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Assessment of common red blood cell pretreatments to yield an accurate serologic antigen phenotype compared with genotype-predicted phenotype
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Communications
Thank You
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The modernist American poet Hart Crane wrote these words in his proem To Brooklyn Bridge. Completed after 14 years in 1883 and considered a modern miracle, a fusing of creative epiphany with industrial power and precision, the Brooklyn Bridge spans the East River in New York, joining the boroughs of Brooklyn and Manhattan. The list of notable artists and writers inspired by the bridge also includes Joseph Stella, an Italian immigrant. For modernists like Stella, the utilitarian and the aesthetic mingled in both art and public works, and the true rendition of a painting’s subject addressed not only its form but its impact. Perhaps for Stella, the geometrically dazzling cables and arches symbolized the tenuous and fantastical vault of immigration itself. He painted Brooklyn Bridge in 1920 and would go on to make the bridge a subject of numerous later paintings. As a measure of his artistic vision, it would span his life. An article in this issue of Immunohematology discusses the use of dithiothreitol (DTT), a reducing reagent used to disrupt the bridging of the disulfide bonds between amino acid residues.

David Moolten, MD
Assessment of common red blood cell pretreatments to yield an accurate serologic antigen phenotype compared with genotype-predicted phenotype

T. Horn, J. Hamilton, J. Kosanke, V.W. Hare, W. Kluver, W. Beres, S. Nance, and M.A. Keller

For patients requiring multiple transfusions and patients with positive direct antiglobulin tests (DATs), an extended red blood cell (RBC) phenotype can provide valuable information and help to determine the risk of forming alloantibodies. In some instances, the phenotype may be used for prophylactic matching. Phenotyping in this patient population is often hindered by the presence of circulating donor cells and/or by a positive DAT. Several methods, such as EDTA glycine acid (EGA) treatment to remove IgG, hypotonic saline wash to separate autologous RBCs, or reticulocyte separation, are often used in these situations to isolate patient RBCs for serologic phenotyping. This study aimed to determine the accuracy of each RBC pretreatment method by comparing serologically determined antigen types with those predicted by RBC genotyping. Forty-eight peripheral blood samples from recently transfused patients were phenotyped for selected antigens in the Rh, Kell, MNS, Duffy, and Kidd systems. Treatment methods for the sample sets were reticulocyte separation (N = 12), EGA (N = 16), and hypotonic saline wash (N = 20). DNA was extracted using standard methods, and genotyping was performed using the HEA BeadChip panel. In addition, 21 samples positive for RBC-bound IgG were EGA-treated up to two times. These samples were analyzed pre- and post-EGA treatment for RBC-bound IgG by tube DAT and by flow cytometry with fluorescein isothiocyanate–labeled anti-human IgG. After reticulocyte separation, 3 of the 12 samples had discordant types with one antigen each: Fy\textsuperscript{a}, N, and K; serologic results were negative compared with genotype-predicted positive phenotype results. The EGA-treated sample set showed one discordant type: Fy\textsuperscript{a}; serologic results were negative compared with genotype-predicted positive phenotype results. Four of the 20 samples had discordant types involving the following antigens: Fy\textsuperscript{b}, N, e, and M; serologic results were negative compared with genotype-predicted positive phenotype results. After EGA treatment of 21 samples, 14 (67%) were negative for RBC-bound IgG by tube DAT, and 7 remained positive. Using flow cytometry, EGA treatment rendered only 4 samples negative, and 17 remained positive. In the antigen testing sample set of 48 samples, 10 of 511 total antigen types tested were discordant. Discordant types were most frequent in the hypotonic saline wash sample set (N = 6). In the flow cytometry sample set, 48 percent of the samples negative by tube DAT after EGA elution had detectable RBC-bound IgG by flow cytometry. These findings suggest that caution should be taken when using phenotype results from all pretreated RBCs and support the use of RBC genotyping to predict RBC antigen expression in samples from recently transfused patients.

**Key Words:** antigen phenotype, microhematocrit separation, EDTA glycine acid (EGA), hypotonic saline wash, RBC genotyping

Red blood cell (RBC) phenotyping is valuable for transfusion management of multiply transfused patients, including the determination of the risk of forming alloantibodies. Extended phenotype matching is most commonly used in situations where there is a need to avoid sensitization in a nontransfused patient\(^1\) or to avoid further alloimmunization in a patient who has already been sensitized.\(^2\)\(^-\)\(^5\) Phenotyping of patients in these situations is often hindered by the presence of circulating donor cells or by a positive direct antiglobulin test (DAT). Because a donor RBC can survive in circulation for up to 120 days, it is not recommended to phenotype individuals who have been transfused in the past 3 months.\(^6\) In addition, phenotyping with certain types of antisera may be hindered in patients with autoantibodies causing a positive DAT. In these scenarios, methods—such as EDTA glycine acid (EGA) treatment to remove IgG, hypotonic wash to separate autologous cells from patients with sickle cell disease, or microhematocrit centrifugation to isolate reticulocytes—are often used in an attempt to obtain a phenotype.\(^6\) The effectiveness of removing IgG from RBCs to obtain DAT-negative RBCs can vary between methods.\(^7\) With the increasing availability of RBC genotyping, more blood banks are using this testing to obtain a predicted RBC phenotype as an alternative to RBC pretreatments followed by serologic antigen typing.\(^8\) A RBC genotyping panel such as the U.S. Food and Drug Administration (FDA)-approved PreciseType Molecular BeadChip\(^9\) (Immucor, Norcross, GA) can predict antigen status for many of the major clinically significant blood group systems (Table 1). Especially in patients with hemoglobinopathies, genotyping is a routine approach to obtaining an extended RBC phenotype.\(^3\)\(^,\)^\(^5\)\(^,\)^\(^10\) It has been previously documented that genotyping can provide more accurate RBC phenotype results than routine serology.\(^11\)
A small study was performed to measure, by tube and flow cytometry, the effectiveness of EGA treatment in removing IgG from RBCs. This study aimed to compare the accuracy of the results obtained from the commonly used RBC pretreatment methods with the results from a genotype-predicted phenotype using the BeadChip platform.

### Materials and Methods

This study was approved by the American Red Cross institutional review board. A total of 48 peripheral blood samples from recently transfused patients were phenotyped for RH, KEL, MNS, FY, and JK antigens (Table 2). RBC pretreatment methods included reticulocyte separation (N = 12), EGA treatment (N = 16), and hypotonic wash (N = 20). Serologic antigen typing was performed by the tube method with licensed antisera from various sources (American Red Cross, Gaithersburg, MD; Immucor). Genomic DNA was extracted from peripheral blood mononuclear cells using DNA Blood Mini Kits (Qiagen, Carlsbad, CA), and genotyping was performed per the manufacturer’s directions using the HEA BeadChip (Immucor) with a 96-well Veriti thermal cycler (Applied BioSystems, Foster City, CA), InSlideOut oven (Boekel Scientific, Feasterville, PA), and Array Imaging System (Immucor). The blood group antigens predicted by the genotyping panel are listed in Table 1. For comparison of effectiveness of EGA treatment by tube-DAT and flow cytometry, 21 samples were tested. Fourteen (67%) samples were negative for RBC-bound IgG by tube DAT and 7 were positive. When tested by flow cytometry, 4 (19%) samples were negative after EGA treatment, and 17 remained positive. Interestingly, of the 17 samples that were positive by flow cytometry, 10 were negative by tube DAT after EGA treatment.

### Discussion

This study aimed to compare antigen types obtained after commonly used RBC pretreatments to RBC genotyping using an FDA-approved test. A total of 10 discordant results were discovered in 48 samples, with discordant types identified in each of the three treatment sets and with each discordant result being a false negative by phenotyping after RBC treatment. Discordant types (N = 6) were most frequently identified in the hypotonic wash sample set (N = 20), with 25 percent of samples being discordant with one or more antigens. The total number of antigens that were tested on these samples was 248, 2.4 percent of which were found to be discordant.
Table 2. Antigen typing results [positive (+), negative (0), or weak (+w)] for reticulocyte separation samples (R-1 through R-12), EDTA glycine acid (EGA)-treated samples (E-1 through E-16), and hypotonic saline wash samples (H-1 through H-20).

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Discordant antigens are highlighted in gray with serologic result presented first and genotype-predicted phenotype after the slash. Antigens not tested by serology are indicated by NT.
One discordant type was noted in the EGA-treated sample set (N = 16), with 6 percent of samples being discordant with one or more antigens and 0.8 percent of antigens tested (N = 116) being discordant. Three discordant types were noted in the reticulocyte-separated sample set (N = 12), with 25 percent of samples being discordant with one or more antigens and 2 percent of total antigens tested (N = 147) being discordant (Figs. 1 and 2).

Two of the 10 total discordant samples (1 reticulocyte separation, 1 EGA treatment) carried the missense variant c.265C>T in the ACKR1 gene, which encodes the FY antigens. This variant causes marked weakening of the Fy(b) antigen (Fy(x) phenotype), which can be weakly agglutinated by some commercial antisera but may be missed by others and is a common cause of Fy(b) typing discrepancies. This scenario may result in interpreting a patient with Fy(x) as Fy(b–) and may complicate provision of antigen-matched RBCs for transfusion-matching protocols requiring FY status. More recently, a similar variant was found that can cause weakening of Fy(a).13

Furthermore, flow cytometry of EGA-treated RBCs suggests that samples negative by tube DAT after EGA elution may still have trace amounts of RBC-bound IgG detectable only by flow cytometry.14 It is therefore critical that controls for residual IgG coating be performed with each sample treated with EGA (if the treatment is intended to remove RBC-bound IgG for the purpose of antigen testing), since the effectiveness of the treatment on removal of IgG may be different for individual patient samples.

A recent survey of current practices for providing blood to patients with warm-reactive autoantibodies (WAA) showed that 75 percent of laboratories surveyed provided phenotype-matched or genotype-matched RBCs for transfusion, with 80 percent of laboratories using RBC genotyping as part of an antibody workup in patients with WAA.15 These findings suggest that caution should be taken when using phenotype results from treated RBCs to confirm suspected antibody specificities or to provide extended matching for future transfusions. This study did not rule out the presence of uncommon variants in the 10 samples with discordant antigen types. The discrepancies described here were associated with false-negative typings after cell treatment. False-negative results may cause the laboratory to misidentify an antibody specificity and distract the tester from identifying the true specificity, especially in patients with variant antigens when the phenotype is being used to help rule in or out certain antibodies.

For example, in our study, one sample was discrepant for e (serologic E+e–, genotype predicted E+e+); if the patient was receiving blood matched for RH antigens, e– blood would have been sourced, increasing the complexity because of the lower incidence of the e– phenotype. If this testing were to be used to provide antigen-matched blood, it could potentially cause delays in blood selection because of the perceived need for more antigens to be negative than is needed.

This study shows the advantages of using genotyping to predict RBC antigen expression and confirms that it is preferable in difficult patient samples. Our results show that RBC manipulation can result in serologic/genotypic antigen discrepancies and suggest that if extrapolated to general clinical use, additional antigen discrepancies—some with significant clinical impact—could be recognized.
Acknowledgments

The authors would like to thank the technical staff at the American Red Cross Immunohematology Reference Laboratories and the National Molecular Laboratory who tested the study samples.

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Anti-Vel alloimmunization and severe hemolytic disease of the fetus and newborn

K.J. Moise Jr., Y. Morales, M.F. Bertholf, S.N. Rossmann, and Y. Bai

Only rare cases of anti-Vel–associated mild-to-moderate hemolytic disease of the fetus and newborn have been previously reported. No case of fetal anemia requiring prenatal therapy has been noted to date. We report such a case recently encountered at our Fetal Center. Strategies are discussed for managing pregnancy complicated with alloimmunization to an antibody to a high-prevalence antigen, including sources of red blood cells for intrauterine transfusions. Immunohematology 2017;33:152–154.

Key Words: anti-Vel, Vel, red blood cell antibody, red blood cell alloimmunization, hemolytic disease of the fetus and newborn, intrauterine transfusion

The Vel red blood cell (RBC) antigen was first described in 1952.1 The antigen is ubiquitous in the general population, with only 0.04 percent of Caucasian individuals failing to exhibit expression.2 Only rare cases of anti-Vel–associated mild-to-moderate hemolytic disease of the fetus and newborn (HDFN) have been previously reported.3–7 The neonatal manifestation of HDFN in these cases was limited to hyperbilirubinemia requiring only phototherapy. No case of fetal anemia requiring prenatal therapy has been noted to date. We report such a case recently encountered at our Fetal Center.

Case Report

The patient was a 31-year-old woman (gravida/para/abortus [GPA] = G2P1000) who was referred to our Fetal Center from a neighboring state for evaluation of Vel alloimmunization. Her past history was significant, with the finding of a positive antibody detection test and identification of anti-Vel during her previous pregnancy. Based on literature reports of only mild-to-moderate HDFN in association with Vel alloimmunization, the patient’s obstetrician had elected to follow the previous pregnancy conservatively. The patient was delivered by vaginal birth at 39 6/7 weeks of gestation. The neonate was noted to exhibit severe anemia and was transferred to a tertiary level nursery. After a stormy neonatal course that was complicated by pulmonary hypertension, the neonate died on day 13 of life.

In the patient’s current pregnancy, her anti-Vel titer was 32 at 11 weeks of gestation. A repeat at our regional blood center indicated a titer of 4. Consideration was given to begin a plasmapheresis/intravenous immune globulin treatment; given the patient’s low titer, however, this was not felt to be indicated. The patient returned to her local maternal-fetal medicine specialist and was followed with weekly middle cerebral artery Doppler ultrasounds to measure the peak systolic velocity (MCA-PSV). She was encouraged to donate autologous RBC units in anticipation of a possible need for intrauterine transfusion later in gestation. The patient was able to donate 4 units; these were kept refrigerated for 3–5 days and then frozen at the local blood center. At 24 weeks of gestation, the MCA-PSV became elevated to 1.89 multiples of the median (MoM), and the patient was referred back to our Fetal Center (>1.5 MoM is indicative of severe fetal anemia8). A repeat MCA-PSV was 2.0 MoM, and the repeat maternal anti-Vel titer was 16. A decision was made to proceed with intrauterine transfusion. Arrangements were made for transport of the previously donated frozen maternal RBC units to our regional blood center. Initial sampling at the time of cordocentesis at 24 1/7 weeks of gestation revealed a fetal hematocrit (Hct) of 11.8 percent (normal 36%) with a reticulocyte count of 14.7 percent (normal <2%). RBC typing indicated the fetus to be Vel+. One unit of maternal autologous RBCs was deglycerolized, irradiated, washed, and reconstituted with normal saline to achieve an Hct of 72 percent in the final blood product. A 60-mL intravascular transfusion combined with a 40-mL intraperitoneal transfusion was performed successfully. A total of five intrauterine transfusions were subsequently undertaken (Fig. 1). The patient donated 3 additional RBC units during the remainder of the pregnancy and maintained an Hct above 32 percent, with oral iron and folate supplementation. The additional RBC units for intrauterine transfusion came from a single local blood donor (2 frozen, 1 fresh) and another rare registry donor (1 fresh). The patient was delivered uneventfully by repeat caesarean section at 37 6/7 weeks of gestation of a 4235-gram male infant with Apgar scores of 8 and 9. The infant was noted to have an Hct of 46.2 percent (normal 53%) with a
Anti-Vel with severe HDFN

A reticulocyte count of 2 percent (normal <7%) and a Kleihauer-Betke stain indicating 3.7 percent fetal cells remaining in the infant's circulation (normal 100%). The neonatal course was benign and required only treatment with bili light therapy for 3 days; the maximum total bilirubin was 10.6 mg/dL (normal 6.5 mg/dL). He was discharged on day 7 of life. The infant was followed weekly by a local pediatric hematologist. He required one top-up transfusion at 5 weeks of age and was released by the pediatric hematologist at 9 weeks of age.

Discussion

Our case illustrates at least three important points. Vel is expressed on fetal RBCs as early as 12 weeks of life; its strength appears to be equivalent to that on adult RBCs. Because alloimmunization to this antigen is so rare and only a few case reports of HDFN have been reported to date, it is difficult to determine a “critical titer” for this entity. Often, anti-Vel is only IgM, which would not present a risk for HDFN because transplacental passage cannot occur. Linz et al. recommended treatment with 2-mercaptoethanol or dithiothreitol to determine whether an IgG component is present. Both IgG and IgM had been detected in our case. A predominance of IgG in our case may well explain the severe degree of HDFN. One should assume that a paternal Vel antigen will be inherited by the fetus, given that Vel is a high-prevalence antigen. Thus, it would appear prudent to institute serial MCA-PSV determinations in the early second trimester in the rare case that anti-Vel is detected during pregnancy.

The strategy in obtaining sufficient RBC units for intrauterine transfusion to treat the HDFN is also an essential element of our case. The high prevalence of Vel makes finding Vel− donors extremely difficult. The patient was evaluated before pregnancy, and no sibling or family member was identified as a potential donor. A search by our regional blood center found only one eligible donor locally. The patient was instructed to undergo autologous donations in the early part of pregnancy and was able to donate 4 units. Iron and folate supplementation was initiated in advance. We previously reported a series of 21 patients who donated 77 units of autologous blood as a source of RBCs for intrauterine transfusion in the pre-HIV testing era. Three of the patients in that series were able to donate 6 units with maintenance of their baseline Hct levels. In our case, we were able to have our patient donate 7 units while maintaining her Hct with hematopoietic pharmacologic supplementation. Because freshly donated RBCs are typically preferred as a source for intrauterine transfusion, we defined the specific time for the final 3 units of autologous donation and arranged allogeneic donation times so that fresh blood was used for the scheduled intrauterine transfusions. Our experience in timed blood preparation would be useful in managing similar patients.

Finally, at delivery, we had multidisciplinary communication. To prepare for the urgent need for blood as well as to prevent wasting the rare blood product, we secured 2 frozen autologous compatible RBC units in our regional blood center before the scheduled cesarean delivery. In the event of an acute postpartum hemorrhage, we developed a plan with our blood bank for the emergency release of ABO-compatible RBCs pending the deglycerolization of these frozen units.
Acknowledgments

The authors would like to thank the blood bank staff at the Memorial Hermann Hospital at the Texas Medical Center and the Gulf Coast Regional Blood Center for their assistance in diagnostic testing and blood procurement and processing.

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Separation of multiple antibodies by adsorption with allogeneic red blood cells

E.M. Ekema

Antibody detection and identification are processes that are commonly performed in the transfusion service before transfusion of allogeneic red blood cells (RBCs). Antibody identification usually follows the discovery of a positive antibody detection test, or other factors such as ABO serum/cell discrepancy or an incompatible crossmatch. Antibody identification is a necessary practice in blood banking to determine the suitability of blood products for transfusion on an individual basis. When the presence of multiple antibodies is suspected, several methods, including neutralization of patient’s plasma, titration, elution, chemical or enzyme treatment of reagent RBCs, and adsorption with allogeneic RBCs, may be used to separate and properly identify other atypical antibodies that are present in a single serum or plasma sample. This review will focus on the use of allogeneic adsorption to identify antibody specificities in a patient’s sample. Immunohematology 2017;33:155–158.

Key Words: multiple antibodies, adsorption, allogeneic red blood cells, antibody identification

Principle

Antibody detection and identification are processes that are commonly performed in the transfusion service before the transfusion of allogeneic red blood cells (RBCs). Antibody identification usually follows the discovery of a positive antibody detection test, or other factors such as ABO serum/cell discrepancy or incompatible crossmatch. Antibody identification is a necessary practice in blood banking to determine blood products that are suitable for transfusion to an individual. Routinely, antibody identification practices in a blood bank comprise the testing of a patient’s plasma against reagent RBCs using standard agglutination and indirect antiglobulin methods. There are, however, instances when identification of multiple antibodies may be complicated and require additional serologic methods. When the presence of multiple antibodies is suspected, several methods—including neutralization of patient’s plasma, titration, elution, chemical or enzyme treatment of reagent RBCs, and adsorption with allogeneic RBCs—may be used to separate and properly identify antibodies that are present in a single serum or plasma sample.

Reagents/Supplies

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<td>• Allogeneic red blood cells positive for antigen to which antibody is directed against, and antigen negative for other suspected antibody(ies)</td>
<td>• 1 mL graduated, disposable pipettes</td>
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<td>• Phosphate-buffered saline</td>
<td>• 37°C incubator</td>
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<tr>
<td>• Proteolytic enzyme</td>
<td>• Test tubes</td>
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<td>• Centrifuge</td>
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Procedural Steps

• Select test RBCs to be used for allogeneic adsorption.
• Wash the test RBCs three to four times with phosphate-buffered saline.
• Remove all supernatant saline from the washed RBCs.
• If enzyme treatment is desired, follow manufacturer’s directions and treat the RBCs.
• Wash the RBCs three to four times after treatment with enzymes.
• Add 1 volume serum or plasma to 1 volume washed, packed RBCs.
• Stopper, gently mix well, and incubate for 30–60 minutes at 37°C.
• Centrifuge for 5 minutes and remove the plasma/serum. Test against fresh sample of adsorbing cells to see if adsorption is complete.
• Repeat the procedure with fresh adsorbing cells if adsorption is incomplete.

RBCs = red blood cells.

Performing pretransfusion testing with a plasma or serum sample that contains multiple antibodies can present serologic challenges. Differentiating and properly identifying antibody specificities that are present in a sample containing multiple antibodies can be achieved by adsorption of patient plasma or serum with allogeneic RBCs. Allogeneic adsorption is used in blood banking to remove and/or separate antibody specificities from a plasma or serum sample with the use of RBCs that express the corresponding antigen and that are antigen-negative for other antibodies.

If the patient’s phenotype is known, adsorptions with allogeneic RBCs that are phenotypically similar to the patient’s RBCs may be used. This process must ensure that adsorption
cannot remove any antibody(ies) that the patient may form based on his or her RBC phenotype. For example, if the patient types as C–, E–, K–, Fy(a–), Fy(b–), Jk(b–), and S–, the adsorbing cells must be negative for these antigens so that, if present, antibodies to these antigens will not be adsorbed onto the adsorbing cells. It is important to note, however, that the use of phenotypically similar cells for adsorption carries the inherent risk of adsorbing out alloantibodies to variant antigens. This method should be used with extreme caution.

A preferred method is the use of differential adsorptions using a set of three different adsorbing cells with known phenotypes. Generally, one cell should be R1R1, another should be R2R2, and the third cell should be rr. Treating the RBCs to be used for allogeneic adsorption with proteolytic enzymes can also be performed to enhance the adsorption process. Treatment with enzymes will destroy certain antigens that are sensitive to enzyme treatment such as antigens in the Duffy and MNS blood group systems.

Application

Adsorption with allogeneic RBCs presents antibody(ies) to the corresponding antigen-positive RBCs under optimal conditions so that the antibody will attach to the antigen, thereby removing the antibody from the serum or plasma.3 Allogeneic adsorption methods can be used to confirm an antibody specificity, separate multiple antibodies present in a single plasma or serum, remove autoantibody to permit the detection of underlying alloantibodies, remove unwanted antibody from serum that is suitable for the preparation of typing reagents, and assist in detecting the presence of weak antigens on RBCs.4

When all or most cells of an antibody panel react at different strengths or different phases in conjunction with a negative autocontrol, the presence of multiple antibodies in the patient’s plasma or serum should be suspected, especially if the reactivity does not fit a single antibody using inclusion and exclusion methods.1–5 It is important to exclude the presence of a possible single antibody to a high-prevalence antigen that may be showing dosage on an RBC panel. If the patient’s phenotype is known or can be obtained, perform testing using a selected cell panel that is similar to the patient’s phenotype. Several selected cell panels may be necessary to resolve the antibody identification issue. There are instances where the separation and identification of multiple antibodies cannot be attained using the methods described here because of insufficient reagent RBCs to exclude or include all common alloantibodies. In this instance, other methods such as adsorption of known antibodies with allogeneic RBCs can be performed, and the adsorbed plasma can then be used for the identification of other antibodies.

In the presence of multiple antibodies in a single plasma or serum sample, allogeneic adsorptions are used to confirm or exclude suspected antibodies against RBC antigens that may be masked by an antibody of known specificity. For example, if the RBCs of a patient type as E– and the patient’s serum contains anti-U, it will be statistically difficult to find sufficient U–E+ RBC samples to confirm or exclude the presence of anti-E. In this scenario, the use of allogeneic adsorptions will be necessary to remove anti-U from the test plasma or serum, leaving the adsorbed plasma with anti-E, if present. The adsorbed plasma can then be tested against E+U+ and E–U+ cells to determine if the patient’s plasma contains anti-E.

If there is no known antibody specificity in the patient’s plasma, differential adsorptions may be necessary to exclude or include all the common RBC antigens.

Procedure

Select group O allogeneic RBCs to be used for adsorption that are positive for the antigen against which the antibody to be removed is directed (for plasma with known antibody specificity) and negative for the other antibodies that are suspected to be present in a single plasma or serum sample. When there is no known antibody specificity in the test plasma and the patient’s phenotype is known, select group O allogeneic RBCs that are phenotypically similar to the patient’s RBCs. Note that the use of phenotypically similar cells for adsorption carries the inherent risk of adsorbing out alloantibodies to variant antigens and should be used with extreme caution. For patients with unknown phenotypes, select a set of three different RBCs with known phenotypes. The group O RBCs selected should be R1R1, R2R2, and rr. One of the cells must be negative for K, another negative for Jk, and the third negative for Jk to ensure that alloantibodies to common RBC antigens will be excluded.6 Adsorption can be performed with RBCs that are untreated or treated with proteolytic enzymes. If adsorption is performed with untreated RBCs, one of the cells selected should be negative for S and Fya or Fyb.7

Obtain an aliquot of the selected RBCs and wash at least three times with saline. After the last wash, centrifuge the RBCs for 5 minutes and remove the supernatant saline using disposable pipettes. Add 1 volume of washed, packed RBCs to 1 volume of the patient’s plasma or serum and mix gently. Incubate at 37°C for 30–60 minutes. Mix the serum or plasma/
cell mixture periodically throughout the incubation period to enhance adsorption.8

After the incubation period has elapsed, centrifuge the serum or plasma/cell mixture for 5 minutes to pack the RBCs. Remove the supernatant fluid (adsorbed plasma) with a disposable pipette and dispense into a clean, properly marked test tube. Discard the RBCs if an eluate will not be prepared. If an elution study is indicated, save the RBCs. An elution study can be performed using the first adsorbing cell, or sets of adsorbing cells, to determine the antibody specificity that has been removed by adsorption. Test an aliquot of the adsorbed plasma or serum with an aliquot of fresh adsorbing cells to see if adsorption is complete. This testing should be performed by the same method that will be used to test the reagent panel cells with the adsorbed plasma. For example, if the adsorbed plasma/reagent cell mixture will be enhanced by low-ionic-strength saline (LISS) or polyethylene glycol (PEG), the same method should be used for testing of the adsorbed plasma with adsorbing cells. If adsorption is complete, no reactivity will be observed. If reactivity is observed, adsorption is incomplete. For incomplete adsorption, repeat the process using a fresh volume of washed, packed RBCs until complete adsorption is attained. Once complete adsorption is observed, test the adsorbed plasma with a panel of reagent RBCs to exclude other underlying alloantibodies.

It is important to know which antibody specificities may be present in each adsorbed plasma. R,R,R allogeneic RBCs adsorb anti-D, -C, and -e and other antibodies to high-prevalence antigens. Specificities left behind are anti-c and -E. R,R,R allogeneic RBCs adsorb anti-D, -C, and -E and other antibodies to high-prevalence antigens. Specificities left behind are anti-C and -E. The rr cells adsorb anti-c and -e and antibodies to high-prevalence antigens. Specificities left behind are anti-D, -C, and -E. In addition to these antibodies, alloantibodies detecting antigens that are absent from the adsorbing cells will also be left behind in the adsorbed plasma. The adsorbed plasma can be tested with or without enhancements such as LISS or PEG.

It is vital to note that adsorption is more effective if the area of contact between the plasma or serum and RBCs is large. This effect can be obtained by using large-bore test tubes (16 × 100 mm) for adsorption.9 To completely remove an unwanted antibody it may be necessary to perform multiple adsorptions, although there is an increased risk of diluting the serum with each successive adsorption, which can lead to weak or false-negative reactions. The number of adsorptions necessary to completely remove an unwanted antibody can be suggestively determined by the strength of reactivity on the initial antibody panel. Theoretically, the total adsorptions needed to completely remove an unwanted antibody is the strength of reactivity on the initial panel plus one. For example, if the initial panel's reactivity is 3+, perform four adsorptions.10 Repeat adsorptions should always be performed with a fresh volume of RBCs and not the RBCs already used for prior adsorptions.

Pretreating the RBCs used for allogeneic adsorptions with proteolytic enzymes will reduce the number of adsorptions needed for complete removal of unwanted antibodies because enzyme treatment of cells enhances the uptake of antibodies against enzyme-resistant antigens. The use of a higher cell-to-serum ratio can help the adsorption process by increasing the amount of antigens able to adsorb the target antibody.6

**Limitations**

Adsorption with allogeneic RBCs does not only remove targeted antibodies but also removes antibodies to high-prevalence antigens when they are present in the test plasma or serum. RBCs used for allogeneic adsorptions are presumed to be positive for all high-prevalence antigens. If the test plasma or serum happens to contain an antibody to any of the high-prevalence antigens, that antibody will be adsorbed onto the RBCs. In this case, the antibody will not be detected in the adsorbed plasma but can be recovered in an eluate prepared from the adsorbing cells.

Adsorption with allogeneic RBCs is a time-consuming process that may take several hours to complete if multiple adsorptions are required, and this step may be contraindicated for a patient who is in critical need of blood transfusion. When allogeneic adsorption is indicated for such a patient, transfusion of phenotype-matched units may be necessary while testing is in process.

Adsorptions pose the risk of diluting the adsorbed plasma, thereby weakening the strength of antibody(ies) present. Weak-reacting antibodies may be completely missed when testing adsorbed plasma. To attenuate the possibility of missing a weak-reacting antibody in an allogeneic adsorbed plasma, the number of adsorptions should be limited to a maximum of six. Increasing the ratio of adsorbed plasma to reagent RBCs during the testing phase increases the chances of identifying weak-reacting alloantibodies.

**Quality Control**

To ensure that allogeneic adsorption is complete, test the adsorbed plasma with a freshly prepared, untreated, 3–5
percent suspension of allogeneic RBCs used for adsorption with or without enhancement. If reactivity persists, adsorption is not complete. A negative result proves that adsorption is complete, and the adsorbed plasma can be used for testing with RBC panels. However, it is important to note the phenotype of the adsorbing cells when testing for completeness. If anti-Jk², for example, is present in the plasma, adsorption with allogenic cells that are Jk(a⁻) will not remove this antibody from the plasma, and the adsorbed plasma will not react with the untreated cells that are Jk(a⁻).

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References

Hemovigilance and the Notify Library

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

Hemovigilance systems allow reporting of adverse occurrences associated with blood transfusion to a central database where events can be reviewed and analyzed for the benefit of patients and donors. Hemolytic and serologic transfusion reactions are among the many types of reactions reported to these systems. The Notify Library, a database of adverse events associated with medical products of human origin, has incorporated hemovigilance into its didactic resources. Students and practitioners are encouraged to use the electronic library and to further enhance this resource through review and recommendation of additional publications in the area of immunohematology. *Immunohematology* 2017;33:159–164.

**Key Words:** biovigilance, hemovigilance, transfusion reactions, hemolytic transfusion reactions, serologic transfusion reactions

Advances in science and health care technology have led to the development of replacement medicine, with many human body components being collected for the preparation of medical products of human origin (MPHO). These collections encompass a wide range of medical products—from cells (e.g., human stem cells from peripheral blood, bone marrow, or cord blood; gametes) and tissues (e.g., corneas, musculoskeletal tissues) to blood and blood components and organs, from anatomical components to secretions (e.g., breast milk) and excretions—all originating from the human body. Donated by a human with the goal of benefitting others, these MPHO have indeed saved and improved human lives through their clinical application.

Vigilance is a powerful tool for improving safety and quality. Vigilance includes monitoring and reporting adverse events and outcomes associated with therapeutic treatments. Originating from hemovigilance for blood components, *biovigilance* is the term used for the monitoring of adverse outcomes associated with all MPHO. Sharing the lessons learned from adverse outcomes can allow for significant process improvements for the greater protection of donors and patients. These benefits apply not only to the institution where the incident occurred but also to other institutions where an identical or similar incident might occur. Detection, investigation, and communication of adverse events provide the transparency and openness that these uniquely sourced medical treatments demand.

The World Health Organization (WHO) promotes the governance of MPHO in a manner that acknowledges their exceptional nature. From donation to the follow-up care of the recipient, MPHO have a shared exposure to risks from breaches of ethical, legal, and safety standards—for example, the risk of disease transmission and consequent morbidity or mortality. Ensuring the protection of the donor, the recipient, and society as a whole requires establishment of globally consensual principles to govern the use of MPHO, such as the noncommercial nature of the human body and its parts and strict traceability associated with vigilance and surveillance. The Notify Library is the first WHO initiative that covers vigilance across the full MPHO scope.

**Hemovigilance**

Hemovigilance of blood transfusions encompasses a set of surveillance procedures that cover the entire collection to transfusion process: from the donor and donation, to the processing of the donor’s blood and its components, to their provision and transfusion to patients, to patient follow-up. Hemovigilance includes the monitoring, reporting, investigation, and analysis of adverse events related to the donation, processing, and transfusion of blood, as well as the development and implementation of recommendations to prevent occurrence or recurrence.

Hemovigilance systems arose as a response to the threat to the blood supply from emerging infections, such as HIV and the hepatitis viruses. Hemovigilance was first developed in Japan and then in France in 1993, featuring mandatory reporting. The UK developed the first voluntary system in 1996, called Serious Hazards of Transfusion (SHOT). Over the years, systematic analyses from these systems and subsequent process improvements have led to enhanced patient safety. In 2002, the European Union (EU) Commission Directive 2002/98/EC set standards of quality and safety for the collection, testing, processing, storage, and distribution of human blood and blood components and amended Directive 2001/83/EC, thereby mandating hemovigilance in the EU. Subsequently, other countries around the world have established hemovigilance systems, adopting either active or passive hemovigilance reporting. Systems engaging in active
hemovigilance require a confirmation of a transfusion reaction or the lack thereof after every transfusion. In 1998, the European Haemovigilance Network was established; in 2009, it evolved into the International Haemovigilance Network to share common definitions, findings, and interventions and to promote transfusion safety worldwide.

The Haemovigilance Working Party of the International Society of Blood Transfusion (ISBT) has developed transfusion reaction definitions (Tables 1 and 2) and imputability criteria (Table 3). Imputability is the strength of the relationship between the transfusion and the adverse transfusion reaction and is usually determined through a thorough investigation of the adverse reaction. Standard definitions for hemolytic transfusion reactions include acute hemolytic transfusion reactions (AHTRs), delayed hemolytic transfusion reactions (DHTRs), and delayed serologic transfusion reactions (DSTRs) (Table 1). Non-hemolytic transfusion reactions are described in Table 2.

**Notify Library**

The Notify Library is a database developed through a collaboration of WHO and the Italian National Transplant Center (CNT). It supports the sharing of published vigilance information for teaching purposes and for greater public transparency on the use of MPHO. The library aims to support three key audiences: the general public (especially potential donors or recipients), health professionals working in donor detection and selection or in the clinical application of MPHO, and health authorities responsible for vigilance systems. It aims to be comprehensive, describing all types of reactions or events that might have teaching value and assist in the estimation of risk.

The core of the Notify Library Web site (www.notifylibrary.org) includes an online publicly accessible relational database of adverse occurrences collected and analyzed by dedicated editorial groups of international experts, regulators, and clinicians. The database is not a vigilance reporting program but rather a collection of information identified primarily by review of published articles in scientific journals and/or books. It also includes case reports from regulatory or professional vigilance programs (gray literature). Sources include events that may have occurred in procurement and processing, as well as in the clinical application, of blood, organs, tissues, and cells used in transfusion, transplantation, and assisted reproduction. For each adverse occurrence type, at least one reference is cited. The project’s collaborating international experts provide a structured analysis, focused in particular on how the adverse occurrence was recognized and how it was shown to have been associated with the donation, processing, or clinical application of MPHO. Categories of occurrences that are analyzed include transmitted infections, malignancies, donor reactions, clinical complications, and process-associated incidents.

Currently, each database entry is given a title and a unique numeric identifier. Through the case analysis, the entry is assigned an adverse occurrence type; an MPHO type; the time to detection; alerting signs, symptoms, and evidence of occurrence; the estimated frequency; demonstration of imputability and imputability grade; keywords; copy of the reference; and any expert comments about the reference or occurrence. To facilitate a structured database search, all cases have been classified according to a taxonomy of two main

**Table 1. Hemolytic transfusion reaction definitions developed by the Haemovigilance Working Party of the International Society of Blood Transfusion**

<table>
<thead>
<tr>
<th>Adverse transfusion reaction</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic transfusion reactions (general)</td>
<td>A hemolytic transfusion reaction is one in which symptoms and clinical or laboratory signs of increased red cell destruction are produced by transfusion. Hemolysis can occur intravascularly or extravascularly and can be immediate (acute) or delayed.</td>
</tr>
<tr>
<td>Acute hemolytic transfusion reaction (AHTR)</td>
<td>AHTR has its onset within 24 hours of a transfusion. Clinical or laboratory features of hemolysis are present. Not all clinical or laboratory features are present in cases of AHTR. Blood group serology usually shows abnormal results but absence of immunological findings does not exclude AHTR. AHTR may also be due to erythrocyte auto-antibodies in the recipient or to non-immunological factors like mechanical factors inducing hemolysis (malfunction of a pump, of a blood warmer, use of hypotonic solutions, etc.).</td>
</tr>
<tr>
<td>Delayed hemolytic transfusion reaction (DHTR)</td>
<td>DHTR usually manifests between 24 hours and 28 days after a transfusion and clinical or laboratory features of hemolysis are present. Signs and symptoms are similar to AHTR but are usually less severe. DHTR may sometimes manifest as an inadequate rise of post-transfusion hemoglobin level or unexplained fall in hemoglobin after a transfusion. Blood group serology usually shows abnormal results.</td>
</tr>
<tr>
<td>Delayed serologic reaction (DSTR)</td>
<td>There is a DSTR when, after a transfusion, there is demonstration of clinically significant antibodies against red blood cells which were previously absent (as far as is known) and when there are no clinical or laboratory features of hemolysis. This term is synonymous with alloimmunization.</td>
</tr>
</tbody>
</table>
Non-hemolytic transfusion reaction definitions developed by the Haemovigilance Working Party of the International Society of Blood Transfusion

<table>
<thead>
<tr>
<th>Adverse transfusion reaction</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile non hemolytic transfusion reaction (FNHTR)</td>
<td>There is a FNHTR in the presence of one or more of the following: fever (≥38°C oral or equivalent and a change of ≥1°C from pretransfusion value), chills/rigors. These may be accompanied by headache and nausea. Symptoms occurring during or within four hours following transfusion without any other cause such as hemolytic transfusion reaction, bacterial contamination or underlying condition. FNHTR could be present in absence of fever (if chills or rigors without fever).</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>An allergic reaction may present only with mucocutaneous signs and symptoms: morbilliform rash with pruritus; urticaria (hives); localized angioedema; edema of lips, tongue, and uvula; periorbital pruritus, erythema and edema; conjunctival edema. Occurring during or within 4 hours of transfusion. In this form it usually presents no immediate risk to life of patient and responds quickly to symptomatic treatment like antihistamine or steroid medications. This type of allergic reaction is called ‘minor allergic reaction’ in many hemovigilance systems. For the purpose of classification this type of allergic reaction would be graded as 1, i.e. non-severe.</td>
</tr>
<tr>
<td>Transfusion associated graft-versus-host disease (TA-GVHD)</td>
<td>TA-GVHD is a clinical syndrome characterized by symptoms of fever, rash, liver dysfunction, diarrhea, pancytopenia, and findings of characteristic histological appearances on biopsy occurring 1–6 weeks following transfusion with no other apparent cause. The diagnosis of TA-GVHD is further supported by the presence of chimerism.</td>
</tr>
<tr>
<td>Post-transfusion purpura (PTP)</td>
<td>PTP is characterized by thrombocytopenia arising 5–12 days following transfusion of cellular blood components with findings of antibodies in the patient directed against the human platelet antigen (HPA) system.</td>
</tr>
<tr>
<td>Transfusion-related acute lung injury (TRALI)</td>
<td>In patients with no evidence of acute lung injury (ALI) prior to transfusion, TRALI is diagnosed if a new ALI is present (all five criteria should be met): 1) Acute onset 2) Hypoxemia of PaO2/FiO2 &lt;300 mmHg, or oxygen saturation of &lt;90% on room air, or other clinical evidence 3) Bilateral infiltrates on frontal chest radiograph 4) No evidence of left atrial hypertension (i.e., circulatory overload) 5) No temporal relationship to an alternative risk factor for ALI, during or within 6 hours of completion of transfusion Alternate risk factors for ALI include: direct lung injury (aspiration, pneumonia, toxic inhalation, lung contusion, near drowning) or indirect lung injury (severe sepsis, shock, multiple trauma, burn injury, acute pancreatitis, cardiopulmonary bypass, drug overdose). Note: It has been suggested by the Toronto TRALI Consensus Panel to add a category of possible TRALI that would have the same definition as TRALI except for the presence of a temporal relationship to an alternative risk factor for ALI (as described earlier). In such a circumstance, TRALI should be indicated with a possible imputability to transfusion. TRALI is therefore a clinical syndrome and neither presence of HLA or HNA antibodies in donor(s) nor confirmation of cognate antigens in recipient is required for diagnosis.</td>
</tr>
<tr>
<td>Transfusion associated circulatory overload (TACO)*</td>
<td>TACO is characterized by any 4 of the following occurring within 6 hours of completion of transfusion: 1) Acute respiratory distress 2) Tachycardia 3) Increased blood pressure 4) Acute or worsening pulmonary edema on frontal chest radiograph 5) Evidence of positive fluid balance. An elevated brain natriuretic peptide (BNP) is supportive of TACO.</td>
</tr>
<tr>
<td>Transfusion associated dyspnea (TAD)</td>
<td>TAD is characterized by respiratory distress within 24 hours of transfusion that does not meet the criteria of TRALI, TACO, or allergic reaction. Respiratory distress should be the most prominent clinical feature and should not be explained by the patient’s underlying condition or any other known cause.</td>
</tr>
<tr>
<td>Hypotensive transfusion reaction</td>
<td>This reaction is characterized by hypotension defined as a drop in systolic blood pressure of ≥ 30 mm Hg occurring during or within one hour of completing transfusion and a systolic blood pressure ≤ 80 mm Hg.</td>
</tr>
<tr>
<td>Haemosiderosis</td>
<td>Transfusion-associated haemosiderosis is defined as a blood ferritin level of ≥ 1000 micrograms/L, with or without organ dysfunction in the setting of repeated RBC transfusions.</td>
</tr>
</tbody>
</table>
Table 2. Non-hemolytic transfusion reaction definitions developed by the Haemovigilance Working Party of the International Society of Blood Transfusion² (continued)

<table>
<thead>
<tr>
<th>Adverse transfusion reaction</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperkalemia</td>
<td>Any abnormally high potassium level (&gt; 5 mmol/L or ≥1.5 mmol/L net increase) within an hour of transfusion can be classified as a transfusion-associated hyperkalemia.</td>
</tr>
<tr>
<td>Unclassifiable complication of transfusion (UCT)</td>
<td>Occurrence of an adverse effect or reaction temporally related to transfusion, which cannot be classified according to an already defined reaction type and with no risk factor other than transfusion and no other explaining cause.</td>
</tr>
</tbody>
</table>

*The definition of TACO is currently undergoing revision by the working party.

HLA = human leukocyte antigen; HNA = human neutrophil antigen; RBC = red blood cell.

Table 3. Imputability criteria developed by the Haemovigilance Working Party of the International Society of Blood Transfusion²

<table>
<thead>
<tr>
<th>Imputability category*</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite (certain)</td>
<td>There is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to the transfusion.</td>
</tr>
<tr>
<td>Probable (likely)</td>
<td>The evidence is clearly in favor of attributing the adverse event to the transfusion.</td>
</tr>
<tr>
<td>Possible</td>
<td>The evidence is indeterminate for attributing the adverse event to the transfusion or an alternate cause.</td>
</tr>
<tr>
<td>Unlikely (doubtful)</td>
<td>The evidence is clearly in favor of attributing the adverse event to causes other than the transfusion.</td>
</tr>
<tr>
<td>Excluded</td>
<td>There is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to causes other than the transfusion.</td>
</tr>
</tbody>
</table>

*Only definite, probable, and possible cases should be used for international comparisons.

Fig. 1 Adverse occurrence type taxonomy (extract). AHTR = acute hemolytic transfusion reaction; DHTR = delayed hemolytic transfusion reaction; DSTR = delayed serologic transfusion reaction; Detr. Imm. = detrimental immunization; GvHD = graft-versus-host disease; PTP = post-transfusion purpura; TRALI = transfusion-related acute lung injury.
groups—adverse occurrence type (harm to a recipient, harm to a donor, harm to a fetus or offspring, risk of harm) and MPHO type (organs, blood, cells, tissues, etc.). Figure 1 provides an extract of the taxonomy for adverse occurrence type. For example, immunological complications are further categorized into AHTRs, allergic reactions, DHTRs, DSTRs, detrimental immunization, graft-versus-host disease, post-transfusion purpura, rejection, and transfusion-related acute lung injury (TRALI). Figure 2 provides the taxonomy applied for MPHO type. This predefined classification enables searching by adverse occurrence type, by MPHO type, or both, as well as by using free text or key words.

Of the current searchable entries in the Notify Library, 68 percent are classified as harm to recipient, 18 percent are classified as harm to donor, 13 percent are classified as risk of harm, and the remaining 1 percent are classified as harm to fetus or offspring. Not surprisingly, among entries relating to recipient harm, 52 percent are subsequently categorized as relating to infection and 17 percent as relating to immunological complications. Of the 331 reports of adverse occurrences associated with the MPHO of blood, 57 percent are related to transfusion of red blood cells, 21 percent to platelets, 11 percent to plasma, 7 percent to whole blood, and 1 percent to granulocytes; the remaining are not specified. Of the 89 percent of blood reports of the occurrence type harm to recipient, 49 percent of reports are immunological in nature (Fig. 3), including DHTRs, AHTRs, DSTRs, TRALI, and allergic reactions.
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Current Notify Library Activities

Competent authorities for blood, tissues, and cells in the 28 countries of the EU have been approached through the Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation (VISTART) Joint Action supported by the European Commission and coordinated by CNT (since VISTART includes a specific Work Package on Vigilance Communication dedicated to the Notify Library). Moreover, a directory of vigilance is being created through the WHO regional offices to associate all competent authorities for MPHO at a global level to facilitate member states’ access and contribution to the Notify project, building a global network of experts who are learning from each other.

Since the beginning of the project, annual consultations have been organized and, thanks to the collaboration of all editorial group members, the database is regularly updated and the Web site continues to improve. Further tools to support vigilance have been developed over the years, including a comprehensive vigilance document available for download through the related section (Notify Booklet, currently in revision for the inclusion of hemovigilance data) and the ability to access a panel of experts providing support and advice on request via the Notify Web site.

Invitation to Join and Contribute

The Notify Library and the team of professionals associated with its creation and maintenance are a global resource in the area of biovigilance that encourages broad global participation in the project. Access is open to the public. Individual users are encouraged to search and review the contents of the Library. Clinical and laboratory practitioners and students are invited to identify information that might be still missing, to refer new cases, propose new entries, involve students and clinicians from domains outside transfusion and transplantation, recommend references, and even join an editorial group to review new cases. All organizations, authorities, or professional societies that work on the safety and quality of MPHO are invited to support and contribute to this didactic initiative.

References


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Anti-M is a frequently detected naturally occurring antibody that has been reported in various clinical settings and also in voluntary donors. We describe here the clinical and laboratory findings of 11 cases with anti-M detected at our center. This report is a retrospective study in which we reviewed our immunohematology laboratory records for cases involving anti-M. Both donor and patient data from a 28-month period (September 2014 to December 2016) were reviewed. During this period, 11 examples of anti-M were detected (8 patients, 1 voluntary whole blood donor, and 1 hematopoietic stem cell donor. Anti-M was also detected in one external quality assessment scheme sample received during this period. In conclusion, anti-M can be detected in various clinical settings. This antibody can be clinically significant; in the laboratory, it can present as a serologic problem such as an ABO group discrepancy or an incompatible crossmatch. After detection, management and course of action is determined by both the antibody characteristics and the clinical setting.

**Materials and Methods**

This report is from a tertiary care hemato-oncology center in eastern India treating patients with hematologic malignancies and solid tumors (i.e., gynecologic, gastrointestinal, head and neck, and soft tissue tumors). Pretransfusion antibody detection testing and crossmatch is performed for all RBC requests. Hematopoietic stem cell transplantations are performed in patients with hematologic as well as nonhematologic disorders. The pretransplant evaluation includes ABO blood grouping, extended RBC phenotyping, antibody detection testing, direct antiglobulin test (DAT), and autocontrol in both donor and recipient. In addition, major and minor crossmatches are performed. ABO grouping is performed by a conventional tube technique (CTT) with anti-A, anti-B, and anti-D reagents (Immucor India, New Delhi, India), and plasma ABO testing is performed using in-house reagent RBCs (A, B, and O). A second anti-D reagent (Tulip Diagnostics, Goa, India) is also tested. Antibody detection testing and identification is performed by the column agglutination technique (CAT) (Biovue System; Ortho Clinical Diagnostics, Raritan, NJ) using anti-IgG glass bead cards and commercially available 3-cell panels (Surgiscreen; Ortho Clinical Diagnostics) and 11-cell panels (Resolve Panel A; Ortho Clinical Diagnostics). Whenever necessary, the same is repeated in neutral or reverse diluent cards at 4°C. DAT, autocontrol, and crossmatch are performed by CAT. Extended phenotyping is performed by CTT using commercially available monoclonal antisera from Ortho Clinical Diagnostics. Titer is performed by the double dilution method using double-dose M+ (M+N–) group O RBCs. Standard validated methods are used.

**Results**

Over a period of 28 months (September 2014 to December 2016), 11 cases of anti-M were detected, comprising eight patients, one donor, one hematopoietic stem cell transplant donor, and one external quality assessment scheme (EQAS) sample containing anti-M. The patient/donor ages ranged from 2.5 to 82 years. Patient diagnoses and clinical profiles of the cases are given in Table 1.
Immunohematologic Features of Anti-M in Patients

An ABO blood group discrepancy was noted in six of the eight patients, where anti-M interfered in the reverse grouping as detected by an extra reaction with O reagent RBCs. In five of these patients, this discrepancy was resolved by repeating the reverse group with a 15-minute incubation at 37°C. In one patient, the discrepancy was resolved by using M– reagent RBCs in reverse grouping.

Specificity of the antibody was determined in seven patients by antibody detection testing and identification at 37°C. In one patient, anti-M was detected at 4°C. A dosage effect of anti-M, showing stronger agglutination strength with double-dose M+ reagent RBCs, was observed in four patients.

Antibody detection testing using serum treated with 0.01 M dithiothreitol (DTT) (Himedia Laboratories, Mumbai, India) was performed on samples from five patients. In all these patients, the agglutination strength decreased but did not disappear, suggesting the presence of both IgG and IgM components in the antibody. In two patients, DTT treatment of the serum could not be performed, and the antibody detection testing was not repeated by a prewarmed test. Hence, whether this finding was a false reaction at 37°C due to high affinity IgM anti-M or a reaction due to an IgG component of anti-M was not determined.

Titration of the anti-M was performed in four cases and was <1 (no reaction in neat plasma) at 37°C in all these cases. Because the test was performed by CTT, it is possible that titration by CAT might have shown different results, since CAT is more sensitive. Phenotyping for M was performed on samples from all eight patients; seven were found to be M– and one was M+. Of the seven M– patients, six had no known history of blood transfusion; it thus appears that, in these patients, the anti-M was naturally occurring. In the seventh patient (who received RBC transfusion earlier [outside our hospital]), the anti-M could not be characterized as immune-stimulated or naturally occurring because the donor of the transfused RBCs was unavailable for M antigen testing. One patient with anti-M (acute lymphoblastic leukemia [ALL] case) was phenotyped as M+ with a positive DAT and autocontrol. This was thus a case of autoanti-M. The immunohematologic profile of these patients is summarized in Table 2.

### Table 1. Clinical profile of the cases

<table>
<thead>
<tr>
<th>Case number</th>
<th>Donor/patient</th>
<th>Age (years)/gender</th>
<th>ABO group/D type</th>
<th>Diagnosis</th>
<th>History of previous blood transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole blood donor</td>
<td>24/F O+</td>
<td>NA</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Stem cell donor</td>
<td>3/F B+</td>
<td>NA</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Patient</td>
<td>3/F O+</td>
<td>Pre B ALL</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Patient</td>
<td>2.5/F A+</td>
<td>Pre B ALL</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Patient</td>
<td>5/M O+</td>
<td>T ALL</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Patient</td>
<td>63/M A+</td>
<td>Squamous cell carcinoma of buccal mucosa</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Patient</td>
<td>24/F O+</td>
<td>Carcinoma breast</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Patient</td>
<td>66/M O+</td>
<td>Multiple myeloma</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Patient</td>
<td>82/F A+</td>
<td>Squamous cell carcinoma of cervix</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Patient</td>
<td>72/M A+</td>
<td>Seminoma testis</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

NA = not applicable; Pre B ALL = Precursor B cell acute lymphoblastic leukemia; T ALL = T cell acute lymphoblastic leukemia.

### Table 2. Serologic profile of anti-M in patients

<table>
<thead>
<tr>
<th>Case number</th>
<th>Presentation</th>
<th>DAT</th>
<th>Class of antibody</th>
<th>Thermal amplitude</th>
<th>Titer</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Antibody detection testing</td>
<td>Positive</td>
<td>IgM ± IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 16</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>4</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>5</td>
<td>ABO group discrepancy</td>
<td>Positive</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>6</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 4</td>
<td>No transfusion</td>
</tr>
<tr>
<td>7</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM ± IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>8</td>
<td>Antibody detection testing</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 8</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>9</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 4</td>
<td>No transfusion</td>
</tr>
<tr>
<td>10</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM</td>
<td>4°C, RT</td>
<td>NP</td>
<td>No transfusion</td>
</tr>
</tbody>
</table>

DAT = direct antiglobulin test; RT = room temperature; RBC = red blood cell; NP = not performed.
**Immunohematologic Features of Anti-M in Donors**

Anti-M was found in one whole blood donor and one hematopoietic stem cell donor. In the whole blood donor, a blood group discrepancy was noted and was resolved when the reverse group was repeated with prewarmed serum. The anti-M was also detected at 4°C and not at 37°C, suggesting possibly only an IgM component. The donor’s RBCs tested as M–.

In the stem cell donor, anti-M was detected during the pre-transplant evaluation. Although both the donor and recipient were group B, D+, the minor crossmatch was incompatible. Antibody identification confirmed anti-M reactive at 37°C, showing a dosage effect. Presence of IgG along with IgM was confirmed by DTT treatment of the serum. The RBCs were phenotyped as M–. Neither this donor nor the whole blood donor had history of blood transfusion, suggesting that the anti-M in both donors was naturally occurring. The immunohematologic profile of these donors is summarized in Table 3.

**EQAS Sample**

During the study period, anti-M was identified in one EQAS sample. This antibody was detected at 37°C and showed dosage. The agglutination strength increased when the testing was repeated at 4°C. DTT treatment of the plasma or antibody titration studies could not be performed nor was a transfusion history supplied with the sample.

**Clinical Outcome of Anti-M in Patients and Donors**

Among the patients with anti-M, five patients required RBC transfusion. As in most of the cases, the IgG component was present along with IgM; hence, the anti-M was considered to be clinically significant, and these five patients received M– RBC units. The patient with ALL who had autoanti-M had clinical as well as laboratory features of hemolysis. The unconjugated bilirubin was increased at 1.5 mg/dL (normal 0–1.1 mg/dL), total bilirubin was 1.8 mg/dL (normal 0.2–1.3 mg/dL), reticulocyte count was 4% (normal 0.5–2.5%), and lactate dehydrogenase was 4039 U/L (normal 313–618 U/L). This patient received injection dexamethasone along with RBC transfusion and responded well, confirmed by an increase in hemoglobin increment from 4.5 to 9.4 g/dL.

The anti-M in the hematopoietic stem cell donor was detected during pretransplant evaluation. Incompatibility between donor’s plasma and patient’s RBCs was detected, and anti-M was then identified. Because the stem cell donor was 3 years of age, a marrow harvest was undertaken. RBC loss in the stem cell donor required transfusion of 1 RBC unit during the harvest. Because the donor had anti-M, group-specific, M–, crossmatch-compatible RBCs were transfused. To further complicate the matter, the stem cell recipient was M+. Because the donor anti-M was clinically significant, the marrow harvest product was plasma-depleted before transfusion to reduce chances of a hemolytic transfusion reaction. Hence, once anti-M is detected, further management of the patient depends on the specific clinical setting.

The donor unit, which had anti-M in the plasma, was separated into RBCs, platelets, and plasma components. The plasma was sent for fractionation, platelet concentrate was transfused to an M– patient, and the RBC unit was transfused to an ABO group-specific patient.

**Discussion**

In patients, anti-M may be detected in various medical as well as surgical settings. Das et al.10 reported three cases of anti-M, all of which were detected during pre-transfusion testing before surgery. In the present study, anti-M was detected in our medical and surgical oncology patients at the time of blood grouping and antibody detection testing. Our population consisted of medical and surgical oncology patients. Generally, anti-M is IgM in nature, optimally reactive below 22°C, and most often causes a blood grouping discrepancy. It may also be detected incidentally during pretransfusion evaluation. If there is no known stimulating event (i.e., RBC transfusion, pregnancy, or other exposure to foreign RBCs), the antibodies are classified as naturally occurring. Autoanti-M has also been reported in the literature. Anti-M is more commonly observed in children.6 In the present study, however, there were only four individuals younger than 10

<table>
<thead>
<tr>
<th>Case number</th>
<th>Presentation</th>
<th>DAT</th>
<th>Class of antibody</th>
<th>Thermal amplitude</th>
<th>Titer</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABO group discrepancy</td>
<td>NP</td>
<td>IgM</td>
<td>4°C and RT</td>
<td>NP</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Minor crossmatch incompatible</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
</tbody>
</table>

DAT = direct antiglobulin test; NP = not performed; RT = room temperature; NA = not applicable; RBC = red blood cell.
years of age (three patients and one stem cell donor). Mathur et al.\textsuperscript{11} described one case of anti-M in a voluntary blood donor where the antibody had a thermal amplitude of 22°C to 37°C but showed no reaction at 4°C. This finding is in contrast to our finding from the whole blood donor, where the anti-M was reactive at room temperature with optimum activity at 4°C. Sacher et al.\textsuperscript{12} described 17 cases of autoanti-M, of which four had symptoms of cold agglutinin disease, such as Raynaud's phenomenon, livedo reticularis, and acrocyanosis, and two had mild hemolysis; the rest were asymptomatic despite having a significant titer of the autoanti-M.

Patients with anti-M have been described by Tandon et al.\textsuperscript{13} and Kaur et al.\textsuperscript{14} These antibodies were identified as IgM, causing ABO grouping discrepancy, with optimum reactivity below 37°C. Anti-M with both IgM and IgG components and wide thermal amplitude (4°C–37°C) were also described. Hence, those authors suggest that when evaluating for the IgG component of anti-M, strict prewarmed conditions must be maintained during antibody identification to avoid interference by high-affinity, high-titer IgG.

Anti-M, which is commonly IgM in nature, can cause complement activation. In 1991, Combs et al.\textsuperscript{15} described an autoanti-M causing hemolysis in vitro by activating complement in CTT at low-ionic strength.

Naturally occurring anti-M with only an IgG component has been reported very rarely.\textsuperscript{6} Anti-M shows a dosage effect, that is, it shows a stronger reaction with RBCs with double-dose expression of M (M+N–) than with RBCs with single-dose expression (M+N+). For this reason, the prevalence of naturally occurring anti-M in blood donors was shown to be 1 in 2500 when testing with M+N– RBCs and 1 in 5000 when using M+N+ RBCs.\textsuperscript{7} In our series, a dosage phenomenon was noted in 6 of the 11 cases described. Anti-M has also demonstrated increased reactivity when tested with serum that has been adjusted to a pH of 6.5.\textsuperscript{3} Beattie and Zuelzer\textsuperscript{16} described two anti-M examples that were identified after acidification of the serum.

Delayed hemolytic transfusion reaction is very rare because of alloanti-M. Sancho et al.\textsuperscript{17}, Alperin et al.\textsuperscript{18}, and Furlong and Monaghan\textsuperscript{19} reported cases of alloanti-M that were undetectable during pretransfusion evaluation but were subsequently detected 5–15 days posttransfusion during investigation of posttransfusion hemolysis. Anti-M has also been implicated in hemolytic disease of the fetus and newborn (HDNF). The clinical effects in HDNF as described in the literature range from severe HDNF with intrauterine death\textsuperscript{20} to cases where the fetus is unaffected.\textsuperscript{21} Like anti-K, anti-M has been reported to cause suppression of erythroid precursors, leading to RBC aplasia and prolonged anemia of the newborn.\textsuperscript{22,23} Hence, anti-M in various clinical settings presents differently and demands different management.

In conclusion, anti-M can have varied clinical presentations and can interfere during blood grouping and crossmatching. To determine the clinical significance of anti-M, elaborate methods such as antibody titration, the determination of antibody class, and thermal amplitude testing may be necessary. In the case of a transfusion requirement for a patient with anti-M, the specific clinical setting along with the antibody characteristics would determine the course of action to be taken. This report also highlights the fact that, in all pretransplant evaluations, a minor crossmatch or antibody screening of the donor must be performed to ensure that a donor antibody is not missed. Lastly, in a stem cell donor, the harvest may require modification to avoid any adverse transfusion reaction in the recipient.

References

Dithiothreitol treatment of red blood cells

C.B. Bub

Dithiothreitol (DTT), a reducing reagent, has multiple applications in blood bank testing. DTT disrupts the bridging of the disulfide bonds between amino acid residues necessary for structural conformation of some proteins and the bonds holding an IgM molecule in the pentameric formation. DTT treatment of red blood cells (RBCs) can denature or modify certain blood group antigens—in particular, those in the Kell, Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock, and Knops systems—and prevent recognition by the corresponding antibodies. It also destroys RBC CD38, allowing DTT-treated RBCs to be used to avoid testing interference by therapeutic anti-CD38 preparations. DTT treatment can be used to disperse spontaneous agglutination of RBCs caused by heavy IgM autoantibody coating that invalidates ABO/Rh cell grouping and direct antiglobulin tests. Immunoheematology 2017;33:170–172.

Key Words: dithiothreitol (DTT), indirect antiglobulin test, Kell system, daratumumab

Principle

Dithiothreitol (DTT) is a reducing agent capable of irreversibly cleaving accessible disulfide bonds when the solution pH is >7. DTT treatment of red blood cells (RBCs) will modify the tertiary structure of protein-based erythrocyte membrane antigens if their confirmation depends on disulfide bonds.1 Antigens of the following blood group systems are destroyed or weakened by 0.2 M DTT treatment: KEL, IN, JMH, YT, LU, MER2, KN, DO, CROM, and LW.2,3 Antibodies directed at antigens in these systems will not react or will be significantly weaker with the treated RBCs.

DTT will also cleave the disulfide bonds that connect the monomeric subunits and the J chain of the IgM antibody pentameric form. When heavy coating of RBCs with IgM autoantibody causes spontaneous agglutination, DTT treatment will disrupt the IgM structure and disperse the agglutination.

Indications

DTT-treated reagent RBCs can be used in antibody identification to suggest the possible blood group specificity of an unidentified antibody based on whether reactivity is affected by the treatment. When a known antibody is directed at a DTT-sensitive antigen, treated RBCs may be used to detect or exclude underlying antibodies to DTT-resistant antigens.

Treatment of autologous RBCs with 0.01 M DTT can resolve spontaneous agglutination due to potent IgM autoantibodies that interfere with ABO/Rh and direct antiglobulin testing.4

Monoclonal anti-CD38 (daratumumab), approved by the U.S. Food and Drug Administration for treatment of multiple myeloma, targets the CD38 antigen on malignant plasma cells. CD38 is also weakly expressed on all normal RBCs, including those in RBC reagents used in pretransfusion testing. This expression complicates the identification of clinically significant RBC antibodies because the plasma/serum of such patients will react with most or all RBCs in antibody detection

### Treatment to Destroy RBC Antigens

**Reagents/Supplies**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• PBS, pH 7.3</td>
<td>• Test tubes</td>
</tr>
<tr>
<td>• 0.2 M DTT</td>
<td>• Pipettes</td>
</tr>
<tr>
<td>• RBC test cell</td>
<td>• Centrifuge</td>
</tr>
<tr>
<td>• K+ RBCs (control cell)</td>
<td>• pH meter</td>
</tr>
<tr>
<td>• Anti-K (reagent or serum/plasma specimen)</td>
<td>• Water bath or 37°C incubator</td>
</tr>
</tbody>
</table>

**RBC = red blood cell; PBS = phosphate-buffered saline; DTT = dithiothreitol.**

### Procedural Steps

#### 0.2 M DTT Preparation

- Dissolve 1 g DTT in 32 mL PBS, pH 8.0. Adjust final pH of DTT to 8.0 with 0.1 M HCl/0.1 M NaOH as needed.
- Aliquot and store at –20°C or colder for up to 12 months.

#### Procedure

- Wash 1 volume of the test RBCs and control RBCs with PBS, pH 7.3. Decant.
- Add 4 volumes of 0.2 M DTT, pH 8.0.
- Incubate at 37°C for 30 minutes.
- Wash three to four times with PBS.
- Resuspend the cells to a 2–5 percent suspension in PBS.
- Test DTT-treated cells with serum containing the antibody in question. Test K+ treated RBCs with anti-K.

**DTT = dithiothreitol; PBS = phosphate-buffered saline; RBCs = red blood cells.**

---

1 Dithiothreitol is a reducing agent capable of irreversibly cleaving accessible disulfide bonds when the solution pH is >7. DTT treatment of red blood cells (RBCs) will modify the tertiary structure of protein-based erythrocyte membrane antigens if their confirmation depends on disulfide bonds. Antigens of the following blood group systems are destroyed or weakened by 0.2 M DTT treatment: KEL, IN, JMH, YT, LU, MER2, KN, DO, CROM, and LW. Antibodies directed at antigens in these systems will not react or will be significantly weaker with the treated RBCs.

2 DTT will also cleave the disulfide bonds that connect the monomeric subunits and the J chain of the IgM antibody pentameric form. When heavy coating of RBCs with IgM autoantibody causes spontaneous agglutination, DTT treatment will disrupt the IgM structure and disperse the agglutination.

3 DTT-treated reagent RBCs can be used in antibody identification to suggest the possible blood group specificity of an unidentified antibody based on whether reactivity is affected by the treatment. When a known antibody is directed at a DTT-sensitive antigen, treated RBCs may be used to detect or exclude underlying antibodies to DTT-resistant antigens.

4 Treatment of autologous RBCs with 0.01 M DTT can resolve spontaneous agglutination due to potent IgM autoantibodies that interfere with ABO/Rh and direct antiglobulin testing.

5 Monoclonal anti-CD38 (daratumumab), approved by the U.S. Food and Drug Administration for treatment of multiple myeloma, targets the CD38 antigen on malignant plasma cells. CD38 is also weakly expressed on all normal RBCs, including those in RBC reagents used in pretransfusion testing. This expression complicates the identification of clinically significant RBC antibodies because the plasma/serum of such patients will react with most or all RBCs in antibody detection.
and identification tests performed by the indirect antiglobulin test.5 Because CD38 configuration depends on disulfide bonds, DTT treatment of reagent RBCs is used to denature the red cell CD38 and avoid this interference.6 A large volume of RBCs can be treated with DTT and stored in Alsever’s solution, since the stability of common RBC antigens has been demonstrated for up to 14 days.7 Serologic investigations within this time period are more efficiently performed when pretreated cells are available.

**Procedure to Destroy RBC Antigens**

In a properly labeled tube, place 1 volume of the patient’s packed RBCs (approximately 50 μL). Wash the RBCs three times with phosphate-buffered saline (PBS), pH 7.3. Decant the supernatant completely after the last wash. Add 4 volumes of 0.2 M DTT (approximately 200 μL) to 1 volume of RBCs to be treated. Mix well, and incubate at 37°C for 30 minutes. Wash the RBCs four times with PBS. If marked hemolysis occurs, repeat the procedure with fresh RBCs and a smaller volume of DTT. Resuspend the RBCs to a 2–5 percent suspension if the testing is performed in the tube or to the concentration required for non-tube test methods. Assess the batch for completeness of treatment using anti-K as described in the quality control section. If the reactivity of the test serum is eliminated, appropriate RBC samples can be treated and tested to exclude other clinically significant alloantibodies.

**Procedure to Destroy Spontaneous Agglutination**

In a properly labeled tube, wash autologous RBCs three times in saline. Washing with warm saline can also aid in removing cold-reacting IgM autoantibodies. Dilute washed RBCs to a 50 percent suspension in PBS. Add an equal volume of 0.01 M DTT. Mix and incubate at 37°C for 15 minutes. Wash treated RBCs at least three times in PBS. Resuspend an aliquot of the RBCs to a 2–5 percent suspension or to the concentration required by the test method being performed. Test the RBCs for removal of the spontaneous agglutination as described in the quality control section.

**Limitations**

As in all serologic procedures, factors such as contaminated materials or inadequate incubation time, temperature, or centrifugation may produce false results. Preparation of the 0.2 M DTT at pH 8.0 is required for irreversible denaturation of antigens.2 Reagent preparations at a lower pH may cause inadequate and reversible reduction of disulfide bonds. Treated RBCs cannot be typed for any antigens destroyed by DTT. Antibodies to antigens destroyed or weakened by DTT cannot be excluded on nonreactive treated RBCs. Because anti-K is the most frequently encountered clinically important antibody in this group, K– RBCs should be selected for transfusion if the patient’s RBCs are K– or the K antigen status is unknown, unless anti-K has been excluded in other tests.

**Quality Control**

When performing treatment to destroy RBC antigens, K+ RBCs should be DTT-treated with each test batch. The treated and untreated K+ RBCs are then tested with anti-K. The treated RBCs should be nonreactive; otherwise, the DTT treatment was not adequate. Other antigens of the Kell system can also serve as controls.1 If dispersal of spontaneous agglutination is being performed, test the treated RBCs using 6 percent albumin. No agglutination should be present if the treatment was successful.
**Summary**

DTT treatment of RBCs is an informative method to aid in antibody identification and to determine whether a serum contains additional alloantibodies when an antibody to a DTT-sensitive antigen is present. It also destroys CD38 on the RBC surface, thereby avoiding interference from anti-CD38 in pretransfusion testing. Donor units typed as K– must be transfused when antibody detection/identification has been performed using DTT-treated RBCs, unless anti-K is excluded by another method.

Spontaneous agglutination of autologous RBCs causing false-positive results in ABO/Rh and direct antiglobulin testing can be resolved by using DTT to degrade the IgM antibody coating the RBC.

**References**


Carolina Bonet Bub, MD, PhD, Hematology and Hemotherapy Physician, Hematology and Cell Therapy Department, Hospital Israelita Albert Einstein, 627 – 3º andar, Bloco E, CEP: 05651-901 Sao Paulo, SP, Brazil.
To Contributors to the 2017 Issues

*Immunohematology* depends on many contributors to make each issue a success: authors who submit reviews, scientific reports, case reports, blood group allele information, book reviews, and letters to the editors; peer reviewers who review each submission for accuracy and completeness of content; medical editors who oversee each submission for medical content and appropriateness for publication; technical editors who ensure that the technical aspects of each submission are correct; and the copy editor, proof reader, and graphic designer who format each issue to ensure that it is aesthetically pleasing to the eye of the reader. Each submission is reviewed and managed by an editorial staff who move the manuscripts through the review/editing process and who determine the acceptability of each for publication, namely, the editor-in-chief, the managing editor, and the medical and molecular editors. Behind the scenes are our administrative staff who process and manage subscriptions, reply to emails in our mailbox, and various other duties that keep the journal on track. In addition, our prestigious editorial board members submit manuscripts and provide peer reviews, guidance in policy, ideas for focused issues, and suggestions for improvement of the journal. Without all of these contributors, *Immunohematology* would not exist. We appreciate the time and effort each contributes to the journal.

The authors are showcased in their publications. The editorial staff, administrative staff, medical and technical editors, and current members of the editorial board are listed in the front of each issue of the journal along with the copy editor, proof reader and graphic designer: our sincerest appreciation to each.

Our peer reviewers did an excellent job this year. We take this opportunity to list each by name below with heartfelt gratitude to all.

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Jeff Trimble, B.Sc., ART(CSMLS)  
Carlos H. Villa, MD, PhD  
F. Bernadette West, MD

Finally, we thank our readers, whose enthusiasm and interest in the journal make it all worthwhile.

Sandra Nance, MS, MT(ASCP)SBB  
Editor-in-Chief  

Cynthia Flickinger, MT(ASCP)SBB  
Managing Editor
Daniels G. The Augustine blood group system, 48 years in the making. *Immunohematology* 2016;32:100–103.

An error was detected in the review of the Augustine blood group system: At<sup>a</sup> was reported as AUG1, when it should have been AUG2; the antigen defined by the antibody produced by the woman with the null phenotype was reported as AUG 2, when it should have been AUG1. Below is the corrected Table 1.

**Table 1. Antigens of the Augustine system and their molecular backgrounds**

<table>
<thead>
<tr>
<th>Name</th>
<th>Antigens</th>
<th>ISBT number</th>
<th>Nucleotides</th>
<th>Exon/intron</th>
<th>Amino acids</th>
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<tbody>
<tr>
<td>—</td>
<td>AUG1</td>
<td>036001</td>
<td>C.589+1G (C)</td>
<td>Intron 6</td>
<td>Splice site mutation</td>
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<tr>
<td>Ata</td>
<td>AUG2</td>
<td>036002</td>
<td>C.1171G (A)</td>
<td>Exon 12</td>
<td>Glu391 (Lys)</td>
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</table>

* *Molecular basis of antigen-negative phenotype provided in parentheses.*
2018 Educational Courses

<table>
<thead>
<tr>
<th>DATES</th>
<th>PROGRAMS</th>
<th>LOCATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 21</td>
<td>Webinar</td>
<td>Online</td>
</tr>
<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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<td>May 17-18</td>
<td>TSEC</td>
<td>Kansas City, KS</td>
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<tr>
<td>June 20</td>
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<td>TSEC</td>
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<td>October 24-26</td>
<td>Hands-on (Serology)</td>
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<td>November 15-16</td>
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<td>December 19</td>
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Email: TSEC@grifols.com; Phone: 1-855-600-7101
Educational Programs 2018

SAVE THE DATE

Dear Colleague,

The Grifols Academy of Immunohematology is pleased to announce its 2018 educational schedule. This extensive educational offering exemplifies Grifols’ commitment to support continued education in the field of transfusion medicine and addresses comments and suggestions provided during past editions. The Grifols Academy of Immunohematology is certified as a provider by the American Society for Clinical Laboratory Science (ASCLS). P.A.C.E.® credits are offered to all our courses.

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: 13.5-11.5 hours P.A.C.E.® credits

Immunohematology workshop (Hands-on)
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

2018 TSEC Schedule*

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<thead>
<tr>
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<th>Date</th>
<th>Level</th>
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<td>March 15-15</td>
<td>Intermediate-Advanced</td>
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<tr>
<td>Kansas City, KS</td>
<td>May 17-16</td>
<td>Intermediate-Advanced</td>
</tr>
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<td>Emeryville, CA</td>
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<tr>
<td>Philadelphia, PA</td>
<td>November 15-16</td>
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*All dates subject to change.

2018 Hands-On Schedule*

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</tr>
<tr>
<td>October 24-26</td>
<td>Serology</td>
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*All dates subject to change.

2018 Webinar Schedule*

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<tr>
<td>August 15</td>
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<tr>
<td>December 10</td>
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</tbody>
</table>

*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.

This program will provide basic information for study and preparation for the SBB/BB exams. Handouts with references, case studies, and practice questions will be provided for both on-site and virtual participants. Individuals may purchase just the handouts for self-study purposes.

**REGISTRATION**
- $200 Onsite Attendance
- $175 Virtual Attendance
- $100 Handouts Only

**14 PACE and Florida CE Broker credits available!**

**DATE**
April 21-22, 2018

**LOCATION**
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**ONSITE PARTICIPATION WILL INCLUDE**
- Lab Math Boot Camp
- Interactive educational opportunities
- Networking dinner!

More information at www.passbbexamreview.org
## AIMS

### Renaissance Glendale Hotel & Spa
April 28-29, 2018
Glendale, Arizona

The Advanced Immunohematology & Molecular Symposium is designed to foster education, networking, and camaraderie among colleagues. With the combined experience of the presenters and attendees, this workshop is an opportunity to capture a broad spectrum of information from experts and colleagues in the Immunohematology field.

### REGISTRATION FEES:

| Advance Registration (before February 28, 2018) | $200.00 |
| Registration (after February 28, 2018) | $250.00 |

Registration for AIMS opens JANUARY 8th!

### SCHEDULE AT A GLANCE

#### Saturday, April 28

<table>
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<tr>
<th>TIME</th>
<th>SESSION NAME (CBBS/SCABB Joint Meeting Final Sessions)</th>
<th>SPEAKER(S)</th>
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<tbody>
<tr>
<td>8:00 AM</td>
<td>MD Roundtable</td>
<td>To Be Announced</td>
</tr>
<tr>
<td>9:15 AM</td>
<td>Administrative &amp; Technical Scientific Award Lectures</td>
<td>Bud Scholl, Geoff Daniels, BSc, PhD, FRCPath</td>
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<tr>
<td>10:45 AM</td>
<td>Karen Williams Memorial Lecture</td>
<td>Richard J. Benjamin MBChB, PhD, FRCPath</td>
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<table>
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<tr>
<th>TIME</th>
<th>SESSION NAME (AIMS Sessions Begin)</th>
<th>SPEAKER(S)</th>
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</thead>
<tbody>
<tr>
<td>1:00 PM</td>
<td>AIMS Begins: RH System Genotyping &amp; Discrepancy Resolutions</td>
<td>Margaret Keller, MD, Katrina Billingsley, MT(ASCP)/SBB, Monica Kalveig, MT(ASCP)/MB, SBB, Gorka Ochoa, MD, PhD</td>
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<tr>
<td>3:30 PM</td>
<td>Next Generation Sequencing &amp; Case Studies #1</td>
<td>William Lane, MD, PhD, Fernado Lerma, SBB(ASCP), Heather vonHartitzach, MT(ASCP)/SBB, Virginia Reyes, M.Ed MT(ASCP)/SBB</td>
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#### Sunday, April 29

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<tr>
<th>TIME</th>
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<th>SPEAKER(S)</th>
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<tr>
<td>8:30 AM</td>
<td>John Moulds Memorial Lecture Training &amp; Competency Assessments</td>
<td>Geoff Daniels, MD, Margaret Keller, PhD, To Be Announced</td>
</tr>
<tr>
<td>10:45 AM</td>
<td>Providing Platelets to the Refractory Patient: Where to Begin?</td>
<td>Kevin Land, MD, Margaret Keller, MD, Hank Hanna, MD</td>
</tr>
<tr>
<td>12:30 PM</td>
<td>Industry Workshop Luncheon (Sponsored by Bloodhub)</td>
<td>To Be Announced</td>
</tr>
<tr>
<td>1:30 PM</td>
<td>Ask the Experts &amp; Blood Group Systems Overview</td>
<td>Geoff Daniels, MD, Margaret Keller, PhD, To Be Announced</td>
</tr>
<tr>
<td>3:45 PM</td>
<td>Case Studies #2</td>
<td>Margaret Keller, MD, Katrina Billingsley, MT(ASCP)/SBB, Sandy Wortman, MT(ASCP)/SBB</td>
</tr>
</tbody>
</table>

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
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
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)CM-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
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)CM 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

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Phyllis Walker, MT(ASCP)SBB, San Francisco

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Marion E. Reid & Christine Lomas-Francis

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Becoming a Specialist in Blood Banking (SBB)

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

Individuals who have an SBB certification serve in many areas of transfusion medicine:
- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?
- Supervisors of Transfusion Services
- Managers of Blood Centers
- LIS Coordinators
- Educators
- Supervisors of Reference Laboratories
- Research Scientists
- Consumer Safety Officers
- Technical Representatives
- Reference Lab Specialists

Why become an SBB?
- Professional growth
- Job placement
- Job satisfaction
- Career advancement

How does one become an SBB?
- Attend a CAAHEP-accredited SBB Technology program OR
- Sit for the examination based on criteria established by ASCP for education and experience.

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

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

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

<table>
<thead>
<tr>
<th>State</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>American Red Cross Blood Services</td>
<td>Pomona, CA</td>
</tr>
<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>
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<td>Indiana</td>
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<td>University Medical Center New Orleans</td>
<td>New Orleans, LA</td>
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<td>Maryland</td>
<td>National Institutes of Health Clinical Center</td>
<td>Bethesda, MD</td>
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<td>The Johns Hopkins Hospital</td>
<td>Baltimore, MD</td>
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<td>Walter Reed National Military Medical Center</td>
<td>Bethesda, MD</td>
</tr>
<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>University of Texas Medical Branch</td>
<td>Galveston, TX</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>BloodCenter of Wisconsin</td>
<td>Milwaukee, WI</td>
</tr>
</tbody>
</table>

Revised October 2017
Reference and Consultation Services

Antibody identification and problem resolution

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

For information, contact:
Mehdizadeh Kashi
at (503) 280-0210

or write to:
Tissue Typing Laboratory
American Red Cross Biomedical Services
Pacific Northwest Region
3131 North Vancouver
Portland, OR 97227

IgA Testing

IgA testing is available to do the following:

- Identify IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

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

For additional information contact:
Sandra Nance (215) 451-4362
or e-mail: Sandra.Nance@redcross.org
or write to:
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Sandra Nance

Immunohematology Reference Laboratory

Immunohematology Reference Laboratory
AABB, ARC, New York State, and CLIA licensed
24-HOUR PHONE NUMBER:
(215) 451-4901
Fax: (215) 451-2538

American Rare Donor Program
24-HOUR PHONE NUMBER:
(215) 451-4900
Fax: (215) 451-2538
ardp@redcross.org

Immunohematology
PHONE, BUSINESS HOURS:
(215) 451-4902
Fax: (215) 451-2538
immuno@redcross.org

Quality Control of Cryoprecipitated-AHF
PHONE, BUSINESS HOURS:
(215) 451-4903
Fax: (215) 451-2538

National Reference Laboratory for Blood Group Serology

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Fax: (215) 451-2538

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Fax: (215) 451-2538
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Fax: (215) 451-2538

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For additional information:
Kathy Kaherl
at (860) 678-2764
e-mail: Katherine.Kaherl@redcross.org

or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Avenue
Farmington, CT 06032
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- Post-transfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

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  - 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

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Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

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

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- Autoimmune neutropenia (AIN)
- Transfusion-related acute lung injury (TRALI)

Methodologies employed:
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- 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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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
      a. List under abstract
   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:
         ● ○ △ ▲ ■
   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-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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