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Sero logic Method Review

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Review

Proceedings from the International Society of Blood Transfusion Working Party on Immunohaematology Workshop on the Clinical Significance of Red Blood Cell Alloantibodies, Friday, September 2, 2016, Dubai

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Management of pregnancy sensitized with anti-Inb with monocyte monolayer assay and maternal blood donation
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To the Editor

The devil is in the details: retention of recipient group A type 5 years after a successful allogeneic bone marrow transplant from a group O donor
L.L.W. Cooling, M. Herrst, and S.L. Hugan
A Wheatfield on a Summer's Afternoon is a backdrop, one of four that Marc Chagall executed in 1942 for the premier of Rachmaninoff's Carmenesque ballet, Aleko, based on the Pushkin poem, The Gypsies. Both the ballet and the poem confront the romantic notion of the “noble savage” who rejects an oppressive society for a truer life apart from it. Aleko, the hero, is a fugitive who, despite his yearning for sanctuary, brings with him a violence and rigidity of thought that drives the narrative to its tragic end. Chagall’s four works largely fail to match events on stage, instead finding within themselves similar primal elements. Here, the sickle in the grain, the fish out of water beneath it, the two suns: one childish, the other a bulls-eye target, and the white branch falling upside down near the couple in the boat whom fish surround, create a wildly liberated dream-like idiom, absurdly comic, and yet tender in its tone. The article by Wilson et al. in this issue of Immunohematology addresses the recovery of autologous sickle cells by hypotonic wash.

David Moolten, MD
Warm autoadsorption using ZZAP

F.M. Tsimba-Chitsva, A. Caballero, and B. Svatora

The masking of clinically significant alloantibodies by warm autoantibodies presents challenges in pretransfusion testing. The adoption of transfusion practices such as the issuing of “least incompatible” red blood cells (RBCs) without a complete antibody workup is potentially unsafe for patients. Several autoadsorption methods can be used to remove autoantibody reactivity. ZZAP treatment of autologous RBCs is an efficient way to prepare the cells for autoadsorption. Autoadsorbed serum or plasma can then be used to remove autoantibody reactivity and identify clinically significant alloantibodies. *Immunohematology* 2018;34:1–3.

Key Words: warm autoadsorption, warm autoantibodies, ZZAP-treated RBCs, autoimmune hemolytic anemia

Principle

Warm autoantibodies (WAAs) present serologic challenges in pretransfusion compatibility testing. These autoantibodies, which are optimally reactive at 37°C, may mask the presence of clinically significant alloantibodies by agglutinating most or all red blood cells (RBCs) tested. Studies have shown that the incidence of clinically significant alloantibodies is higher in patient sera containing WAAs than in multiply transfused patients without autoimmune hemolytic anemia (AIHA).1–4 Some blood banks have implemented the policy of transfusing “least incompatible” non-phenotypically matched RBCs, and others transfuse least incompatible RBCs that are phenotypically matched for either the full phenotype or only for the common antigens in the Rh blood group system and for K. The transfusion of least incompatible non-phenotypically matched units without exclusion of other alloantibodies poses a major risk to patients with WAAs because hemolysis secondary to undetected alloantibodies can be falsely attributed to an increasing severity of AIHA.2

Some blood banks have implemented the policy of transfusing “least incompatible” non-phenotypically matched RBCs, and others transfuse least incompatible RBCs that are phenotypically matched for either the full phenotype or only for the common antigens in the Rh blood group system and for K. The transfusion of least incompatible non-phenotypically matched units without exclusion of other alloantibodies poses a major risk to patients with WAAs because hemolysis secondary to undetected alloantibodies can be falsely attributed to an increasing severity of AIHA.2

The detection of clinically significant alloantibodies can be achieved through the removal of autoantibody from the patient’s plasma or serum. Autoantibody removal can be achieved by adsorbing the plasma or serum onto autologous RBCs (provided the patient has not been transfused in the last 3 months).5 Alternatively, if the patient has been recently transfused or autologous RBCs are limited, allogeneic RBC adsorption may be performed.5–7 Autologous adsorption is preferred because of the risk of adsorbing an alloantibody to a high-prevalence antigen onto allogeneic adsorbing RBCs.

Indications

To effectively achieve the autoadsorption of WAAs, initial preparation of the patient’s RBCs is required. In vivo adsorptions occur at 37°C, and all the antigen sites on the patient’s RBCs may be blocked with immunoglobulin.5 Methods for immunoglobulin dissociation may include partial heat elution at 45°C, gentle heat elution at 56°C, treatment with proteolytic enzymes (papain and/or ficin), treatment with chloroquine diphosphate, or treatment with a ZZAP reagent (mixture of a proteolytic enzyme and the sulfhydryl reagent dithiothreitol [DTT]).5,8

### Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
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<tbody>
<tr>
<td>0.2 M DTT</td>
<td>1-mL graduated pipettes</td>
</tr>
<tr>
<td>1% cysteine-activated papain or 1% ficin</td>
<td>37°C water bath</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>Large-bore test tubes</td>
</tr>
<tr>
<td>Patient’s autologous RBCs</td>
<td>Calibrated centrifuge</td>
</tr>
<tr>
<td>DTT = dithiothreitol; RBCs = red blood cells.</td>
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</table>

### Procedural Steps

RBC Treatment

- Mix 2 volumes of ZZAP and 1 volume of packed RBCs.
- Incubate mixture.
- Wash to remove ZZAP.
- Centrifuge to pack RBCs.
- Remove supernatant.

Adsorption

- Mix serum/plasma and ZZAP-treated packed RBCs.
- Incubate mixture.
- Centrifuge to pack RBCs.
- Harvest serum/plasma.

RBCs = red blood cells.
Treatment of the adsorbing RBCs with ZZAP has been shown to be one of the most effective methods for pretreating adsorption RBCs. It simultaneously removes bound IgG and enhances autoantibody uptake during the adsorption process. In addition to removing IgG from coated RBCs, ZZAP also removes bound IgM and complement (approximately 35% of patients with WAAs present with cold autoantibodies reactive at room temperature).

ZZAP will destroy some antigen sites on RBCs. Enzymes remove sialic acid from the RBCs and will destroy some antigens, including M, N, S, s, Fy\(^a\), Fy\(^b\), En\(^a\), Ge, JMH, Ch/Rg, and In\(^b\). DTT will alter Yt\(^a\), JMH, Kn\(^a\), McC\(^a\), Yk\(^a\), LW\(^a\), and LW\(^b\), as well as all Kell, Lutheran, Dombrock, and Cromer blood group antigens. Alloantibodies and autoantibodies with a specificity to these altered antigens will remain in the plasma or serum after the adsorption process with ZZAP-treated RBCs.

The number of adsorptions needed to adequately remove the autoantibody usually correlates with the strength of WAA reactivity against panel RBCs. In some instances, additional adsorptions may be necessary.

**Procedure**

**Preparation of ZZAP**

Combine 0.5 mL of 1 percent cysteine-activated papain, 2.5 mL of 0.2 M DTT, and 2 mL of pH 7.3 phosphate-buffered saline. In place of papain, 1 mL of 1 percent ficin can be combined with 2.5 mL of 0.2 M DTT and 1.5 mL of pH 7.3 phosphate-buffered saline. If the enzyme used to make ZZAP is prepared in-house, the stock solution must have the reactivity and incubation time standardized by the in-house procedure after each preparation. If using a commercially prepared enzyme solution, follow the manufacturer’s directions.

**RBC Treatment**

RBCs may be unwashed before ZZAP treatment, but 1 mL packed RBCs must be obtained. Combine packed RBCs and ZZAP at a 1:2 ratio, mixing well (e.g., 1 mL packed RBCs to 2 mL ZZAP). Incubate the mixture for 30 minutes at 37°C, mixing periodically. After incubation, wash the RBCs at least three times with saline to remove the ZZAP (additional washes may be necessary to obtain a clear supernatant). The final wash is centrifuged a minimum of 5 minutes at 900–1000g to pack the RBCs and allow for removal of as much saline as possible. This step is important, since excess residual saline may dilute the plasma or serum during adsorption. Suctioning with a small-bore pipette or using filter paper may be helpful.

**Autologous Adsorption**

Combine plasma or serum with ZZAP-treated autologous RBCs at a 1:1 ratio (equal volumes of both); the volume of ZZAP-treated autologous RBCs may be increased, however, to enhance antibody uptake. Incubate the mixture at 37°C for 10–60 minutes in a water bath, mixing periodically.

After incubation, centrifuge the mixture to pack the RBCs. Carefully remove as much adsorbed plasma or serum as possible without disturbing the packed RBCs. After removing the adsorbed plasma or serum, discard the adsorbing RBCs; additional autologous adsorptions should be performed using a fresh aliquot of ZZAP-treated autologous RBCs.

The number of autologous adsorptions needed to remove the original reactivity generally correlates with the strength of reactivity observed with reagent panel RBCs tested by an indirect antiglobulin test with any test method. Generally, 1+ reactivity will be removed with one autologous adsorption, 2+ reactivity removed with two autologous adsorptions, and so on. Additional autologous adsorptions may be necessary regardless of this guideline.

Adsorbing more than four times should be avoided to prevent dilution of underlying alloantibodies. It is good practice to test the adsorbed plasma or serum after the first autologous adsorption (if original 1+ reactivity) or after the second autologous adsorption (if original 2+ reactivity) against group O reagent panel RBCs or direct antiglobulin test–negative autologous RBCs to determine the need for additional adsorptions. If reactivity remains, perform additional autologous adsorptions until the extra reactivity is removed. Once the autoantibody is fully adsorbed, the adsorbed plasma or serum can then be tested against any panel RBCs to investigate for underlying alloantibodies.

**Limitations**

Autologous adsorptions should not be performed when the sample to be tested consists of a mixture of transfused and autologous RBCs. If a patient has been transfused within the last 3 months, transfused RBCs may adsorb a clinically significant alloantibody as well as the WAA. Known transfusion history of the patient is thus of great importance.

In addition to determining the patient’s transfusion history, the volume of patient RBCs should be assessed. Patients who have a low hematocrit may not be able to provide
enough RBCs to perform adequate autologous adsorptions. In these cases, the use of allogeneic RBCs may be used in lieu of autologous RBCs.\textsuperscript{1}

Adsorptions, whether autologous or allogeneic, can dilute a weak-reactive alloantibody to undetectable levels. For every adsorption, the dilution factor increases. It is imperative to completely remove saline after the final wash to reduce the risk of alloantibody dilution.

The use of ZZAP can have limitations. If the autoantibody is specific to a DTT- or enzyme-sensitive antigen, it will not be adsorbed out and can continue to mask alloantibodies.\textsuperscript{11}

\textbf{Acknowledgments}

The authors thank Kelly Kezeor, MT(ASCP), Document Developer—Hospital Program Coordinator, American Red Cross, Midwest Region, for providing technical assistance and expertise and for her critical reading and editing of the manuscript.

\textbf{References}


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A brief overview of clinical significance of blood group antibodies

M.J. Gandhi, D.M. Strong, B.I. Whitaker, and E. Petrisli

This review was derived from a presentation made on September 2, 2016 for the first Academy Day presented by the Working Party on Immunohematology at the International Society of Blood Transfusion (ISBT) Congress in Dubai. The focus of this review is to provide a brief overview of the clinical significance of blood group antibodies. Blood group antibodies can be naturally occurring (e.g., anti-A and anti-B through exposure to naturally occurring red blood cell [RBC] antigen-like substances) or can occur via exposure to foreign (donor) RBC antigens through previous transfusions, transplants, or exposure to fetal RBCs during or after pregnancy. However, not all blood group antibodies are clinically significant. Clinically significant blood group antibodies can cause adverse events after blood component transfusion or transplantation and/or can cause hemolytic disease of the fetus and newborn. Immunohematology 2018;34:4–6.

Key Words: blood group antibodies, alloantibodies, autoantibodies, hemolytic transfusion reactions

Red blood cells (RBCs) carry numerous protein and carbohydrate antigens on their surface. In 2016, the International Society of Blood Transfusion recognized 346 RBC antigens.1 These antigens can provoke an immune response that may lead to the development of antibodies. Such antibodies, directed at RBC antigens, can be produced through exposure to naturally occurring RBC antigen-like substances or exposure to foreign (donor) RBC antigens through previous transfusions, transplants, or exposure to fetal cells during or after pregnancy. Naturally occurring anti-A and anti-B are the only RBC antibodies normally found in human serum or plasma. All other antibodies are unexpected and can be divided into alloantibodies (an antibody to an antigen that an individual lacks) and autoantibodies (an antibody to an individual’s own antigen[s]). The role of blood group antibodies in blood transfusion, transplantation, hemolytic disease of the fetus and newborn (HDFN), and, in some cases, hemolytic anemia are reviewed in previous publications and summarized here.2–5

Blood Component Transfusion

The presence of antibodies directed against the blood group antigens in the transfused component (major incompatibility) or antibodies in the transfused product directed against the recipient’s blood group antigens (minor incompatibility) may lead to the destruction of RBCs and manifest as a hemolytic transfusion reaction (HTR). In HTRs, hemolysis may be intravascular or extravascular.

Intravascular HTR occurs rapidly, with cell destruction within a few minutes. This event is caused by IgM antibodies that activate the complement pathway, which leads to formation of a membrane-activating complex that punctures the RBC membrane and subsequently releases hemoglobin into the plasma, with severe signs and symptoms of hemolysis (described subsequently). The most common antibodies causing intravascular HTR are of the ABO blood group system (anti-A, anti-B, and anti-AB), but antibodies of other blood group systems have also been occasionally implicated.

Extravascular HTRs can be immediate (occurring within a few hours of transfusion) or delayed (occurring within a few days of transfusion) and are caused by IgG antibodies that do not bind complement (e.g., antibodies to Rh system antigens), or that bind complement at levels that do not lead to the formation of membrane activating complex (e.g., antibodies to Kidd or Duffy system antigens). RBCs coated with IgG or C3b are phagocytized by the macrophages in the spleen or sequestered by the liver macrophages. Usually, immediate extravascular HTRs occur in the presence of antibodies to ABO blood group antigens and have similar signs as intravascular HTRs but are milder.

Delayed transfusion reactions (DTRs) usually occur in patients who were previously alloimmunized to an antigen but whose antibody titers have dropped to levels not detected by serologic tests or to levels insufficient to produce immediate hemolysis. In DTRs, the presence of the antigen in the
transfused product results in an anamnestic response within a few days, resulting in the clearance of the donor’s RBCs. These reactions may present with no clinical signs and may only be detected by serologic methods (delayed serologic transfusion reactions) or as delayed hemolytic transfusion reactions (DHTRs) with mild signs and few symptoms. These reactions may also result in fever, fall in hemoglobin levels, jaundice, and hemoglobinuria. In some cases, immune destruction of transfused RBCs may have no obvious pathological effects, yet these are detrimental because they reduce the efficacy of the transfusion. Severe manifestations (e.g., disseminated intravascular coagulation, renal failure) are rarely seen.

**Destruction of Autologous RBCs (Autoimmune Hemolytic Anemias)**

Autoimmune hemolytic anemias are a group of disorders in which the malfunction of the body’s immune system leads to development of antibodies against the individual’s own RBCs (autologous) and may lead to destruction of RBCs with release of hemoglobin into the plasma. These disorders can be idiopathic or secondary to a disease (e.g., systemic lupus erythematosus, lymphoma) or to drugs (e.g., alpha methyldopa, penicillin), the mechanisms of which are poorly understood and are reviewed elsewhere.6–10 These antibodies frequently react with all RBCs tested, although in some cases, specificity to antigens of high prevalence can be determined. Certain clinical conditions may also be associated with autoantibodies (e.g., antibodies to the I antigen after viral infection or autoantibodies to the P antigen in paroxysmal cold hemoglobinuria).

**Transplantation**

Many blood group antigens are expressed on tissues other than RBCs. ABO antigens are expressed on most tissues and, because anti-A and anti-B occur naturally in the plasma, these antigens play a major role in transplantation. In solid organ transplantation, antibodies in the recipient directed at donor antigens result in immune reactions that may cause rejection of the transplanted organ. In hematopoietic stem cell transplantation (HSCT), hematopoietic cells can be transplanted from a donor of any blood group to a recipient of any blood group. Consequently, HSCT can result in a matched transplant from an ABO-compatible donor or a mismatched transplant from a donor with antigens to which the recipient has antibodies (major mismatch), from a donor who will produce antibodies to the recipient’s RBCs (minor mismatch), or from a bidirectional mismatch in which both the donor’s and recipient’s RBCs produce antibodies against each other’s RBCs. In a mismatched HSCT, the donor’s hematopoietic cells will give rise to a new blood group in the recipient. Such mismatched HSCTs can result in immediate hemolysis at the time of graft infusion (more common with marrow transplants containing large amounts of RBCs) or delayed complications, such as hemolysis at the time of engraftment, pure RBC aplasia, delayed engraftment, or failure to engraft because of the presence of ABO antibodies or passenger lymphocytes.11

**Hemolytic Disease of the Fetus and Newborn**

HDFN is a potentially fatal alloimmune reaction caused by maternal antibodies that cross the placenta to the fetus, where they initiate immune destruction of RBCs in utero or during the neonatal period. These maternal antibodies are restricted to IgG because antibodies of other classes are not transported across the placental barrier. Maternal antibodies may have developed in response to prior exposure to paternal antigens [e.g., anti-D due to exposure of D− mother to a D+ fetus] or through previous blood transfusions [e.g., anti-K and -Jkα in a K−, Jk(a−) woman]. Clinically, the severity of HDFN varies from mild neonatal jaundice that can be treated by phototherapy, to severe in utero anemia requiring intrauterine and/or post-delivery transfusions, to fetal or neonatal death.

**Classifying RBC Antibodies**

RBC antibodies can be directed at any of the more than 300 known RBC antigens, although not all of them may cause the reactions just discussed. One clinical approach is to classify these antibodies into four categories: (1) clinically significant antibodies; (2) clinically insignificant antibodies; (3) antibodies that are clinically insignificant unless they are reactive at 37°C; and (4) antibodies whose clinical significance is unknown or that have occasional case reports of being clinically significant (Table 1). For evaluation of antibodies in this last category, curated literature can be found at the Notify Library Web site (www.notifylibrary.org), an online publically accessible database of adverse outcomes collected and analyzed by editorial groups of international experts, regulators, and clinicians.12
Table 1. Selected RBC antibodies and their clinical significance

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tr>
<td>Clinically significant</td>
<td>Clinically insignificant</td>
<td>Clinically significant when reactive at 37°C</td>
<td>Unknown or variable clinical significance</td>
</tr>
<tr>
<td>ABO</td>
<td>Chi, Rg</td>
<td>Le, Le</td>
<td>Yt</td>
</tr>
<tr>
<td>D, C, c, E, e</td>
<td>Xg</td>
<td>M, N</td>
<td>Gy</td>
</tr>
<tr>
<td>K, k</td>
<td>Os</td>
<td>P1</td>
<td>Hy</td>
</tr>
<tr>
<td>Fy, Fy</td>
<td>Kn</td>
<td>Lu</td>
<td>Sd</td>
</tr>
<tr>
<td>Jk, Jk</td>
<td>McC, Yk</td>
<td>A1</td>
<td>Ge</td>
</tr>
<tr>
<td>S, s</td>
<td>JMH</td>
<td>Bg</td>
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</tr>
<tr>
<td>Vel</td>
<td>Lu</td>
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Agreement does not exist in the literature for all antigens in all categories, and this list is not inclusive of all alloantibodies to blood group antigens. RBC = red blood cell.

Incidence of RBC Alloimmunization, HTRs, and Role of Hemovigilance

It is estimated that RBC antibodies occur in less than 0.3 percent of normal blood donors, although the rate of alloimmunization increases in hospitalized patients who have received a blood transfusion. This rate is significantly higher in certain patient populations (e.g., transfusion-dependent patients with sickle cell disease or thalassemia). Alloimmunization was shown to be the third leading cause of transfusion-associated deaths in the United States and the UK. Data are generated from ongoing hemovigilance studies reported by various national agencies such as the Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA), the UK’s Serious Hazards of Transfusion (SHOT) annual report, and the Hemovigilance Activity Report of the French National Agency for Medicine and Health Product Safety. The Notify Library has only recently begun to add cases from the literature and reporting authorities; nevertheless, it serves as a ready resource for reviewing alloimmunization related to RBC antigens resulting from transfusion, pregnancy, and transplantation.

References

18. The Notify Library has only recently begun to add cases from the literature and reporting authorities; nevertheless, it serves as a ready resource for reviewing alloimmunization related to RBC antigens resulting from transfusion, pregnancy, and transplantation.

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Management of pregnancy sensitized with anti-In\textsuperscript{b} with monocyte monolayer assay and maternal blood donation

R. Shree, K.K. Ma, L.S. Er and M. Delaney

Maternal red blood cell (RBC) alloantibodies can cause hemolytic disease of the fetus and newborn (HDFN). Although much is described about common antibodies associated with HDFN, management of a pregnancy complicated by a maternal rare antibody presents several challenges related to assessment of fetal anemia risk, availability of blood for transfusion to the mother and/or the fetus or newborn if needed, and planning for delivery in the case of maternal hemorrhage. Here we report the laboratory medicine workup of a patient who presented for obstetrical care in the United States in the third trimester and had a rare antibody (anti-In\textsuperscript{b}). Prenatal antibody detection testing demonstrated maternal anti-In\textsuperscript{b} in a 28-year-old woman (gravida 4 para 1021). Ultrasound could not rule out fetal anemia. Monocyte monolayer assay was performed to assess for the clinical significance of the anti-In\textsuperscript{b} and revealed that the antibody may be capable of causing accelerated clearance of antigen-positive RBCs. A local and national query revealed that no appropriate units of RBCs were available for either the mother or neonate. Given this information, serial maternal autologous blood donations were performed, and a comprehensive care plan with a multidisciplinary approach for delivery, neonatal management, and preparation for delivery in the case of maternal hemorrhage was developed. Published data and our experience suggest that maternal blood donation appears to be a safe and effective way to manage mothers who cannot safely use the community blood supply. Involvement of obstetric, transfusion medicine, anesthesia, and neonatology providers was imperative for a favorable outcome. The antibody did not cause clinically significant anemia in this infant. Immunohematology 2018;34:7–10.

Key Words: blood groups, hemolytic disease of the fetus and newborn, red blood cell antibody

Maternal non-D red blood cell (RBC) alloantibodies are detected in 1.5–2.5 percent of pregnancies and can cause hemolytic disease of the fetus and newborn (HDFN).\textsuperscript{1} The risk of HDFN is not uniform for all antibodies; thus, management varies based on identification of the antibody and its strength (titer). Because transplacental transfer of antibodies is necessary to cause hemolysis of fetal cells, IgG antibodies (and not IgM antibodies) are implicated in HDFN. Although much is described about common antibodies associated with HDFN (anti-K, -c, -E, -Fy\textsubscript{a}, -Fy\textsubscript{b}, etc.), management of a pregnancy complicated by a maternal rare antibody presents several challenges related to assessment of fetal anemia risk, availability of blood for transfusion to the mother and/or the fetus or newborn if needed, and planning for delivery in the case of maternal hemorrhage.

Here we report the laboratory medicine workup of a patient who presented for obstetrical care in the United States in the third trimester and had a rare antibody (anti-In\textsuperscript{b}). This case is unique in that advanced laboratory methodologies were used to identify the rare antibody and assess for the potential risk of HDFN, given very limited published data. We also describe successful serial maternal autologous blood donations for banking and multidisciplinary preparation for possible hemorrhage at the time of repeat cesarean delivery.

Case Report

A 28-year-old Bangladeshi woman (gravida 4 para 1021) at 28 weeks of gestation presented to establish obstetrical care with a generalist obstetrician, at which time she was found to have a positive antibody detection test. She had previously delivered a full-term infant via primary cesarean delivery in Bangladesh due to fetal distress negating a vaginal delivery; no complications were noted. Her subsequent pregnancies included an early first-trimester miscarriage and an ectopic pregnancy treated with methotrexate. She had the same partner for all pregnancies and denied a history of transfusion. Prenatal care occurred in Bangladesh until 28 weeks of gestation, and records (including laboratory evaluations) were not available for review.

Immunohematologic Evaluation and Results

At her first obstetrical visit in the United States, her blood type was found to be group A, D+. The antibody detection test was positive with all screening cells (Immucor, Norcross, GA) at the antihuman globulin phase of testing (Anti-IgG; Ortho Clinical Diagnostics, Raritan, NJ). An antibody identification panel was performed by tube indirect antiglobulin test (IAT) using reagent panel RBCs (Immucor), ficin-treated RBCs (Immucor) as well as dithiothreitol (DTT)-treated (Sigma,
St. Louis, MO) reagent RBCs. The patient’s plasma was found to react with all reagent panel RBCs tested except those that were treated with ficin or DTT. The patient’s plasma was negative with her own RBCs (autocontrol), and the direct antiglobulin test (DAT) was negative using polyspecific AHG (Anti-IgG–C3b,C3d; Immucor). All alloantibodies to common RBC antigens were excluded using the ficin- and DTT-treated panel RBCs. To confirm the possibility of an antibody against a high-prevalence antigen, the laboratory verified the patient’s ethnicity to be of Southeast Asian descent. Knowing the serologic characteristics of the antibody and ethnic background of the patient, preliminary deduction of the antibody was thought to be anti-In^b, an antibody to a high-prevalence antigen. The patient’s plasma was further tested with two examples of rare reagent In(b−) RBCs and was found to be nonreactive. Typing the patient’s RBCs for In^b was not performed because reagent anti-In^b was not available. Because of this reagent unavailability, the specimen was sent to another reference laboratory to confirm the presence of anti-In^b. Eventually, the patient’s RBCs were found to be In(b−) with three examples of anti-In^b unlicensed reagents. The titer (saline tube-IAT) of the anti-In^b was found to be 4.

To prepare for the possibility of hemorrhage at delivery and potential transfusion needs of the fetus, a national search of the American Rare Donor Program managed by the American Red Cross was conducted for rare RBCs, but this search revealed no available blood donors. Next, maternal autologous blood donations were performed to bank blood for delivery with a planned repeat caesarean. To perform autologous blood collection, the threshold for blood donation had to be lowered after agreement of the transfusion and obstetrical physicians. In addition, careful assessment for underlying anemia was undertaken, and the patient was given intravenous and oral iron supplementation to prepare for surgery.

The patient was subsequently referred to the maternal–fetal medicine clinic for consultation at 34 weeks and 6 days of gestation to assess the risk of fetal anemia. Ultrasound evaluation revealed a singleton fetus with concordant biometry, normal amniotic fluid volume, an estimated fetal weight of 3297 g, and no evidence of hydrops. Middle cerebral artery peak systolic velocity (MCA PSV) was 78.0 cm/s (1.54 multiples of the median [median: 51.1 cm/s]). Repeat maternal titer was 2, and an additional weak antibody (anti-P1) was also detected. Anti-P1 was only demonstrable with ficin-treated panel RBCs and, therefore, not indicated for titration study. A monocyte monolayer assay (MMA) was performed using group O In(b+) and group A In(b−) RBCs to assess for the clinical significance of anti-In^b. The percentages of reactive monocytes using In(b+) RBCs with and without fresh complement were 3.2 and 4.2 percent, respectively (normal range 0–3%). Results greater than 3 percent suggest that the antibody may be capable of causing accelerated clearance of antigen-positive RBCs. Serial prenatal antibody titrations were carried out at 2 weeks apart; a significant rise of anti-In^b titer was not observed.

An attempt to confirm fetal anemia via amniotic fluid measurement of ΔOD450 was unsuccessful given maternal obesity, a large anterior placenta, no accessible fluid pocket, and maternal discomfort. Although concern for fetal anemia was low, given the limited information known about this antibody, delivery was scheduled for 38 weeks of gestation via repeat caesarean. At the time of her maternal–fetal medicine consultation, the patient was already scheduled to complete one autologous blood donation. The patient’s cousin and sister also presented for blood typing. Both were noted to be In(b+); thus, they were not appropriate candidates for blood donation. To consider whether the mother’s In(b−) blood could be used for transfusion to the neonate, ABO genotyping was performed on a maternal sample; the result was group A homozygous, suggesting that the baby would be group A or AB based on known ABO inheritance patterns; the blood type of the baby’s father was unknown. Neonatal and anesthesia consults were obtained, and, given the lack of available blood bank resources and a reasonable maternal hematocrit (Hct) of 34% (normal range for third trimester pregnancy 30–40%), the patient donated another unit of autologous blood to be banked for her procedure and to be used for the neonate if necessary. Cell salvage was arranged for as well as preparations for possible hysterectomy, if necessary.

**Recommended Therapy**

The patient underwent an uncomplicated repeat caesarean delivery at 38 weeks of gestation and delivered a live-born male infant weighing 3907 g, with Apgar scores of 7 and 8. Cord milking was performed at the time of birth to reduce the risk of neonatal anemia. Estimated maternal blood loss from delivery was 500 mL. The patient’s Hct fell to 29 percent from 31 percent preoperatively. The neonatal Hct at birth was 41 percent and improved to 46 percent (normal range 42–65%) at discharge, without transfusion. Total bilirubin at birth was 1.8 mg/dL (normal range 0.2–1.3 mg/dL), and reticulocyte count was normal at 3.2 percent (normal range 3.0–7.0%). The patient and infant were discharged in stable condition on postoperative day 3.
The neonatal blood type was group A, D+. Neonatal RBCs were not typed for In\textsuperscript{b} because there was no anti-In\textsuperscript{b} antiserum available. Furthermore, cord cells have weak expression of In\textsuperscript{b}. DAT and IAT were negative (Table 1).

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient’s results</th>
<th>Normal reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematology</strong>\textsuperscript{*}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>11.1 g/dL</td>
<td>9.5–15.0 g/dL</td>
</tr>
<tr>
<td>Hct</td>
<td>34%</td>
<td>30–40%</td>
</tr>
<tr>
<td>RBC count</td>
<td>3.95 × 10\textsuperscript{12}/mm\textsuperscript{3}</td>
<td>2.71–4.43 × 10\textsuperscript{12}/mm\textsuperscript{3}</td>
</tr>
<tr>
<td>MCV</td>
<td>86 fl</td>
<td>81–99 fl</td>
</tr>
<tr>
<td>WBC count</td>
<td>9.30 × 10\textsuperscript{12}/mm\textsuperscript{3}</td>
<td>5.9–16.9 × 10\textsuperscript{12}/mm\textsuperscript{3}</td>
</tr>
<tr>
<td>Platelet count</td>
<td>195 × 10\textsuperscript{12}/L</td>
<td>146–429 × 10\textsuperscript{12}/L</td>
</tr>
<tr>
<td><strong>Transfusion medicine</strong>\textsuperscript{†}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO, D</td>
<td>Group A, D+</td>
<td></td>
</tr>
<tr>
<td>Antibody detection test</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>ABO genotyping</td>
<td>A/A</td>
<td></td>
</tr>
<tr>
<td>MMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With complement</td>
<td>3.2%</td>
<td>0–3%</td>
</tr>
<tr>
<td>Without complement</td>
<td>4.2%</td>
<td>0–3%</td>
</tr>
<tr>
<td><strong>Fetal/Neonatal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ultrasoundography</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCA PSV</td>
<td>78.0 cm/s</td>
<td>Median: 51.1 cm/s</td>
</tr>
<tr>
<td><strong>Hematology</strong>\textsuperscript{‡}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct</td>
<td>41%</td>
<td>42–65%</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>1.8 mg/dL</td>
<td>0.2–1.3 mg/dL</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>3.2%</td>
<td>3.0–7.0%</td>
</tr>
<tr>
<td><strong>Transfusion medicine</strong>\textsuperscript{‡}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO, D</td>
<td>A, D+</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>IAT</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

*At 35 weeks of gestation.
†At 28 weeks of gestation.
‡At birth.

Hb = hemoglobin; Hct = hematocrit; RBC = red blood cell; MCV = mean corpuscular volume; WBC = white blood cell; DAT = direct antiglobulin test; MMA = monocyte monolayer assay; MCA PSV = middle cerebral artery peak systolic velocity; IAT = indirect antiglobulin test.

**Discussion**

The In antigens are part of the Indian blood group and were first described in 1973.\textsuperscript{2} In\textsuperscript{a} is a low-prevalence antigen present in approximately 0.1 percent of white individuals, 4 percent of Indian individuals, and approximately 11 percent of Middle Eastern populations.\textsuperscript{3} Conversely, In\textsuperscript{b} is a high-prevalence antigen noted in all populations. As such, the phenotype In(b–) is quite rare across multiple ethnicities and has been noted in approximately 2 of 700 Indian blood donors. Two additional high-prevalence antigens, INFI and INJA, were added to the Indian blood group system in 2007 after their identification. In\textsuperscript{a} and In\textsuperscript{b} are encoded by CD44, differ by 1 base pair, and are carried on the surface glycoprotein CD44. IN3 and IN4 are also encoded by CD44.\textsuperscript{4}

Anti-In\textsuperscript{b} is an IgG antibody based on studies by Joshi\textsuperscript{5} and Ferguson and Gaal.\textsuperscript{6} In vivo, this antibody has been shown to cause a hemolytic reaction with fetal cord blood.\textsuperscript{6} In limited older reports, anti-In\textsuperscript{b} was not associated with HDFN, although maternal titers were low (≤4) in all of these cases.\textsuperscript{7} A more recent Australian case report identified a woman presenting with preterm labor and chorioamnionitis at 16 weeks of gestation to have anti-In\textsuperscript{b} on routine blood typing. Similar to our case, no In(b–) units were available, necessitating the use of intravenous iron and erythropoietin in the setting of excessive bleeding at the time of placental removal. Given the gestational age in that case, however, no assessment for possible HDFN was considered necessary.\textsuperscript{8}

Thus far, anti-In\textsuperscript{b} has not been implicated in HDFN, although case numbers are low and, in some instances, this finding may be affected by low antibody titers. It is postulated that the risk of HDFN is likely low in these cases either because anti-In\textsuperscript{b} does not readily cross the placenta, or because the antibody may be neutralized by CD44 on placental macrophages, inhibiting transfer to the fetus.\textsuperscript{6,7,9} Although the literature suggests that HDFN would be unlikely in this case, in vivo experiments suggest an ability to cause hemolysis. As such, we chose to assume that this fetus may be at risk for anemia. In such cases, ΔOD\textsubscript{450} testing is useful to evaluate fetal anemia; if unable to test for it, little else is available to detect clinically significant anemia in the absence of hydrops, since measurements of MCA PSV are less reliable at later gestational ages.\textsuperscript{10}

Prior reports have implicated anti-In\textsuperscript{b} in both immediate and delayed hemolytic transfusion reactions.\textsuperscript{5,6} Because of this information and because there were no appropriate In(b–) blood units available locally or nationally, MMA testing was performed to assess the clinical significance of the antibody and risk of hemolysis. The percentages of reactive monocytes with In(b+) RBCs with fresh complement and without fresh complement were 3.2 and 4.2 percent, respectively, suggesting the potential for the antibody to clear circulating...
RBCs. Although the results were slightly over the threshold of positive, we felt it was prudent to recommend transfusion of ABO- and D-compatible In(b−) RBCs units if the patient required transfusion. Of note, MMA testing is not routinely recommended for monitoring of HDFN.

In this case, maternal autologous blood donation was recommended in anticipation for surgery and for possible neonatal use. Autologous blood donation during pregnancy has previously been described in the setting of alloimmunization and intrauterine transfusion. In our experience, this approach appears to be a safe procedure in these critical scenarios. Over the last 10 years, our center has collected and/or stored RBC units for several women of childbearing potential who were not able to use the community blood supply because of the presence of an antibody that makes them incompatible with most of the population (Table 2). After storage, these units are kept frozen until expiration (10 years) in case they are needed for use by the patient. Although testing of family members to locate appropriate blood donors is recommended and should be considered when possible, family donors often do not share the same blood type, as was the case with the described patient.

A multidisciplinary approach involving transfusion services, anesthesia, neonatology, and maternal–fetal medicine was essential in this case. Extremely limited RBCs were potentially to be shared by both the mother and neonate, and thus additional measures (cell salvage, expeditious operating, and access to alternative hematologic products [i.e., platelets, fresh frozen plasma, cryoprecipitate]) were paramount aspects of this case requiring thoughtful planning and feedback from the multiple services.

Ultimately, our patient underwent an uncomplicated repeat caesarean delivery with minimal blood loss, and her neonate did not have anemia. Engagement of our multidisciplinary team members resulted in a favorable outcome in this case. Here, we have described the management of a maternal rare antibody in the setting of extremely limited resources requiring maternal autologous blood donations, preparation for possible fetal anemia, and preparation for possible maternal hemorrhage.

References


Table 2. Maternal blood group types requiring autologous blood donation at our center over the past 10 years

<table>
<thead>
<tr>
<th>Blood Group Type</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>In(b−)</td>
<td>ENDA−</td>
</tr>
<tr>
<td>H− (Bombay)</td>
<td>Para Bombay</td>
</tr>
</tbody>
</table>
A review of in vitro methods to predict the clinical significance of red blood cell alloantibodies

S.J. Nance

This review was derived from a presentation made on September 2, 2016, for the first Academy Day presented by the Working Party on Immunohematology at the International Society of Blood Transfusion (ISBT) Congress in Dubai. The focus of this review is on the clinical significance of alloimmunization in transfusion—specifically, the parameters that contribute to a clinically significant alloantibody. The areas of focus were as follows: Introduction, Technical Aspects, and Indications and Limitations. Each section contains a brief review of selected literature and experiential knowledge. Case reports are needed to collect data on current outcomes of incompatible transfusion. The ISBT Working Party on Rare Donors has developed a form to capture case-specific information. *Immunohematology* 2018;34:11–15.

Key Words: RBC alloantibodies, clinical significance, ISBT Academy Day

Introduction

When transfusion was first implemented, little was known about the alloantibodies produced as a side effect of the procedure. Dr. Philip Syng Physick from Philadelphia is given credit for the first human blood transfusion in 1795. Although Dr. Physick did not publish the event, Dr. De Wees credits him with the transfusion in a comment in a journal article in 1825 for the event 30 years earlier.¹ In 1818, British obstetrician James Blundell performed the first successful transfusion of human blood (4 mL) to a patient for the treatment of postpartum hemorrhage.²

From these early times emerged the discipline of transfusion medicine and, within this discipline, immunohematology. The study of the immune response to transfusion has become an integral part of the treatment of patients. Fewer than 10 percent of first-time transfusion recipients make alloantibodies, compared with reported percentages of up to 40 percent in multiply transfused patients. Only a fraction of patients will form antibodies to the foreign red blood cells (RBCs) they receive which subsequently may destroy a fraction of or all of the foreign RBCs; this possibility negates the positive effects of transfusion. Thus, alloimmunization is considered a significant side effect. Only for certain categories of patients in selected centers is it common practice to match the antigens of the patient and of the blood for transfusion before antibodies are made. It is remarkable that this significant side effect of transfusion has not resulted in a uniform goal of phenomatching or genomatching all patients receiving RBC transfusions.

Defining the clinical significance of RBC alloantibodies has been pursued for many years, and today we still do not know how to predict the outcome of transfusion for an individual patient. Organizations have developed their own definitions; the one that is most suitable is that of the British Committee for Standards in Haematology Blood Transfusion Task Force³: Clinically significant antibodies are those that are capable of causing patient morbidity due to accelerated destruction of a significant portion of transfused RBCs. Other groups, like the AABB, have defined a clinically significant antibody as "an antibody that is frequently associated with hemolytic disease of the fetus and newborn, hemolytic transfusion reaction, or a notable decrease in survival of transfused red cells."⁴ The pathogenic activity of RBC alloantibodies varies from that of no clinical evidence of RBC destruction to destruction of transfused allogeneic RBCs in an acute or delayed hemolytic transfusion reaction (DHTR). There is also an alloimmune response that results in serologic evidence of new alloantibody formation or detection of antibody bound to transfused RBCs (positive direct antiglobulin test) but no clinical evidence of destruction of transfused RBCs. This event has been termed a delayed serologic transfusion reaction (DSTR). Other pathogenic evidence is seen in the destruction of fetal or newborn RBCs by maternal alloantibodies (termed hemolytic disease of the fetus and newborn [HDFN]) and in transplants.
(destruction of transplanted tissue [transplant rejection] and destruction of cells of the recipient by donor immune cells in graft-versus-host disease). The phenomenon of destruction of not only the transfused RBCs but also of the patient’s autologous RBCs in a hyperhemolysis event has also been described but will not be covered here.

The U.S. Food and Drug Administration Department of Regulation compiles and releases annual statistics on death associated with transfusion. In the most recent data posted from 2014, tracked information included deaths due to hemolytic transfusion reaction (HTR) from ABO and non-ABO antibodies. In 2014, there were eight fatalities attributed to HTRs—down from a high of over 25 reported in 2002; four of these deaths were caused by ABO antibodies and four were caused by non-ABO antibodies. The non-ABO antibodies associated with the deaths in 2014 included one newly identified anti-C, one anti-Jkα, and two ascribed to a category of “other.” The anti-C was identified in a post-transfusion sample, and the anti-Jkα death was due to a mislabeled unit. One case classified as “other” was of a warm-reactive autoantibody with concomitant anti-Jkα and anti-Fyα and a new anti-M (determined to not be implicated) with no other newly identified alloantibodies. The final reported case was a patient with sickle cell disease with multiple preexisting known antibodies who developed hyperhemolysis with no additional alloantibodies detected.

In the UK, the Serious Hazards of Transfusion (SHOT) hemovigilance data are tracked in three categories: transfusion reactions that may not be preventable, transfusion reactions that are possibly or probably preventable with improved practice and monitoring, and adverse events caused by errors. In these three categories, from 1996 to 2013, there were over 2500 reports in the first category, over 500 in the second, and over 1500 in the last category attributed to D/anti-D errors. For the years 1996–2013, there were 13,141 total adverse transfusion events reported, with anti-D alloimmunization being approximately 1500 cases, with 500 HTRs. In looking at hemovigilance data from France from 2008 to 2013, there were 11,625 reports of alloimmunization, with 3617 occurring in patients aged older than 80 years, and the highest rate was found in patients aged 55–79 years.

### Technical Aspects

The technical aspects regarding the determination of the significance of RBC alloantibodies are based on experiential information, what textbooks report, and selected research studies.

Of foremost importance in considering the significance of a RBC alloantibody is determining whether the antibody is reactive solely at room temperature or body temperature (37°C). It is most generally practiced that if an alloantibody is not reactive at body temperature, then the specificity does not need to be considered in the selection of blood for transfusion. If the antibody is reactive at body temperature, however, then, in the absence of other studies showing or predicting the antibody to be clinically insignificant, one should transfuse antigen-negative blood.

Standard practice is to select blood for transfusion that is negative for the antigen, if the antibody is to a common antigen that has been known to be associated with transfusion reactions (e.g., anti-C, anti-K, anti-Jkα). See Table 1 for predictions of clinical significance of antibodies based on specificity. The literature is not absolute on clinical significance of all antibodies detected with methods used more than 20 years ago. The literature is also not strong on antibody specificity and clinical significance using methods currently used in transfusion services for antibody detection, that is, automated methods (that were not in use at the time of the reports on specific antibody clinical significance). There is a global initiative to collect the literature that is known in this area and have it available in an electronic online database called the Notify Library.

Consideration for selection and identification of antibody techniques should be given to determining whether an antibody is IgM or IgG when there is risk to a fetus or newborn through the presence of a maternal antibody. If an IgM antibody is present, there is no risk to the fetus because

| Table 1. Predictions of clinical significance by antibody specificity |
|------------------------|------------------------|
| Usually clinically significant: ABO, D, C, c, E, e, K, k, Fyα, Fyβ, Jkα, Jkβ, S, s, U, Doα, Doβ, Vel |
| Clinical significance variable by patient: Ytα, Ge, Gyβ, Hy |
| Clinically significant if reactive at 37°C: Leα, Leβ, M, N, P1, Luα, Luβ, A1 |
| Usually clinically insignificant: Chα, Rgα, Xgα, Bg, Caα, Knα, McCα, Ykα, JMH, Sdα, “HTLA-like” |

Agreement does not exist in the literature for all antigens in all categories, and this list is not inclusive of all alloantibodies to blood group antigens.
IgM antibodies do not cross the placenta. IgG antibodies do cross the placenta, however, and it is important to monitor these antibodies in the pregnant mother, particularly anti-D. From a transfusion perspective, if an IgM antibody is reactive in strict prewarmed testing at 37°C by agglutination, then the transfusionist should use blood selected and tested to be negative for the antigen. IgM antibodies seldom react at 37°C, but, when they do, they can be detected by agglutination at 37°C or at the antihuman globulin (AHG) phase by the anti-C3 component of polyspecific AHG reagent if complement has been activated. IgG antibodies are seldom detected at 37°C by agglutination because, although IgG antibodies commonly sensitize reagent RBCs at 37°C, this sensitization cannot be visualized by macroscopic examination at that temperature, but only visualized at the AHG phase through the binding of anti-IgG. Antibodies to common antigens reactive at 37°C and detected in the AHG phase are important to consider in the selection of blood.

**Indications and Limitations**

Although Table 1 lists commonly encountered specificities and groups them into categories of clinical significance, it is important to recognize that these categories are not always going to be correct for 100 percent of the antibody specificities identified. It is especially important to publish and register the outcomes of incompatible transfusions in the Notify Library because detection methods have changed since the early publications on the outcome of incompatible transfusions for commonly encountered antibodies. Additionally, there is literature to support the idea that not all antibodies have the same destruction potential. For example, Garratty et al. noted that, of 559 patients studied, there were common antibodies that were reported to cause a DHTR with clinical signs of transfused RBC destruction. As an example, in 95 patients with anti-Jk⁺, 45 patients (47%) were reported to have a DHTR and 50 (53%) a DSTR. Thus, although a significant percentage of the patients with anti-Jk⁺ demonstrated positive serologic reactions (DSTR), no clinical signs of RBC destruction were seen in these patients. Table 2 shows more of the data reported. Of interest, for the antibodies shown in Table 2, some specificities (e.g., anti-E, -Jk⁺, -K, -Fy⁺, and -Jk⁻) have been associated with transfusion reactions. In total, in 65 percent of these cases, DSTR was the outcome reported rather than HTR or DHTR. Thirty-five percent of these antibody specificities did cause DHTR, however, so these specificities should be considered clinically relevant for transfusion, with the recognition (again) that not all patients will have the same outcome. It is important to consider all possible outcomes in making decisions for transfusion. A test with a 100 percent positive predictive value to predict the outcome for each patient transfused would be highly useful. It would also be helpful to know which patients will form antibodies.

**Table 2. DHTR versus DSTR for some commonly encountered antibody specificities**

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Total</th>
<th>DHTR (n)</th>
<th>DSTR (n)</th>
<th>% DSTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>184</td>
<td>47</td>
<td>137</td>
<td>74</td>
</tr>
<tr>
<td>Jk⁺</td>
<td>95</td>
<td>45</td>
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<td>53</td>
</tr>
<tr>
<td>Fy⁺</td>
<td>62</td>
<td>26</td>
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<td>K</td>
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<td>46</td>
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</tr>
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<td>Fy⁻</td>
<td>12</td>
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<td>8</td>
<td>67</td>
</tr>
<tr>
<td>Jk⁻</td>
<td>17</td>
<td>3</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>559</td>
<td>197</td>
<td>362</td>
<td>65</td>
</tr>
</tbody>
</table>

Adapted from Garratty. DHTR = delayed hemolytic transfusion reaction; DSTR = delayed serologic transfusion reaction.

The strength of reactivity and degree of destruction of transfused-incompatible RBCs have not been well studied for their relevance to transfusion outcomes, but in this author’s experience, when there is an antibody that cannot be identified, or an antibody to an antigen of high prevalence, a 4+ reactive antibody is regarded more seriously than one that is less than 1+. However, the outcomes of transfusion have not been correlated with a measurement of strength of reactivity. The method used can influence the strength of reactivity, since different methods have different sensitivity levels. Experience shows that not all antibodies that are weakly reactive are clinically insignificant, nor are all strongly reactive antibodies clinically significant. Zupanska et al. studied the in vitro phagocytosis of RBCs with known amounts of RBC-bound IgG, with the outcome that there was a relationship to monocyte phagocytosis and the number of RBC-bound IgG molecules, although there was variation between individuals. The correlation between in vivo hemolysis and the amount of RBC-bound IgG has also been studied by flow cytometry.

The IgG subclasses of IgG alloantibodies have been studied for their association with clinical outcomes. It is known that IgG1, IgG2, and IgG3 subclasses activate complement and interact with macrophage Fc receptors. It is thought that antibodies of the IgG4 subclass should not cause RBC destruction because they do not activate complement or interact with macrophage Fc receptors. Nevertheless, because most antibodies are IgG1 with or without other subclasses, it
is difficult to use IgG subclass information for the prediction of the clinical significance of RBC alloantibodies. It has been reported that some antibodies may not be able to be subclassed by a standardized capillary test, possibly because reactions with antisera to IgG subclasses are weaker than with anti-IgG.\(^{14}\) Zupanska et al.\(^ {13}\) reported that there was a higher degree of phagocytosis with fewer IgG3 molecules than with IgG1 molecules. That report also contained interesting still photographs of phagocytosis taken from a videotape of the testing as it progressed. The commentary that accompanied the photographs reported that the RBCs with bound IgG adhered to the monocytes for a considerable length of time before phagocytosis, but that once phagocytosis began, consumption of the RBCs was rapid.

In vitro tests have been reported that seek to predict the clinical significance of an antibody present in a patient who is pregnant or who needs transfusion. For pregnant patients with IgG antibodies, it is often desired to perform noninvasive tests to seek information rather than turn immediately to invasive tests. Often, titers are requested to provide an indication of the strength of the reactivity. Only anti-D has been studied effectively in an attempt to use a titer result to decide on invasive procedures; other antibody specificities are less well studied, and a titer endpoint has not been correlated to pregnancy outcome. Antibody-dependent cell-mediated cytotoxicity (ADCC) and chemiluminescence assays have been reported to be best for predicting HDFN. The ADCC assay detects RBC lysis by quantitating the released \(^{51}\)Cr by a gamma counter. One limitation to this method is that antibodies to enzyme-sensitive antigens cannot be studied because enzyme-treated RBCs are used in the test.\(^ {16,17}\) Chemiluminescence reports results based on released oxygen radicals that cause luminol to emit light.

For patients who need transfusion, the monocyte monolayer assay (MMA) has been used to predict the clinical significance of antibodies.\(^ {18-21}\) Commonly, the MMA is used in the United States when no blood of the requested blood type can be found domestically before making an international request. A negative MMA indicates that random RBCs (untyped for the indicated antigen) can be transfused for the immediate transfusion event. If more transfusions are needed later, the MMA must be performed with a current sample as close to the time of transfusion in the event that the antibody’s characteristics have changed. Historically, but not often used today, are the in vivo tests: the \(^{51}\)Cr red cell survival study described by Mollison\(^ {22}\) and the in vitro measure of in vivo survival by differential agglutination and flow cytometry,\(^ {23}\) both of which require the transfusion of incompatible blood and then the measurement of the survival of the transfused aliquot.

Clearly, more data would be useful in making transfusion decisions when antigen-negative blood is not readily available. The previously mentioned Notify Library will collect peer-reviewed publications on transfusion outcomes involving blood group specificities. Additionally, the ISBT Working Party on Rare Donors recently launched a form, Outcome of Incompatible Transfusion: Case Study Report (Fig. 1), on the ISBT Web site to track outcomes of incompatible transfusions.\(^ {24}\) This form is intended for use when a patient has an antibody identified and an incompatible transfusion is given deliberately or by mistake. The purpose is to track the outcome by antibody specificity to provide information to clinicians who must make a transfusion decision when blood is needed and not available.

![ISBT Outcome of Incompatible Transfusion: Case Study Report](image1)

**Fig. 1** International Society of Blood Transfusion Working Party on Rare Donors form: Outcome of Incompatible Transfusion: Case Study Report. Reprinted from Nance et al.\(^ {24}\)
Evaluations from the Academy Day showed that the attendees appreciated the concentrated review and the discussions about the clinical significance of alloantibodies.

References


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Recovery of autologous sickle cells by hypotonic wash

E. Wilson, K. Kezeor, and M. Crosby

It is important to isolate autologous red blood cells (RBCs) from transfused RBCs in samples from recently transfused patients to ensure that accurate serologic results are obtained. Typically, this isolation can be performed using methods that separate patient reticulocytes from transfused, older donor RBCs. Patients with sickle cell disease (SCD), however, characteristically have RBCs with altered membrane and morphological features, causing their RBCs to take on a sickle-shape appearance different from the biconcave disc-shape appearance of “normal” RBCs. These characteristics enable the use of hypotonic saline solution to lyse normal RBCs while allowing “sickle cells” to remain intact. Because many patients with SCD undergo frequent transfusions to treat their condition, the use of hypotonic saline solution provides a rapid method to obtain autologous RBCs for serologic testing from this patient population using standard laboratory equipment and supplies. *Immunohematology* 2018;34:16–18.

**Key Words:** cell separation method, sickle cell, autologous cell recovery, hypotonic

**Introduction**

Sickle cell disease (SCD) affects millions of people throughout the world. It is one of the most common inherited blood disorders and can be seen most prevalently in people of African descent. It is characterized by abnormally shaped (sickle-shaped) red blood cells (RBCs), which are removed from circulation and destroyed at increased rates, leading to anemia. The underlying abnormality of the “sickle cell” RBC is the presence of abnormal hemoglobin (Hb S). When deoxygenated, Hb S becomes relatively insoluble and forms aggregates with other Hb molecules within the RBC. These aggregates develop into long chains that distort the RBC into a sickled shape, causing impaired RBC flow through blood vessels.1

One of the most common treatments for patients with SCD is blood transfusion. Blood samples from patients with SCD who have been recently transfused may present challenges when performing serologic testing in the blood bank. Antigen typing results are invalid on blood samples up to 3 months after transfusion because of multiple populations of RBCs in

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**Preparing 0.3% hypotonic saline**

**Reagents/Supplies**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• NaCl</td>
<td>• 1-L container</td>
</tr>
<tr>
<td>• Distilled water OR</td>
<td></td>
</tr>
<tr>
<td>• 0.9% normal saline</td>
<td></td>
</tr>
</tbody>
</table>

**Procedural Steps**

- Add distilled water to 3 g NaCl to final volume of 1 L OR
- Add 1 volume of 0.9% normal saline to 2 volumes of distilled water

---

**Performing hypotonic wash**

**Reagents/Supplies**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 0.9% NaCl normal saline</td>
<td>• Test tubes (10 × 75, 12 × 75, or 16 × 100 mm)</td>
</tr>
<tr>
<td>• 0.3% NaCl hypotonic saline</td>
<td>• Disposable pipettes</td>
</tr>
<tr>
<td>• Packed EDTA RBCs from recently</td>
<td>• Calibrated centrifuge</td>
</tr>
<tr>
<td>transfused patient with SCD</td>
<td></td>
</tr>
</tbody>
</table>

RBCs = red blood cells; SCD = sickle cell disease.

**Procedural Steps**

- Add four to five drops of RBCs to a test tube.
- Fill the tube with 0.3% hypotonic saline. Invert to mix.
- Centrifuge the tube at 1000g for 1 minute or at calibrated high speed for centrifuge in use. Remove supernatant.
- Repeat fill/centrifuge steps with 0.3% hypotonic saline at least six times, or until all gross hemolysis clears.
- Remove as much residual saline as possible after the last wash.
- Fill the tube with 0.9% normal saline. Invert to mix.
- Centrifuge the tube at high speed. Remove supernatant.
- Repeat fill/centrifuge steps with 0.9% normal saline at least twice.
- Centrifuge the tube on the last wash at 200g for 2 minutes or at calibrated low speed for centrifuge in use. Remove all supernatant.
- Add 0.9% normal saline to the washed, packed RBCs to a 2–5% suspension.

RBCs = red blood cells.
circulation. Direct antiglobulin test (DAT) results may also be falsely negative depending on the quantity and frequency of transfusions a patient receives. Therefore, it is imperative to separate the patient’s autologous RBCs from the transfused RBCs before performing serologic testing to obtain accurate results. Methods to separate autologous RBCs from transfused RBCs include microhematocrit centrifugation, hypotonic wash, and use of density gradients.  

RBCs containing Hb S are resistant to osmotic stress and can be quickly separated from transfused cells when washed with a hypotonic saline solution. These recovered autologous RBCs are useful in performing phenotyping, DAT, and autoadsorption procedures.

**Principle**

Microhematocrit cell separation methods separate autologous RBCs from transfused RBCs based on RBC density. High-speed centrifugation causes younger, less dense RBCs to rise to the top of the microhematocrit tube, whereas older, heavier RBCs separate to the bottom. Sickle cells are quite dense; therefore, microhematocrit centrifugation is not effective for separating autologous RBCs from transfused donor RBCs in this patient population.

There is a strong relationship between the morphology of sickle cells and the osmolarity of the suspending solution. RBCs from patients with SCD, either Hb SS or SC, are resistant to lysis by hypotonic saline. RBCs from individuals without SCD (Hb AA) and individuals with the Hb S trait will lyse when washed with the same concentration of hypotonic saline. This lysis occurs because of the spherical shape of the RBCs, which allows them to swell in hypotonic solutions. An increase in membrane permeability of RBCs containing Hb S promotes calcium entry as well as loss of potassium chloride and water, inducing cellular dehydration and the resultant sickling. Hyperconcentration of Hb S is associated with membrane permeability and, consequently, resistance to hypotonic hemolysis.

**Indications**

If RBC transfusion has occurred in a patient with sickle cell anemia (Hb SS or SC disease) and autologous RBCs are desired for further testing, a hypotonic saline wash can be performed to isolate patient autologous RBCs. Lack of an active sickle crisis episode does not preclude use of the hypotonic wash method to separate autologous RBCs from transfused RBCs.

**Procedure**

Add four to five drops of packed EDTA RBCs from a recently transfused patient with SCD to a test tube. If a larger volume of RBCs is desired for use in adsorption studies, a 16 × 100 mm test tube can be used to facilitate adequate washing. Wash the RBCs by filling the tube with a large volume of 0.3 percent NaCl and invert to mix. Centrifuge the tube at 1000g for 1 minute (or at the high-speed setting) in a calibrated centrifuge. Repeat this process approximately six times or until all gross hemolysis clears, removing as much of the supernatant as possible after the last wash. Wash the remaining RBCs twice with 0.9 percent NaCl to restore tonicity. Centrifuge the last wash at 200g for 2 minutes (or at the low-speed setting) in the calibrated centrifuge and remove the supernatant. The lower centrifugation speed will aid in removal of residual stroma. Suspend the washed RBCs in 0.9 percent NaCl to a 2–5 percent concentration. RBCs are now ready for use in antigen typing, DAT studies, or autoadsorption procedures.

**Limitations**

Mixed-field reactivity noted when performing antigen typing of harvested autologous sickle cells may be due to incomplete lysis of the transfused RBCs. Additional washes with hypotonic saline may be warranted in these cases. White blood cells (WBCs) may not be readily lysed by the hypotonic saline solution. Patients with an elevated WBC count may cause a sticky RBC button when autologous RBCs are tested.

**References**


Manuscripts

The editorial staff of Immunohematology welcomes manuscripts pertaining to blood group serology and molecular genetics for consideration for publication. We are especially interested in review articles, case reports, papers on platelet and white cell serology, scientific articles covering original investigations or new blood group alleles, papers on molecular testing, and papers on new methods for use in the blood bank. To obtain instructions for submitting scientific articles, case reports, and review articles, see Instructions for Authors in every issue of Immunohematology or e-mail a request to immuno@redcross.org. Include fax and phone numbers and e-mail address with all manuscripts and correspondence. E-mail all manuscripts to immuno@redcross.org.

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The devil is in the details: retention of recipient group A type 5 years after a successful allogeneic bone marrow transplant from a group O donor

ABO-incompatible (ABOi) hematopoietic stem cell transplants (HSCTs) can present challenges in the blood bank. During transplantation, patients receive components that are ABO-compatible with both the donor graft and recipient; this practice can strain group O red blood cell (RBC) inventories. In addition, there are risks for acute hemolysis at the time of infusion and in the early post-transplant period. In ABO major-incompatible bone marrow HSCTs, which contain significant quantities of donor RBCs that are ABOi with recipient plasma, it is common to perform a RBC depletion of the bone marrow in an effort to minimize hemolysis at the time of infusion. Furthermore, patients with high-titer ABO antibodies may undergo a prophylactic, pre-transplant plasma exchange to further reduce the risk of acute hemolysis, delayed RBC engraftment, and pure RBC aplasia. ABO minor-incompatible HSCTs, in which donor plasma is ABOi with the recipient, have less risk for hemolysis at the time of infusion but can result in transient hemolysis approximately 10–21 days post-transplant, especially in patients undergoing nonmyeloablative HSCT and/or patients who have not received methotrexate for graft-versus-host-disease (GVHD) prophylaxis. In these patients, viable donor B-lymphocytes in the graft may expand and produce ABO antibodies capable of hemolyzing patient RBCs.

ABOi HSCTs can also present serologic challenges post-engraftment, particularly for ABO grouping. After full engraftment, the recipient’s ABO forward type should convert to that of the donor. The back type, however, is often discrepant because of missing ABO antibodies. Specifically, the patient will lack ABO antibodies against the patient’s original ABO-group RBCs. Donor RBCs are usually detected within 3–6 weeks after HSCT but can be significantly delayed, especially with group A/group O major-incompatible HSCT, high-titer ABO antibodies in the recipient, and nonmyeloablative, reduced-intensity conditioning (RIC) chemotherapy. At some point after gaining evidence of stable engraftment and transfusion independence, the patient’s ABO blood group is officially changed to that of the HSCT donor. At our institution, a patient may be considered for an ABO blood group change 1 year after ABOi HSCT, provided that (1) the patient has no clinical or laboratory evidence of disease relapse, (2) the patient has not received any transfusions for at least 3 months, (3) there is no evidence of mixed-field agglutination in the forward typing, (4) a direct antiglobulin test is negative, and (5) an isoagglutinin crossmatch of recipient plasma against the donor ABO-group RBCs is negative (tube method; 15 minutes at room temperature incubation, and indirect antiglobulin test [15 minutes at 37°C incubation, anti-IgG]). Patients meeting these criteria are referred to the medical director, who approves any change in patient ABO group in the patient’s medical record.

Clinically, allogeneic HSCT engraftment is typically monitored through molecular chimerism studies, which measure the percentage of white blood cells (WBCs) that are either donor-derived or recipient-derived using a set of unique molecular markers (e.g., microsatellite variable number tandem repeats). An increase in the percentage of recipient WBCs post-HSCT may indicate graft rejection, disease relapse, or GVHD. In an ABOi HSCT, a reemergence of the patient’s original pre-HSCT ABO group can also be a sign of possible graft loss with restoration of recipient hematopoiesis. We describe a male child who underwent an ABO minor-incompatible HSCT who retained his original ABO group, with no typing discrepancies, despite molecular evidence of full donor engraftment.

The patient (group A, D+) was initially diagnosed with severe combined immunodeficiency (SCID) at 13 months of age after developing life-threatening disseminated varicella infection after routine vaccination. Genetic studies showed that the patient had the classic, X-linked form of SCID due to a nonsense mutation in the IL2RG gene (interleukin 2 receptor gamma subunit: C241>T; 81Gln>stop). Immunological studies showed hypogammaglobulinemia and low CD3+ T-cells (321/μL; normal 2100–6200/μL) and CD56+ NK-cells (25/μL; normal 180–920/μL) but elevated CD19+ B-cells (7786/μL; normal 720–2600/μL), consistent with a T–B+NK– SCID phenotype. Immuno-hematology testing showed a group A RBC phenotype but no evidence of anti-B or other isoagglutinins in plasma (tube, 30 minutes at 4°C). The patient also had evidence of transplacental maternal T-cell engraftment without a history or evidence of GVHD. At 15 months of age, the patient underwent a human leukocyte antigen (HLA)-matched (10/10), ABO minor-incompatible (group O donor/group A recipient), unrelated donor allogeneic HSCT (Table 1, patient 1). At the time of transplant, the patient still had evidence of a systemic viral infection as well as a possible pulmonary infection. For transplant, the patient received

IMUNOHEMATOLOGY, Volume 34, Number 1, 2018
serotherapy with antithymocyte globulin (ATG, 8 mg/kg), followed by infusion of group O, plasma-reduced, donor bone marrow, for a final transplant dose of 6.1 × 10⁶ CD34 cells/kg and 4.1 × 10⁶ total nucleated cells (TNC)/kg. The patient required only one group O RBC transfusion post-transplant. There was no GVHD or pancytopenia after transplant, with normal WBC (8–10 K/μL) and platelet (340–700 K/μL) counts during the first 30 days post-HSCT. At 1-month post-transplant, the patient had molecular evidence of engraftment (TNC, 81% donor). ABO typing showed mixed-field agglutination with both group A and group O RBCs. Engraftment studies 4 months after HSCT showed 100 percent donor lymphocytes (CD3+), normal NK-cell and T-cell numbers, normal T-cell function with evidence of endogenous thymopoiesis, and a normal repertoire TCRß (T-cell receptor beta chain) rearrangement. The patient continued to show defects in humoral immunity consistent with failure to engraft donor B-cells and low immunoglobulin levels requiring monthly intravenous immunoglobulin (IVIG) replacement. Isoagglutinin testing showed evidence of trace anti-B, although it was unclear whether the anti-B was passively acquired from his monthly IVIG prophylaxis. Repeat isoagglutinin testing 1 year post-HSCT was normal, with anti-B detected by tube method at immediate spin (1+), 15 minutes at room temperature (2+), and 30 minutes at 4°C (2 to 3+). Cold agglutinins reactive with group O (1+) and group A1 (1+) RBCs were also present after 4°C incubation.

At a 5-year follow-up visit, a routine type and screen was ordered. By automated gel method, the patient’s RBCs typed as group A (4+), D+ (4+) with anti-B (2+) identified in plasma, consistent with the patient’s pre-transplant blood type (A+). These results were confirmed by tube method (anti-B, 2 to 3+). There was no evidence of mixed-field agglutination or group O HSCT-donor RBCs. The patient had normal T-cell (2604/μL), mildly decreased NK-cell (120/μL), and increased B-cell (3602/μL) numbers. The patient’s immunoglobulin levels remained abnormal with pan-hypoimmunoglobulinemia requiring monthly IVIG infusions (IgM = 32 mg/dL, normal 40–190 mg/dL; IgA < 5 g/dL, normal 35–250 mg/dL; IgG = 482 mg/dL, normal 440–1190 mg/dL; IgE < 2 kU/L, normal 0–150 kU/L). Repeat molecular studies at this time again showed 100 percent donor engraftment for CD3+ lymphocytes; however, CD33+ myeloid cells were 100 percent recipient- or host-derived.

We were initially surprised and confused by the patient’s ABO typing results. We had expected that the patient would forward type as group O, consistent with the bone marrow donor, but reverse type as group A. The presence of a clear, unambiguous group A typing result was highly worrisome, implying that the patient may have rejected his graft. This presumption was quickly disproven by recent laboratory studies showing normal T-cell function and 100 percent donor CD3+ lymphocyte engraftment. The CD33+ engraftment results, which showed 100 percent recipient cells, explained the retention of the patient’s original ABO group. CD33+ myeloid cells arise from the same hematopoietic progenitor as megakaryocytes and erythroblasts, indicating that all erythropoiesis was of recipient origin.

The presence of donor lymphoid, but not myeloid, engraftment reflects several unique features surrounding HSCT in SCID patients. SCID is characterized by defects in T-cell responsiveness and proliferation, accompanied by impaired humoral immunity with hypogammaglobulinemia and absent ABO antibodies. Because of their profound cellular immunodeficiency, these patients are not considered at risk for graft rejection and commonly undergo HSCT with little or no "conditioning" chemotherapy or radiation. HSCT without conditioning is standard for HSCT when using HLA-matched siblings (matched sibling donor [MSD]) and maternal haploidentical donors, especially if there is evidence of maternal transplacental engraftment or if the patient has an active infection at the time of transplant. Serotherapy or immunotherapy is recommended for patients receiving an HLA-matched unrelated donor (MUD) which was the case for our patient. Serotherapy (ATG, fludarabine, or alemtuzumab) is provided to ablate any residual patient lymphoid cells as well as to provide GVHD prophylaxis. Chemotherapy, whether myeloablative or RIC, is generally used for a patient undergoing HSCT with a partial HLA-matched, unrelated donor or a patient with a history of graft rejection requiring a second HSCT.

Current reviews on HSCT for SCID rarely mention issues related to ABO and engraftment, because 50 percent of all HSCTs are done without any conditioning and maintenance of recipient myelopoiesis. It should be noted, however, that 20–40 percent of SCID patients exhibit myeloid chimerism (normal 6–100% donor CD33+ cells), even without conditioning chemotherapy. Interestingly, some degree of myeloid chimerism is desirable, because it is strongly associated with donor B-cell engraftment and IVIG independence. Factors favoring non-lymphoid donor engraftment include the use of conditioning chemotherapy and the occurrence of post-transplant GVHD.

Only two case studies have specifically examined donor erythroid engraftment in SCID patients. Bielorai et al. reported complete, multi-lineage engraftment in three SCID patients undergoing HLA-matched HSCTs from family members, including
two patients who received ABOi HSCTs. None of the patients had received serotherapy or conditioning chemotherapy pre-HSCT. Post-transplant, all three patients developed mild acute GVHD during the first month. Both patients with ABOi HSCTs converted to the donor’s ABO group (O→A; B→AB). Rubocki et al.\(^\text{10}\) described another case of multi-lineage engraftment, including donor RBCs, in a SCID patient who received an HLA-matched marrow transplant from her sister. Although there was no chemotherapy or serotherapy administered pre-HSCT, the patient did develop acute GVHD at 5 weeks, followed by a bout of chronic GVHD at 11 months. At 6 months, the patient had 100 percent donor cells, including evidence of erythroid engraftment. For the latter, the authors were able to show a change in the pre-transplant RBC phenotype (patient: Jk(a−), E+ to donor Jk(a+), E−).

We subsequently performed a retrospective study of HSCTs for SCID performed at our institution between 1994 and 2017. Including the index patient, a total of seven HSCTs were identified, of which five were ABOi (Table 1; 2 MSD, 3 MUD). Two patients were previously transplanted but failed to engraft, resulting in a second HSCT (patient 3) and third HSCT (patient 2). Surprisingly, the majority (4 of 5) of the patients received some form of serotherapy and conditioning chemotherapy, even patients with sibling donors (MSD, patients 4 and 5). Only one patient developed GVHD post-transplant (chronic type). All five patients had evidence of donor T-cell engraftment with normal T-cell counts and function (not shown). In three patients, there is evidence of donor myelopoiesis based on CD33 (40–85% donor) or TNC (100%). More importantly, all four patients who underwent conditioning chemotherapy had evidence of donor RBC engraftment. In one patient with 40 percent donor CD33 cells, both group A and group O RBCs were present, consistent with mixed chimerism.

In summary, we present the unusual engraftment chimerism that can be observed in children undergoing allogeneic HSCT for SCID. Unlike other HSCT populations, these children are often transplanted with little or no conditioning, leading to donor T-cell engraftment but retention of recipient myelopoiesis and erythropoiesis. SCID patients who undergo conditioning chemotherapy will show donor RBC engraftment at rates similar to other allogeneic ABOi transplants.\(^2\)\(^–\)\(^4\)

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Table 1. Relationship between conditioning and post-transplant ABO type in children undergoing ABOi, allogeneic HSCT for SCID

<table>
<thead>
<tr>
<th>Patient information</th>
<th>Transplant donor and conditioning</th>
<th>Post-transplant outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Age at HSCT (months)</td>
<td>Patient ABO/D</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>A+</td>
</tr>
<tr>
<td>2*</td>
<td>12</td>
<td>AB+</td>
</tr>
<tr>
<td>3†</td>
<td>6</td>
<td>O+</td>
</tr>
<tr>
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<td>4</td>
<td>O+</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>O+</td>
</tr>
</tbody>
</table>

*Underwent three HSCTs with same bone marrow donor. First HSCT (5 months; ATG, fludarabine) and second HSCT (7 months; fludarabine, cytoxan) complicated by failure to engraft. Third HSCT at age 12 months was successful but complicated by chronic GVHD.
†Underwent one prior cord HSCT with failure to engraft, followed by a successful HSCT with bone marrow from a different donor.
‡Mixed-field (mf) agglutination observed on RBC grouping 3 years post-HSCT. Donor group A RBCs predominate with a minor fraction of group O RBCs present.
§Post-engraftment molecular studies performed on the TNC fraction. Flow cytometry fractionation (CD3, CD33) not done at the time of testing.

ABOi = ABO-incompatible; HSCT = hematopoietic stem cell transplant; SCID = severe combined immunodeficiency; GVHD = graft-versus-host-disease; RBCs = red blood cells; MUD = matched unrelated donor; ATG = anti-thymocyte globulin; RIC = reduced-intensity conditioning; mf = mixed-field; MSD = matched sibling donor; TNC = total nucleated cells; NA = not applicable.

References

September 11, 2018

37th Annual Immunohematology and Blood Transfusion Symposium
The Department of Transfusion Medicine, Clinical Center, National Institutes of Health (NIH) and the American Red Cross are co-hosting this symposium on the NIH campus in Bethesda, Maryland, USA. There is no registration fee, but advance registration is encouraged. Contact Karen Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184, e-mail: kbyrne@cc.nih.gov or visit the Web site: http://www.cc.nih.gov/dtm/research/symposium.html

September 12, 2018

8th Annual Red Cell Genotyping Symposium: Red Cell Genotyping 2018: Patient Care
The Department of Transfusion Medicine, Clinical Center, National Institutes of Health (NIH) and the BloodCenter of Wisconsin are co-hosting this symposium on the NIH campus in Bethesda, Maryland, USA. For information, registration fee, and advance registration, contact Phyllis Kirchner, BloodCenter of Wisconsin, P.O. Box 2178, Milwaukee, WI 53021-2178, e-mail: Phyllis.kirchner@bcw.edu or visit the Web site: www.bcw.edu/rcg2018.

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# 2018 Educational Courses

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<th>PROGRAMS</th>
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<td>Webinar</td>
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<tr>
<td>March 15-16</td>
<td>TSEC</td>
<td>Nashville, TN</td>
</tr>
<tr>
<td>April 11-13</td>
<td>Hands-on (Molecular)</td>
<td>San Marcos, TX</td>
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<tr>
<td>May 17-18</td>
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<tr>
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<td>Emeryville, CA</td>
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<tr>
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Educational Programs 2018

SAVE THE DATE

Dear Colleague,

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Transfusion Science Educational Course (TSEC)
This course reviews the pathophysiology of alloimmunization, with an emphasis on the integration of serological and molecular methods for blood group antibody identification and compatibility testing. In addition, factors influencing transfusion decisions of the alloimmunized patient are discussed. Interactive case studies provide the opportunity to enhance practical case resolution skills.

Faculty: Recognized experts in the field of immunohematology, blood group genomics and transfusion medicine

Target level: Intermediate-advanced
Continuing education credits: 10.5-11.5 hours P.A.C.E.® credits

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This course presents the molecular basis of blood group diversity and applies various molecular techniques to interrogate red cell antigens polymorphisms. During three days, participants will split their time between lectures and hands-on practice in our state-of-the-art training laboratory in San Marcos, TX, where advanced serological and molecular techniques will be used to solve complex cases.

Target level: Advanced
Continuing education credits: 14-17 hours P.A.C.E.® credits

Webinars
This one hour online course targets current trends and innovative practices relevant to blood bank laboratory and transfusion medicine.

Target level: General
Continuing education credits: 1 hour P.A.C.E.® credit

Cost
These programs are offered FREE of charge. Each participant bears the cost for their own travel and accommodation when required for attendance.

For registration and other information, please email: TSEC@grifols.com

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<th>2018 TSEC Schedule*</th>
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*All dates subject to change.

<table>
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<th>2018 Hands-On Schedule*</th>
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<td>July 11-13</td>
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<td>October 24-26</td>
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<tr>
<td>August 15</td>
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<tr>
<td>December 19</td>
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*All dates subject to change.
PREP for the SBB/BB EXAM

The PASS Blood Bank Exam Review (formerly known as the Last Chance Review) is designed for individuals preparing to take the ASCP Board of Certification Examination in the Specialists in Blood Banking or Blood Banking category and for physicians who are preparing for the American Board of Pathology Examination in Blood Banking/Transfusion Medicine.

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# SCHEDULE AT A GLANCE

## Saturday, April 28

**TIME** | **SESSION NAME** (CBBS/SCABB Joint Meeting Final Sessions) | **SPEAKER(S)**
--- | --- | ---
8:00 AM | MD Roundtable | To Be Announced
9:15 AM | Administrative & Technical Scientific Award Lectures | Bud Scholl
10:45 AM | Karen Williams Memorial Lecture | Richard J. Benjamin MBCHB, PhD, FRCPaht

**TIME** | **SESSION NAME** (AIMS Sessions Begin) | **SPEAKER(S)**
--- | --- | ---
1:00 PM | AIMS Begins: RH System Genotyping & Discrepancy Resolutions | Margaret Keller, PhD
3:30 PM | Next Generation Sequencing & Case Studies #1 | William Lane, MD PhD

## Sunday, April 29

**TIME** | **SESSION NAME** | **SPEAKER(S)**
--- | --- | ---
8:30 AM | John Moulds Memorial Lecture Training & Competency Assessments | Geoff Daniels, BSc, PhD, FRCPaht
10:45 AM | Providing Platelets to the Refractory Patient: Where to Begin? | Kevin Land, MD
12:30 PM | Industry Workshop Luncheon (Sponsored by Bloodhub) | To Be Announced
1:30 PM | Ask the Experts & Blood Group Systems Overview | Geoff Daniels, PhD FRCPaht; Kevin Land, MD; Margaret Keller, MD; Hank Hanna, MD; Gorka Ochoa, MD, PhD; William Lane, MD, PhD; Virginia Reyes, MT(ASCP) SBB; Michael Gannett, MLS(ASCP) Lorena Aranda, MSHS, BB, MT, SBB, (ASCP cm
3:45 PM | Case Studies #2 | Margaret Keller, MD

AIMS registration includes the final sessions of the CBBS/SCABB Joint Meeting.

Visit www.scabb.org for the most up to date information on AIMS.
The Department of Transfusion on Medicine Specialist in Blood Bank Technology Program

The National Institutes of Health (NIH) Clinical Center, America’s research hospital, is located on the NIH campus in Bethesda, Maryland. Through clinical research, clinician-investigators translate laboratory discoveries into better treatments, therapies, and interventions to improve the nation’s health.

The Department of Transfusion Medicine (DTM) Specialist in Blood Bank (SBB) Training Program was established in 1966. Many of its graduates are now technical supervisors, education coordinators, quality assurance specialists, or reference technologists at some of the nation’s finest blood banks and transfusion services. Others have joined commercial companies in reference and education capacities. The program is a 1-year course (July–July) in advanced blood bank technology. The NIH Clinical Center Blood Bank, SBB Technology Program is accredited by the Commission on Accreditation of Allied Health Education Programs (www.caahep.org) upon the recommendation of the AABB Committee on Accreditation of SBB Programs.

The curriculum includes formal and informal teaching sessions covering basic and advanced serological techniques, blood donations, genetics, molecular immunohematology, viral disease testing, blood preservation and storage, component therapy, apheresis, hazards of transfusion, immunology, human leukocyte antigen (HLA) and transplantation, blood bank administration, and other relevant topics. Participation is encouraged at monthly departmental blood bank Journal Club presentations, Laboratory Services Section’s continuing education opportunities, and weekly transfusion medicine conferences. Students complete rotations in the DTM Laboratory Services Section, which includes an AABB-Accredited Immunohematology Reference Laboratory and an ASHI-accredited HLA Typing Laboratory. A rotation in the Blood Services Section of the DTM provides experience in donor recruitment, screening, phlebotomy, and apheresis procedures. Experience in infectious disease testing and hematopoietic transplantation is obtained through rotations in both the Infectious Diseases and Cell Processing Sections of the DTM. Rotations off-site address neonatal/pediatric transfusion practices and specialized testing procedures, such as IgA and monocyte monolayer assays.

With the guidance of a senior DTM staff member, each student is required to pursue an in-depth project suitable for presentation and/or publication. The project may concern a research issue in blood banking, a case study with a review of the literature, or an educational project.

Interested applicants should contact:

Karen M. Byrne, MDE, MT(ASCP)SBB
NIH Clinical Center, Department of Transfusion Medicine
Building 10, Room 1C711
10 Center Drive, MSC 1184
Bethesda, MD 20892-1184
Phone: 301-451-8645
Kbryne@cc.nih.gov
The Johns Hopkins Hospital Specialist in Blood Bank Technology Program

The Johns Hopkins Hospital was founded in 1889. It is located in Baltimore, MD, 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 2 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 Web site at http://pathology.jhu.edu/department/divisions/transfusion/sbb.cfm for additional information.

Contact:  Lorraine N. Blagg, MA, MLS(ASCP)SM SBB
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, MD 21287

Phone (410) 955-6580
Fax (410) 955-0618
Web site: http://pathology.jhu.edu/department/divisions/transfusion/index.cfm
Masters of Science (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 2018 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 a Diploma in Transfusion and Transplantation Sciences or a Certificate in Transfusion and Transplantation Sciences.

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 2018. For additional information and requirements, please visit our Web site 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 Web site: http://www.rushu.rush.edu/admiss/hlthadm.html

Contact: Yolanda Sanchez, MS, MLS(ASCP) SBB, Director, by e-mail at Yolanda_Sanchez@rush.edu or by phone at 312-942-2402 or Denise Harmening, PhD, MT(ASCP), Director of Curriculum, by e-mail at Denise_Harmening@rush.edu
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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

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Sandra J. Nance

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Immunohematology

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www.bloodgroups.info
www.sbbpocketbook.com
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?

<table>
<thead>
<tr>
<th>Supervisors of Transfusion Services</th>
<th>Managers of Blood Centers</th>
<th>LIS Coordinators</th>
<th>Educators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervisors of Reference Laboratories</td>
<td>Research Scientists</td>
<td>Consumer Safety Officers</td>
<td></td>
</tr>
<tr>
<td>Quality Assurance Officers</td>
<td>Technical Representatives</td>
<td>Reference Lab Specialists</td>
<td></td>
</tr>
</tbody>
</table>

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.

Facilities with CAAHEP-accredited programs, onsite or online, are listed below.

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

<table>
<thead>
<tr>
<th>California</th>
<th>American Red Cross Blood Services</th>
<th>Pomona, CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Academic Center at OneBlood</td>
<td>St. Petersburg, FL</td>
</tr>
<tr>
<td>Illinois</td>
<td>Rush University</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Indiana</td>
<td>Indiana Blood Center</td>
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</tr>
<tr>
<td>Louisiana</td>
<td>LifeShare Blood Center</td>
<td>Shreveport, LA</td>
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<td></td>
<td>University Medical Center New Orleans</td>
<td>New Orleans, LA</td>
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<tr>
<td>Maryland</td>
<td>National Institutes of Health Clinical Center</td>
<td>Bethesda, MD</td>
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<td></td>
<td>The Johns Hopkins Hospital</td>
<td>Baltimore, MD</td>
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<td></td>
<td>Walter Reed National Military Medical Center</td>
<td>Bethesda, MD</td>
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<tr>
<td>Texas</td>
<td>University Health System and Affiliates School of Blood Bank Technology</td>
<td>San Antonio, TX</td>
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<td></td>
<td>University of Texas Medical Branch</td>
<td>Galveston, TX</td>
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<tr>
<td>Wisconsin</td>
<td>BloodCenter of Wisconsin</td>
<td>Milwaukee, WI</td>
</tr>
</tbody>
</table>

Revised October 2017
Diagnostic testing for:
- Neonatal alloimmune thrombocytopenia (NAIT)
- Post-transfusion 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 (PSIFT)
- Solid-phase red cell adherence (SPRCA) assay
- Molecular analysis for HPA-1a/1b

**For further information, contact:**

**Platelet Serology Laboratory** (215) 451-4205
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

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**National Neutrophil Serology Reference Laboratory**

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**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
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St. Paul, MN 55107

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- Paternity testing/DNA

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Mehdizadeh Kashi
at (503) 280-0210

or write to:

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For additional information contact:

Sandra Nance (215) 451-4362
or e-mail:
Sandra.Nance@redcross.org

or write to:

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ATTN: Sandra Nance

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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 patients’ 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...) and 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: \( \bullet \), \( \bullet \), \( \triangle \), \( \Delta \), \( \square \).
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.)

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
A. For describing an allele that 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-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 References 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*DIVa-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.
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