
The assays were validated in terms of specificity with another commercial reagent (Human MNS, Duffy, Kell, Dombrock, Diego, Kidd and Lutheran blood type genotyping kit, PCR-SSP, Tianjin Super Biotechnology Developing Co. Ltd., China). PCR assays were performed to ascertain the specificity with 1 cycle at 96°C for 2 minutes followed by 5 cycles of 96°C for 20 seconds and 68°C for 60 seconds; 10 cycles of 96°C for 20 seconds, 65°C for 45 seconds, and 72°C for 30 seconds; and 15 cycles of 96°C for 20 seconds, 62°C for 45 seconds, 72°C for 30 seconds, and 72°C for 2 minutes. The final PCR products were separated by 2 percent agarose gels that were stained with ethidium bromide.

In addition, the validity of the melting curve analysis was also verified by sequencing. Five samples of Dombrock phenotype (DO) that included 2 DO*01/DO*02, 1 DO*01/DO*01, and 2 DO*02/DO*02 defined by our laboratory-developed method were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit in an ABI 3730 analyzer (Applied Biosystems, Inc., Foster City, CA).

**Results**

All assays performed best with a DNA input of 10 ng, which showed the highest and best-defined peaks. For all reactions, there was no measurable influence of different users or amplification instruments, underscoring the reproducibility of this approach. In all cases, the genotypes were easily identified by the differences of melting curves. The genotype results for the Do(a–b+) phenotype are shown in Figure 1A [Do(a–)] and 1B [Do(b+)], and results for Di(a–b+) are shown in Figure 1C [Di(a–)] and 1D [Di(b+)], as well as Lu(a–b+) and Ss(S–s+) in Figure 2. To determine the specificity of the approach based on the melting curve analysis, we compared the DO*01/DO*02, DI*01/DI*02, LU*01/LU*02, and GYPB*03/GYPB*04 blood groups with the determined genotype. It revealed a 100 percent genotype correlation with the different genotypes (Table 2). Genotypes of 300 blood donors were fully consistent with the data obtained by PCR-SSP. The sequencing results further supported the validity of this typing method (Fig. 3). The obtained genotype distribution is in complete concordance with existing data for the China population (Table 3).

**Discussion**

There are many ways to genotype for blood groups. Some papers described a genotyping method by high-resolution melting curve analysis to predict red cell antigen expression. Genotyping can also be performed by allele-specific fluorescence. Some closed-tube fluorescent methods for blood group genotyping do not require probes, but most methods are based on PCR and use fluorescent oligonucleotide probes. Chen et al. reported that the Ael and Bel blood types were rapidly detected by real-time PCR and melting curve analysis. In their methods, the real-time PCR was performed in the Lightcycler thermal cycler and the allele-specific melting behavior of fluorophore-labeled hybridization probe was used to detect Ael- and Bel-specific genotyping. However, labeled probes are costly when compared with real-time PCR with SYBR Green I. Homogeneous, closed-tube methods for blood group genotyping that do not require a separation step are attractive for their simplicity and containment of amplified products. Genotyping of SNPs by high-resolution melting curve analysis in products has been reported. We developed a blood group
Validation of a blood group genotyping method based on allelic discrimination by melting curve analysis that is suitable for the different requirements in an immunohematologic setting. As the costs for reagents remain the same, differences exist in consumables. The cost of this laboratory-developed high-resolution melting curve assay is approximately one-third the cost of the commercial SSP-PCR genotyping kit in our laboratory. From an economical point of view, the melting curve analysis offers a platform for rare blood group molecular screening from a large number of samples. The detection of blood groups in a closed-tube reaction by unique melting behavior of the primer resulting from sequence variations saves on reagent costs.

Novaretti et al. described a real-time PCR and melting curve analysis method for Diego blood group genotyping. We also tried to perform the testing using one tube for both alleles of DO*01/DO*02, DI*01/DI*02, LU*01/LU*02, and GYPB*02/GYPB*04, but we were unable to identify both alleles because the product’s size and melting temperature are high owing to the similarity of sequences between the two alleles.

Ansart-Pirenne et al. described FY*02.M real-time PCR with melting curve analysis associated with a complete one-step real-time FY genotyping. Forty-seven samples were studied by real-time PCR, based on fluorescence resonance energy transfer (FRET) technology. Obviously, although the method of real-time PCR and FRET-PCR is useful for the correct typing of blood donors, it is less suitable for use in the screening of rare blood groups because of the technical skills and precision instruments required.

We were unable to validate the assay for the LU c.230A SNP. For future work, we will consider obtaining commercially
Fig. 2. Melting curve analysis of LU*01/LU*02 and GYPB*03/GYPB*04. The genotype results of the Lu(a–b+) phenotype are shown in panels A and B. A: Lu(a–), no reaction for LU*01, only the ß-actin peak identified. B: Lu(b+), both the ß-actin and LU*02 peaks are visible. The genotype results of GYPB*03/GYPB*04 (S–s+) are shown in panels C and D. C: S–, no reaction for GYPB*03, only the human growth hormone (HGH) peak identified. D: s+, both the HGH and GYPB*04 peaks are visible.

Fig. 3. Sequencing results (reverse) for the Dombrock blood group. The Dombrock genotype of donors A, B, and C, tested by the new method, is DO*01/DO*01, DO*01/DO*02, and DO*02/DO*02, respectively. The arrow indicates nucleotide position at 7975(NG_007477.1), and the genotypes of donors A, B, and C have the base C, C/T, and T at 7975 of the DO gene, respectively. Dombrock genotypes of these three donors were consistent with the data of sequencing.
available DNA (such as from Coriell Cell Repositories) from individuals of other ethnic groups to include in genotyping assay validations. The laboratory-developed high-resolution melting curve method is a medium throughput assay. The advantages of this real-time PCR method are its rapid PCR performance and the fact that the whole amplification and detection process is performed in a closed system, therefore minimizing the contamination risk. The software-operated calculation of melting points enhances the certainty of the results. Methods applied in screening for rare blood groups must show a high degree of robustness. The local genotype and allele distribution for all examined blood groups mirror the known occurrence in a Chinese population as described before.1,5 In summary, the combination of real-time PCR and melting curve analysis provides a useful tool for blood group determination. The genotyping process can be finished within 90 minutes after DNA purification. We suggest that this particular molecular methodology may be successfully adopted as a method for screening a large number of samples for rare blood groups, and we foresee the further extension of real-time PCR assays for routine genotyping.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Melting curve analysis (cases)</th>
<th>PCR-SSP (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do(a+b+)</td>
<td>DO<em>01/DO</em>02</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Do(a+b−)</td>
<td>DO<em>01/DO</em>01</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Do(a−b+)</td>
<td>DO<em>02/DO</em>02</td>
<td>273</td>
<td>273</td>
</tr>
<tr>
<td>Di(a+b+)</td>
<td>DI<em>01/DI</em>02</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Di(a+b−)</td>
<td>DI<em>01/DI</em>01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Di(a−b+)</td>
<td>DI<em>02/DI</em>02</td>
<td>292</td>
<td>292</td>
</tr>
<tr>
<td>Lu(a+b+)</td>
<td>LU<em>01/LU</em>02</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lu(a+b−)</td>
<td>LU<em>01/LU</em>01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lu(a−b+)</td>
<td>LU<em>02/LU</em>02</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Ss(S++)</td>
<td>GYPB<em>03/GYPB</em>04</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Ss(S−)</td>
<td>GYPB<em>03/GYPB</em>03</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ss(S−−)</td>
<td>GYPB<em>04/GYPB</em>04</td>
<td>290</td>
<td>290</td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction; SSP = sequence-specific primer.

### Acknowledgments

This work was financially supported by the Scientific Research Foundation of Chengdu Health Bureau (no. 201112).

### References


### Table 3. DO*01/DO*02, DI*01/DI*02, LU*01/LU*02, and GYPB*03/GYPB*04 distribution among China and Chengdu populations

<table>
<thead>
<tr>
<th>Area</th>
<th>DO*01</th>
<th>DO*02</th>
<th>DI*01</th>
<th>DI*02</th>
<th>LU*01</th>
<th>LU*02</th>
<th>GYPB*03</th>
<th>GYPB*04</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>0.1027 (292)</td>
<td>0.8973 (292)</td>
<td>0.0566 (299)</td>
<td>0.9990 (299)</td>
<td>0 (114)</td>
<td>1.0000 (114)</td>
<td>0.0225 (111)</td>
<td>0.9950 (111)</td>
</tr>
<tr>
<td>Chengdu†</td>
<td>0.0900 (300)</td>
<td>0.9100 (300)</td>
<td>0.0600 (300)</td>
<td>0.9400 (300)</td>
<td>0 (300)</td>
<td>1.0000 (300)</td>
<td>0.0333 (300)</td>
<td>0.9666 (300)</td>
</tr>
</tbody>
</table>

†These data were detected by the melting curve method.
Dear Colleague,

Grifols is pleased to announce the 2015 edition of its Transfusion Science Education Course (TSEC) to be offered in the USA. This intense two-day course focuses on the molecular and biochemical basis of newly discovered blood group systems, and analyzes the clinical application of serology and DNA, in mitigating the risk of red cell alloimmunization and platelet refractoriness. Interaction between internationally renowned lecturers and the audience, using radio frequency transmitters, provides a unique teaching-learning experience. The intended audience includes advanced level technologists, supervisors, transfusion medicine specialists, blood bank medical directors. Registration is free, however, space is limited. Grifols is approved as a provider of continuing education programs in the clinical laboratory sciences by the ASCLS P.A.C.E.® Program. This course awards 12.5 CE credits.

Locations hosting the 2015 TSEC are:
1. May 11 – 12 / Los Angeles, CA
2. August 17 – 18 / Indianapolis, IN
3. November 16 – 17 / Raleigh, NC

For registration and other information, please contact kelly.norton@external.grifols.com.

Best Regards,

GRIFOLS

Your attendance at these training courses is neither conditioned upon any obligation for you to use, recommend, promote or purchase Grifols products nor is it intended to influence you to do so.
Masters (MSc) in Transfusion and Transplantation Sciences  
at  
The University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2015 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following the MSc. The syllabus is organized jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

- Scientific principles of transfusion and transplantation
- Clinical applications of these principles
- Practical techniques in transfusion and transplantation
- Principles of study design and biostatistics
- An original research project

Application can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:  
http://ibgrl.blood.co.uk/MSc/MscHome.htm

For further details and application forms please contact:

Dr Patricia Denning-Kendall  
University of Bristol  
Paul O’Gorman Lifeline Centre  
Department of Pathology and Microbiology  
Southmead Hospital  
Westbury-on-Trym, Bristol BS10 5NB, England  
Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: p.a.denning-kendall@bristol.ac.uk.
Online Specialist in Blood Bank (SBB)
Certificate and Masters in Clinical Laboratory
Management Program
Rush University
College of Health Sciences

Continue to work and earn graduate credit while the Rush University SBB/MS program prepares you for the SBB exam and the Diplomat in Laboratory Management (DLM) exam given by ASCP Board of Certification! (Please note acceptable clinical experience is required for these exams.)

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 2015. For additional information and requirements, please visit our website at: www.rushu.rush.edu/cls/

Rush University is fully accredited by the Higher Learning Commission (HLC) of the North Central Association of Colleges and Schools and the SBB Certificate Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP).

Applications for the SBB/MS Program can be submitted online at the following website: http://www.rushu.rush.edu/admiss/hlthadm.html

Contact: Yolanda Sanchez, MS, MLS(ASCP) SBB, Director, by email at Yolanda_Sanchez@rush.edu or by phone at 312-942-2402 or Denise Harmening, PhD, MT(ASCP), Director of Curriculum by email at Denise_Harmening@rush.edu
The Johns Hopkins Hospital Specialist in Blood Bank Technology Program

The Johns Hopkins Hospital was founded in 1889. It is located in Baltimore, Maryland, on the original founding site, just 45 minutes from Washington, DC. There are approximately 1,000 inpatient beds and another 1,200 outpatient visits daily; nearly 600,000 patients are treated each year.

The Johns Hopkins Hospital Transfusion Medicine Division is one of the busiest in the country and can provide opportunities to perform tasks that represent the entire spectrum of Immunohematology and Transfusion Medicine practice. It provides comprehensive support to all routine and specialized areas of care for surgery, oncology, cardiac, obstetrics, neonatal and pediatric, solid organ and bone marrow transplant, therapeutic apheresis, and patients with hematological disorders to name a few. Our intradepartment Immunohematology Reference Laboratory provides resolution of complex serologic problems, transfusion management, platelet antibody, and molecular genotype testing.

The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is an onsite work-study, graduate-level training program for certified Medical Technologists, Medical Laboratory Scientists, and Technologists in Blood Banking with at least two years of full-time Blood Bank experience.

The variety of patients, the size, and the general intellectual environment of the hospital provide excellent opportunities for training in Blood Banking. It is a challenging program that will prepare competent and knowledgeable graduates who will be able to effectively apply practical and theoretical skills in a variety of employment settings. The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP). Please visit our website at http://pathology.jhu.edu/department/divisions/transfusion/sbb.cfm for additional information.

Contact: Lorraine N. Blagg, MA, MLS(ASCP)CMSBB
Program Director
E-mail: lblagg1@jhmi.edu
Phone: (410) 502-9584

The Johns Hopkins Hospital
Department of Pathology
Division of Transfusion Medicine
Sheikh Zayed Tower, Room 3100
1800 Orleans Street
Baltimore, Maryland 21287

Phone (410) 955-6580
Fax (410) 955-0618
Web site: http://pathology.jhu.edu/department/divisions/transfusion/index.cfm
Reference and Consultation Services

Antibody identification and problem resolution

- HLA-A, B, C, and DR typing
- HLA-disease association typing
- Paternity testing/DNA

For information, contact:
Mehdizadeh Kashi
at (503) 280-0210
or write to:
Tissue Typing Laboratory
American Red Cross Biomedical Services
Pacific Northwest Region
3131 North Vancouver
Portland, OR 97227

CLIA licensed, ASHI accredited

IgA/Anti-IgA Testing

IgA and anti-IgA testing are available to do the following:
- Identify IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

Our ELISA for IgA detects protein to 0.05 mg/dL.

For additional information contact:
Cynthia Flickinger at (215) 451-4909
or e-mail:
Cynthia.Flickinger@redcross.org
or write to:
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Cynthia Flickinger

IgA/Anti-IgA Testing

- Effective tool for screening large volumes of donors
- Gel diffusion test that has a 15-year proven track record:
  Approximately 90 percent of all donors identified as IgA deficient by this method are confirmed by the more sensitive testing methods

For additional information:
Kathy Kaherl
at (860) 678-2764
e-mail:
Katherine.Kaherl@redcross.org
or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Ave.
Farmington, CT 06032

National Reference Laboratory for Blood Group Serology

Immunohematology Reference Laboratory
AABB, ARC, New York State, and CLIA licensed
24-hour phone number:
(215) 451-4901
Fax: (215) 451-2538

American Rare Donor Program
24-hour phone number:
(215) 451-4900
Fax: (215) 451-2538
ardp@redcross.org

Immunohematology
Phone, business hours:
(215) 451-4902
Fax: (215) 451-2538
immuno@redcross.org

Quality Control of Cryoprecipitated-AHF
Phone, business hours:
(215) 451-4903
Fax: (215) 451-2538
Advertisements, cont.

The Discovery and Significance of the Blood Groups

Marion Reid
Ian Shine

A must for the bookshelf of every blood bank.
A superb and beautiful book.

John Gorman MD,
Lasker Award 1980

The authors convey the excitement of scientific discovery so effectively on every page.

S. Gerald Sandler, MD
Professor of Medicine and Pathology,
Georgetown University Hospital

It's more like reading an eye-witness account than a scientific textbook. Wonderfully readable, and a great addition to the books on blood groups.

Phyllis Walker, MT(ASCP)SBB,
San Francisco

Marion E. Reid
Christine Lomas-Francis

BLOOD GROUP ANTIGENS & ANTIBODIES

A guide to clinical relevance
Technical tips

A landmark book . . . Every MD and clinical transfusion service should have their own personal copy.

Sandra J. Nance

The most valued and useful resource in my blood banking and immunohematology library . . . it is highly readable and an enjoyable, painless way to update your information about blood group antigens.

Immunohematology

Blood Group Antigens & Antibodies
by Marion Reid & Christine Lomas-Francis
Paperback/5.4" x 8.4"/214 pp./$25.00
ISBN 978-1-59572-103-7

AVAILABLE NOW! To order, call 718-784-9112 or visit www.bloodgroups.info www.sbbpocketbook.com
Diagnostic testing for:
- Neonatal alloimmune thrombocytopenia (NAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

Test methods:
- GTI systems tests
  - detection of glycoprotein-specific platelet antibodies
  - detection of heparin-induced antibodies (PF4 ELISA)
- Platelet suspension immunofluorescence test (PSIFIT)
- Solid phase red cell adherence (SPRCA) assay
- Monoclonal immobilization of platelet antigens (MAIPA)
- Molecular analysis for HPA-1a/1b

For further information, contact:
Platelet Serology Laboratory (215) 451-4205
Cynthia Flickinger (215) 451-4909
Cynthia.Flickinger@redcross.org
Sandra Nance (215) 451-4362
Sandra.Nance@redcross.org

American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

National Neutrophil Serology Reference Laboratory

Our laboratory specializes in granulocyte antibody detection and granulocyte antigen typing.

Indications for granulocyte serology testing include:
- Alloimmune neonatal neutropenia (ANN)
- Autoimmune neutropenia (AIN)
- Transfusion-related acute lung injury (TRALI)

Methodologies employed:
- Granulocyte agglutination (GA)
- Granulocyte immunofluorescence by flow cytometry (GIF)
- Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:
- HLA (PRA) Class I and Class II antibody detection

For further information, contact:
Neutrophil Serology Laboratory (651) 291-6797
Randy Schuller (651) 291-6758
Randy.Schuller@redcross.org

American Red Cross Biomedical Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107

CLIA licensed
What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services
Managers of Blood Centers
LIS Coordinators
Educators
Supervisors of Reference Laboratories
Research Scientists
Consumer Safety Officers
Reference Lab Specialists
Quality Assurance Officers
Technical Representatives

Why become an SBB?

- Professional growth
- Job placement
- Job satisfaction
- Career advancement

How does one become an SBB?

- Attend a CAAHEP-accredited SBB Technology program OR
- Sit for the examination based on criteria established by ASCP for education and experience.

However: In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam.

Conclusion: The BEST route for obtaining an SBB certification is . . . to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

Contact the following programs for more information:
A. For describing an allele which has not been described in a peer-reviewed publication and for which an allele name or provisional allele name has been assigned by the ISBT Working Party on Blood Group Allele Terminology (http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/)

B. Preparation

1. Title: Allele Name (Allele Detail)
   ex. RHCE*01.01 (RHCE*ce48C)

2. Author Names (initials and last name of each (no degrees, ALL CAPS)

C. Text

1. Case Report
   i. Clinical and immunohematologic data
   ii. Race/ethnicity and country of origin of proband, if known

2. Materials and Methods
   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 patient names or hospital numbers.

3. Results
   Complete the Table Below:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Allele Name</th>
<th>Nucleotide(s)</th>
<th>Exon(s)</th>
<th>Amino Acid(s)</th>
<th>Allele Detail</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>e weak</td>
<td>RHCE*01.01</td>
<td>48G&gt;C</td>
<td>1</td>
<td>Trp16Cys</td>
<td>RHCE*ce48C</td>
<td>1</td>
</tr>
</tbody>
</table>

   Column 1: Describe the immunohematologic phenotype (ex. weak or negative for an antigen).
   Column 2: List the allele name or provisional allele name.
   Column 3: List the nucleotide number and the change, using the reference sequence (see ISBT Blood Group Allele Terminology Pages for reference sequence ID).
   Column 4: List the exons where changes in nucleotide sequence were detected.
   Column 5: List the amino acids that are predicted to be changed, using the three-letter amino acid code.
   Column 6: List the non-consensus nucleotides after the gene name and asterisk.
   Column 7: If this allele was described in a meeting abstract, please assign a reference number and list in the Reference section.

4. Additional Information
   i. Indicate whether the variant is listed in the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/); if so, provide rs number and any population frequency information, if available.
   ii. Indicate whether the authors performed any population screening and if so, what the allele and genotype frequencies were.
   iii. Indicate whether the authors developed a genotyping assay to screen for this variant and if so, describe in detail here.
   iv. Indicate whether this variant was found associated with other variants already reported (ex. RHCE*ce48C,1025T is often linked to RHD*DNa-2)

D. Acknowledgments

E. References

F. Author Information
   List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable.
I. GENERAL INSTRUCTIONS
Before submitting a manuscript, consult current issues of Immunohematology for style. Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. 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
   8. Figures

B. Preparation of manuscript
   1. Title page
      a. Full title of manuscript with only first letter of first word capitalized (bold title)
      b. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
      c. Running title of ≤40 characters, including spaces
      d. Three to ten key words
   2. Abstract
      a. One paragraph, no longer than 300 words
      b. Purpose, methods, findings, and conclusion of study
   3. Key words
   4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
      a. Introduction — Purpose and rationale for study, including pertinent background references
      b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
      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 patient’s names or hospital numbers.
      d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
      e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
   5. Acknowledgments: Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
   6. References
      a. In text, use superscript, Arabic numbers.
      b. Number references consecutively in the order they occur in the text.
   7. Tables
      a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of…) use no punctuation at the end of the title.
      b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
      c. Place explanation in footnotes (sequence: *, †, ‡, §, **, ††).
   8. Figures
      a. Figures can be submitted either by e-mail or as photographs (5 ×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, place first author’s name and figure number on back of each glossy submitted.
      c. When plotting points on a figure, use the following symbols if possible:
         \( \bullet \ \cdot \ \triangle \ \square \ ).
   9. Author information
      a. List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable. Provide e-mail addresses of all authors.

III. EDUCATIONAL FORUM
A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:
   1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
   2. Annotated conference proceedings
B. Preparation of manuscript
   1. Title page
      a. Capitalize first word of title.
      b. Initials and last name of each author (no degrees; all CAPs)
   2. Text
      a. Case should be written as progressive disclosure and may include the following headings, as appropriate
         i. Clinical Case Presentation: Clinical information and differential diagnosis
         ii. Immunohematologic Evaluation and Results: Serology and molecular testing
         iii. Interpretation: Include interpretation of laboratory results, correlating with clinical findings
         iv. Recommended Therapy: Include both transfusion and nontransfusion-based therapies
         v. Discussion: Brief review of literature with unique features of this case
         vi. Reference: Limited to those directly pertinent
         vii. Author information (see II.B.9.)
         viii. Tables (see II.B.7.)
   3. Key words
   4. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR
A. Preparation
   1. Heading (To the Editor)
   2. Title (first word capitalized)
   3. Text (written in letter [paragraph] format)
   4. Author(s) (type flush right; for first author: name, degree, institution, address [including city, state, Zip code and country]; for other authors: name, degree, institution, city and state)
   5. References (limited to ten)
   6. Table or figure (limited to one)

Send all manuscripts by e-mail to immuno@redcross.org
From the publishers of *Immunohematology*

A

**Comprehensive Laboratory Manual**

Featuring—

- Over 100 methods—just about every method used in a reference lab.
- Eleven chapters discussing problems faced by blood group serologists and the procedures and methods that can be used to solve them.
- An extra set of the methods to use at the bench, printed on durable waterproof paper.
- See business reply order card enclosed in this issue or order on the Web at redcross.org/immunohematology