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

**Clinical significance of antibodies to antigens in the International Society of Blood Transfusion collections, 700 series of low-incidence antigens, and 901 series of high-incidence antigens**

C. Lomas-Francis

SEROLOGIC METHOD REVIEW

**Adsorption of cold agglutinins with rabbit red blood cells**

A. Cobaugh

ORIGINAL REPORT

**Mixed-field agglutination observed in column agglutination testing is not always associated with the A₃ subgroup**

N. Anukul, N. Leetrakool, P. Tanan, P. Palacajornsuk, and P. Klangsinsirikul

SEROLOGIC METHOD REVIEW

**Low-ionic-strength saline solution–antiglobulin test (LISS-AGT)**

L. Walker

ORIGINAL REPORT

**Rh and Kell blood group antigen prevalence in a multi-ethnic cohort in Nigeria: implications for local transfusion service**

A.S. Adewoyin, G.M. Lee, T.A. Adeyemo, and O.A. Awodu

TO THE EDITORS

**Clinically significant naturally occurring anti-N and anti-S in a blood donor: a rare finding**

S. Malhotra, G. Negi, and A.K. Tiwari
Franklin Carmichael belonged to the Group of Seven, landscape painters from 1920 to 1933, who devoted themselves to the establishment of an artistic idiom unique to Canada. While influenced by European Impressionism, this group of men hoped to break from it with a focus on the expansive and rugged Canadian landscape. They ascribed to the northern wilderness a transcendental spirituality; this visual rendering they saw as an important purpose of their art. Carmichael’s work features both simplicity of form and a bold use of color. He completed North Shore, Lake Superior, in 1927. Lake Superior, the coldest of the Great Lakes, has an average temperature below 40 degrees Fahrenheit. An article in this issue discusses the adsorption of cold agglutinins with rabbit red blood cells.

David Moolten, MD
This article reviews information regarding the clinical significance of antibodies to antigens in the blood group collections, the 700 series of low-incidence antigens, and the 901 series of high-incidence antigens. Antibodies to many of the antigens in these groups are rarely encountered, meaning that available information is limited. For a few, the clinical significance—the potential to cause reduced survival of transfused antigen-positive red blood cells, a hemolytic transfusion reaction (e.g., anti-AnWj, anti-Emm), or hemolytic disease of the fetus and newborn (e.g., anti-Kg, anti-HJK)—has been documented. Many other specificities have so far been benign (e.g., anti-Cs\(^a\), anti-M\(^1\)).

**Key Words:** clinical significance, antibodies to red blood cell (RBC) antigens, International Society of Blood Transfusion (ISBT) collections, low-incidence antigens, high-incidence antigens

When an antibody, directed at an antigen expressed on red blood cells (RBCs), is present in the plasma of a patient who requires transfusion, the clinical significance of the antibody must be assessed to determine if blood lacking the corresponding antigen should be selected for transfusion.\(^1\) In general, an antibody is considered to have clinical relevance if it has been associated with acute or delayed hemolytic transfusion reactions (HTRs) or with a notable shortening in the survival of transfused RBCs or if it has caused hemolytic disease (or anemia) of the fetus and newborn (HDFN). Information relating to the clinical significance is based on previous experience with a particular antibody specificity. Thus, correct identification of the antibody specificity is essential. Although the need to identify the antibody specificity is obvious, at times identifying the (correct) antibody specificity may not be a simple task.

Antigens that are authenticated by the International Society of Blood Transfusion (ISBT) Working Party for Red Cell Immunogenetics and Blood Group Terminology fall into one of four classifications: blood group systems, collections, series of low-incidence antigens, and series of high-incidence antigens.\(^2\) This review summarizes relevant information about the clinical significance of antibodies to antigens in the ISBT collections (the 200 series), the 700 series of low-incidence antigens, and the 901 series of high-incidence antigens. In the interest of space, few original references are cited. The reader wishing for more details is referred to Daniels\(^3\) and Reid et al.\(^4\)

### Collections (ISBT 200 Series)

A collection consists of two or more antigens that are related serologically, biochemically, or genetically but that do not fit the criteria required for blood group system status. A blood group system is defined as consisting of one or more antigens controlled at a single gene locus or by two or more very closely linked homologous genes with little or no observable recombination between them. Table 1 shows information for the six currently established collections: Cost, Ii, ER, GLOB, “unnamed,” and MN CHO (MNS carbohydrate antigens). When the antigens in a collection become assigned to a system, the collection becomes obsolete; there are now seven obsolete collections: 201 (Gerbich), 202 (Cromer), 203 (Indian), 204 (Auberger), 206 (Gregory), 211 (Wright), and 212 (Vel).\(^2\)

#### Cost Blood Group Collection: Anti-Cs\(^a\) and Anti-Cs\(^b\)

This collection contains two antigens: Cs\(^a\) and Cs\(^b\). Cs\(^a\) has a high prevalence as the antigen is expressed on RBCs of more than 98 percent of people in most populations; in black
populations, the prevalence is slightly lower (95%). Cs\textsuperscript{b} is polymorphic (prevalence of 34%). Only one example of anti-Cs\textsuperscript{b} (an IgG antibody) has been reported; thus, there is no information regarding its clinical significance.

Anti-Cs\textsuperscript{a} is an IgG antibody that is detected by antiglobulin methods but has not been implicated in transfusion reactions or HDFN. Although not clinically significant, anti-Cs\textsuperscript{a} may cause a delay in transfusion because the specificity can be difficult to identify. Anti-Cs\textsuperscript{a} reactivity has many of the features of antibodies to Knops blood group antigens; indeed, five of the original Cost antigens are now part of the Knops system. The challenges of working with anti-Cs\textsuperscript{a} include that many are weakly reacting antibodies that are mostly found in patients with multiple antibodies. Furthermore, the Cs\textsuperscript{a} antigen has variable expression on RBCs from different people; thus, the reactivity pattern may be confusing. There appears to be a phenotypic association between Cs\textsuperscript{a} and Yk\textsuperscript{a}, as RBCs of approximately 12 percent of whites and 15 percent of blacks with the Yk(a–) phenotype are also Cs(a–). Because Cs\textsuperscript{a} is one of the antigens that is suppressed on RBCs with the dominant Lu(a–b–) phenotype [encoded by In(Lu)], it is perhaps not too surprising that anti-Cs\textsuperscript{a} has on occasion initially been misidentified as anti-AnWj. Anti-AnWj, in contrast to anti-Cs\textsuperscript{a}, is a specificity that has on occasion caused transfusion reactions (see later section “Anti-AnWj”). The typical recommendation for patients with anti-Cs\textsuperscript{a} is to use the “least incompatible” (a term that has generated much discussion) RBC unit(s) for transfusion.

**Ii Blood Group Collection: Anti-i**

Anti-i recognizes the i antigen; i is now the only antigen in the Ii collection because the I antigen was promoted to I blood group system status.\textsuperscript{5} The i antigen is on unbranched carbohydrate chains of repeating N-acetyllactosamine units on glycolipids and glycoproteins on RBCs and on proteins in plasma. All RBCs express some i antigen, and the expression is variable, with RBCs from neonates having the strongest expression. It follows, therefore, that all anti-i are autoantibodies. In healthy people, autoanti-i is rare and primarily an IgM antibody weakly reactive at lower temperatures (4–10°C). In pathological situations, autoanti-i can have a high titer and a wide thermal range, react at 37°C, bind complement, and be IgG or a mix of IgG and IgM. Autoanti-i is pathologically significant in cold agglutinin syndrome and mixed-type autoimmune hemolytic anemia. The antibody is often found in plasma from patients with infectious mononucleosis or other lymphoproliferative disorders (e.g., Hodgkin’s disease) and occasionally causes hemolysis. Anti-i has been reported to occur in 64 percent of patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).\textsuperscript{6} Because all RBCs express some i antigen, i– blood is not available for transfusion. Mostly this is not an issue, although acute intravascular hemolysis occurred in a patient with anti-i after transfusion of 2 RBC units that were compatible in the immediate spin crossmatch. With regard to HDFN, maternal autoanti-i can cross the placenta and has caused mild neonatal jaundice or resulted in the RBCs of the newborn to react in the direct antiglobulin test (DAT).

**ER Blood Group Collection: Anti-Er\textsuperscript{a}, Anti-Er\textsuperscript{b}, and Anti-ER3**

This collection consists of two antigens of high prevalence (Er\textsuperscript{a} and ER3) and one of low prevalence (Er\textsuperscript{b}). The antibodies directed at these antigens are rare, so information about their clinical significance is limited. All three specificities have been found to be IgG antibodies that react by antiglobulin methods.

Anti-Er\textsuperscript{a} has caused reduced RBC survival but no overt HDFN. Clinical HDFN has not been reported, but anti-Er\textsuperscript{a} has been associated with neonatal RBCs being positive in the DAT.
The only reported patient with anti-ER3 experienced a mild transfusion reaction. No data are available for HDFN, since the only example of anti-ER3 was made by a male patient. Because Er(a−) or ER−3 blood is unlikely to be found, unless the patient has a compatible sibling, it is recommended by some that “serologically least incompatible” blood should be used with extra caution. Only one example of anti-Er(k) is reported; therefore, its clinical significance with regard to transfusion is not known. This antibody has not caused clinical HDFN but was associated with a positive DAT.

**GLOB Blood Group Collection: Anti-LKE**

Anti-LKE recognizes the high-incidence antigen, LKE, now the only antigen in the GLOB collection. LKE is located on a globoside molecule that has additional galactose (Gal) and N-acetyl neuraminic acid residues. The expression of LKE on RBCs varies: 80–90 percent of people have RBCs with strongly expressed LKE, and 10–20 percent of people have RBCs with weakly expressed LKE. The LKE− phenotype is found in only 1–2 percent of the population. Therefore, anti-LKE is rare; only six examples have been reported, and some appear to be “non–red cell immune.” They are IgM antibodies with optimal reactivity at room temperature or lower and may bind complement. One antibody was associated with posttransfusion hemolysis, but there have been no reports of HDFN. The antibody can be mistaken for anti-P as RBCs with the rare p, P1k, and P2k phenotypes do not react, and anti-LKE may be difficult to identify as RBCs typed for LKE are not routinely available. LKE− blood is not available for transfusion; thus, “serologically least incompatible” blood is given.

**Unnamed Blood Group Collection: Anti-Lec and Anti-Le4**

Despite their name, the determinants recognized by these antibodies are not the product of transferases encoded by Lewis (FUT3) genes. However, like Lewis antigens, Lec and Le4 antigens are adsorbed onto RBCs. Anti-Lec reacts with an antigen expressed on Le(a−b−) RBCs from adult non-secretors, whereas anti-Le4 reacts with an antigen expressed on Le(a−b−) RBCs from adult secretors. These antibodies have no clinical significance with regard to transfusion.

**MN CHO Collection**

The antigens of the MN CHO collection (Table 1) are expressed on glycoporphin A (GPA) molecules that have altered levels of glycosylation in that the amount of N-acetyl neuraminic acid or N-acetyl-D-glucosamine is different from that present on “conventional” GPA. Some antigens are associated with altered GPA, whereas others are associated with altered GPA. The antibodies that detect these determinants are considered to be non–red cell immune and of no clinical significance, but can complicate antibody identification. The optimal reactivity of these antibodies is at room temperature, and most are likely to be IgM, although available information is limited. For readers wishing to learn more about these antigens, please refer to Daniels, Issitt and Anstee, or Dahr et al.

**Low-Incidence Antigens (ISBT 700 Series)**

Antigens in the 700 series occur with an incidence of less than 1 percent in most populations studied and do not have the criteria to be included in an established blood group system or collection. Inheritance of the antigen through at least two generations must be demonstrated for the antigen to join the 700 series. In the clinical setting, antibodies to antigens in the 700 series are usually found because the antibody has caused HDFN or a transfusion reaction if an electronic crossmatch was used. These antibodies may also be found when a patient’s serum or plasma reacts with a single RBC sample during compatibility testing. Before the introduction of monoclonal blood typing reagents, many antibodies to low-incidence antigens were found as contaminants in blood typing reagents. Currently, there are 17 antigens in the 700 series. Some of the antibodies to these antigens are red cell immune, whereas others appear to be “naturally occurring” (or non–red cell immune). Antibodies to low-incidence antigens rarely cause a problem for transfusion as compatible blood is readily available. Several antibodies have caused HDFN, but this is a rare occurrence because of the low prevalence of these antigens that may have been identified in only one family; they are summarized in Table 2.

**High-Incidence Antigens (ISBT 901 Series)**

Antigens in the 901 series occur with an incidence of greater than 90 percent in most populations studied and cannot be included in an established blood group system or collection. This series was originally numbered as the 900 series but was renumbered as the 901 series in 1988 after many of the antigens were relocated to systems or collections. To be included in this series, it must be demonstrated that the antigen is lacking from the RBCs of at least two siblings (i.e., that the antigen-negative phenotype is genetically determined).
Currently, there are six antigens in the 901 series, and they are shown in Table 3.

Ant-Sd

Anti-Sd\textsuperscript{a} detects an antigen with a prevalence of about 91 percent. The strength of Sd\textsuperscript{a} antigen expression is variable, from barely detectable, such that some RBCs may erroneously be considered as Sd(a−), to high levels of Sd\textsuperscript{a} expression [termed Sd(a++)] that results in non-group A\textsubscript{1} RBCs being agglutinated by Dolichos lectin. Anti-Sd\textsuperscript{a} is easily recognized because it causes a characteristic mixed-field agglutination of orange refractile agglutinates that may look like bunches of grapes when viewed microscopically. Urine (human or guinea pig) that contains Tamm-Horsfall glycoprotein can be used to inhibit anti-Sd\textsuperscript{a}. The antibodies are mostly IgM, but some are IgG; they often react at lower temperatures and by antiglobulin methods. Although anti-Sd\textsuperscript{a} is not generally considered a transfusion hazard, and Sd(a−) RBCs are not required for transfusion, serologically least incompatible RBCs should be selected to avoid Sd(a++) RBCs.

<table>
<thead>
<tr>
<th>ISBT number</th>
<th>Name</th>
<th>Symbol</th>
<th>Clinical significance of antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>901008</td>
<td>...</td>
<td>Emm</td>
<td>See text</td>
</tr>
<tr>
<td>901009</td>
<td>Anton</td>
<td>AnWj</td>
<td>See text</td>
</tr>
<tr>
<td>901012</td>
<td>Sid</td>
<td>Sd\textsuperscript{a}</td>
<td>Not considered to be (may increase titer) but two suspected HTRs associated with RBCs with unusually strong expression of Sda [Sd(a++)]</td>
</tr>
<tr>
<td>901014</td>
<td>...</td>
<td>PEL</td>
<td>The four makers of anti-PEL (or the related specificity anti-MTP) were transfused; three were pregnant; no indication of HDFN; normal RBC survival with one anti-PEL but potential reduced survival with anti-MTP</td>
</tr>
<tr>
<td>901015</td>
<td>...</td>
<td>ABTI</td>
<td>No evidence of HDFN; no data for transfusion reactions; first three probands were Israeli</td>
</tr>
<tr>
<td>901016</td>
<td>...</td>
<td>MAM</td>
<td>See text</td>
</tr>
</tbody>
</table>

*The antibodies in this table may occur as IgM, IgG, or a mix of both.
†Severe HDFN requiring intrauterine or exchange transfusion.
‡Few = 1–5 examples; many = >13 examples.
ISBT = International Society of Blood Transfusion; HDFN = hemolytic disease of the fetus and newborn; DAT = direct antiglobulin test.
**Anti-Emm**

The Emm antigen is carried on a glycosylphosphatidylinositol-linked protein in the RBC membrane. Because the Emm− phenotype is rare, it follows that few examples of the antibody have been reported. IgG anti-Emm are more common than IgM (four out of five) and, although the original anti-Emm reacted in direct testing at 4°C, most of these antibodies react optimally by antiglobulin methods. Some anti-Emm have bound complement. There is no information regarding HDFN as six of the reported anti-Emm were found in untransfused males. Until 2013, anti-Emm had not been implicated in a transfusion reaction, possibly because the patients were not transfused.

In 2013, Takahashi et al.\(^\text{10}\) reported the case of a 58-year-old man who had never been transfused but who was in urgent need of transfusion because of massive bleeding from an abdominal stab wound. Pretransfusion testing demonstrated an antibody that was reactive with all panel cells but nonreactive with the autologous RBCs. Thirty minutes after transfusion of a crossmatch-incompatible unit of blood, the patient experienced a drop in blood pressure and hematuria. Because his hemoglobin was 5.5 g/dL, another 2 units of blood were transfused, and his vital signs became stable. A transfusion on the third day was uneventful. On day 6, after receiving 30 mL blood, the patient vomited, had cola-colored urine (total bilirubin 6.1 mg/dL, lactate dehydrogenase LDH 912 U/L), and the transfusion was stopped. No further transfusions were administered, and the patient recovered with iron therapy. After transfusion, his RBCs reacted in the DAT: 1+ on day 1, 2+ on day 3, and negative on day 7 (the day after the acute HTR occurred), suggesting that no transfused RBCs remained in circulation. The antibody in the patient’s plasma reacted in saline at 4°C (2+), by albumin–direct antiglobulin test (IAT) (2+), polyethylene glycol–IAT (2+), and papain–IAT (3+), and was identified as anti-Emm. The antibody had IgG1 and IgG3 components and was shown to fix complement. Before transfusion, the antibody titer was 16 by saline–IAT and rose to 128 by day 10.

**Anti-AnWj**

Anti-AnWj has been an intriguing specificity from the beginning and can at times be tricky to identify. An apparent alloantibody to an antigen called Anton, which was thought to be an antigen in the Lutheran system, was reported in 1982. A year later, an autoantibody called anti-Wj was reported, and in 1985, it was shown that both antibodies detected the same antigen (now named AnWj).\(^\text{4}\) The AnWj antigen is absent or only weakly expressed on RBCs of the dominant Lu(a−b−) phenotype encoded by In(Lu). Lu(a−b−) dominant-type RBCs not only have greatly reduced expression of Lutheran antigens, but also of AnWj, Indian, Knops, Cs\(^a\), MER2, and P1 blood group antigens.\(^\text{3,4}\) This antigen suppression is the result of heterozygosity for any one of several inactivating changes in KLF1, the gene that encodes erythroid Krüppellike factor (EKLF). These changes cause a general reduction of transcription of several erythroid genes and reduced levels of gene products such as the Lu glycoprotein. Therefore, if a patient’s plasma does not react with RBCs of the dominant Lu(a−b−) phenotype, it cannot be assumed that the reactivity is directed at a Lutheran antigen. Furthermore, antibody identification may also be complicated by the fact that the level of suppression on dominant-type Lu(a−b−) RBCs varies so that some RBC samples may react weakly with some examples of anti-AnWj.

Antibodies with AnWj specificity are predominantly autoantibodies but may appear as an alloantibody because of transient (often long-term) suppression of AnWj on the RBCs of the antibody maker. The antibody is frequently found in association with lymphoma (Hodgkin’s and non-Hodgkin’s) and other lymphoid malignancies, immunologic disorders, and autoimmune hemolytic anemia. The only two reported examples of alloanti-AnWj were found in two Arab Israeli sisters with a history of multiple pregnancies but no transfusions.\(^\text{11}\) Anti-AnWj has not been associated with HDFN, however. The fact that pregnancy appeared to be the immunizing event for the sisters is somewhat puzzling, since fetal cells express almost no AnWj antigen and RBCs from cord samples type AnWj−.

Some patients with anti-AnWj tolerate “random” units,\(^\text{12}\) whereas others do not and require blood with the dominant Lu(a−b−) phenotype. The antibody has been implicated in severe HTRs, and its hemolytic potential was substantiated by monocyte monolayer assay (MMA) or in vivo RBC survival studies.\(^\text{3,11}\) Xu et al.,\(^\text{14}\) in an informative review article, described the case of a 56-year-old woman with aplastic anemia with anti-AnWj identified in her plasma. Transfusion of “random” units resulted in an acute HTR. The anti-AnWj activated complement, and only C3 was detected on the patient’s RBCs when tested in the DAT. Blood from donors with the dominant Lu(a−b−) phenotype was well tolerated. There is a limited supply of blood with this rare phenotype, however, and long-term provision can be difficult, if not impossible. Similar to the case described by Xu et al.,\(^\text{14}\) a patient with anti-AnWj and a diagnosis of acute myeloid leukemia was transfused with...
a "random" unit, matched for common antigens. About 100 mL was transfused before the procedure was stopped. The patient suffered from an HTR, and a follow-up MMA at a later date showed that the anti-AnWj was expected to be clinically significant. The patient’s RBCs, when tested in the DAT, were coated with C3 only, and an eluate prepared from the RBCs was nonreactive. Attempts to reduce the anti-AnWj production by giving rituximab were not successful in this case.

Because dominant type Lu(a−b−) donors are rare, it is important to determine if a patient with anti-AnWj can or cannot tolerate blood that is not AnWj−. The tiny amount of AnWj on dominant type Lu(a−b−) RBCs does not appear to affect RBC survival. RBCs with the very rare recessive or X-linked Lu(a−b−) phenotypes fully express AnWj and should not be used for patients with anti-AnWj (in the unlikely event that these rare types are available).4

**Anti-MAM**

Anti-MAM is an extremely rare antibody, and the MAM antigen has a high incidence (greater than 99%) in all populations. The antibody was first reported in 1993 and assigned to the 901 series of high-incidence antigens in 1999. MAM antigen is expressed on cord RBCs, lymphocytes, granulocytes, monocytes, and probably on platelets.

Four of the five reported examples of anti-MAM were identified during pregnancy in mothers who had never been transfused. One of the mothers was of Irish and Cherokee descent, and the four other antibody makers were all of Arab descent. The anti-MAM were IgG antibodies; antibodies from two patients were subtyped as IgG1 and IgG3, and one also had an IgG2 component. Anti-MAM has been associated with clinical HDFN and/or thrombocytopenia. Of the four babies born to mothers with anti-MAM, the course of each pregnancy was slightly different, although the RBCs of the newborns all were reactive in the DAT. One baby had no clinical HDFN but experienced fetal and neonatal thrombocytopenia and required platelet transfusion. Another had severe HDFN and required intranuclear RBC transfusion. In addition, this baby presented with fetal and neonatal thrombocytopenia, but platelet transfusion was not required. A third baby had mild HDFN requiring ultraviolet phototherapy because of elevated bilirubin levels but no fetal or neonatal thrombocytopenia. A fourth baby required no RBC transfusion, platelets, or phototherapy. The platelet count of the fourth baby was slightly reduced (132,000/µL [normal range 150,000–450,000/µL]), but by 8 weeks of age, the count had increased to 305,000/µL.16

With regard to transfusion, the results of the MMA suggest that anti-MAM has the potential to substantially reduce the survival of transfused MAM+ RBCs.17,18 Ideally, MAM− RBCs should be transfused, but, because of their extreme rarity, cautious transfusion of serologically “least incompatible” RBCs may be the only option.

**Conclusions**

The antibodies made to some antigens in the ISBT blood group collections, 700 series, and 901 series have established and documented clinical significance, either for transfusion or for HDFN, but for others the information is limited. When antigen-negative blood is not available and information regarding the clinical significance of an antibody is lacking, it is best to proceed with caution, since any antibody reactive by IAT may have clinical potential. At the same time, the risk of not transfusing versus a potential transfusion reaction must be considered, since a patient is more likely to die from lack of blood than from a transfusion reaction. Clinical significance is mostly based on previous experience with a particular specificity. Thus, to predict clinical significance, the antibody specificity must be correctly identified. At times, this can be a challenge. In vitro tests such as the MMA may also be informative.

**References**

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Adsorption of cold agglutinins with rabbit red blood cells

A. Cobaugh

Cold-reactive autoagglutinins may mask the presence of underlying clinically significant alloantibodies. Adsorption with rabbit red blood cells (RBCs) or stroma can remove cold autoagglutinins found in the patient’s plasma/serum that are directed towards antigens expressed on the surface of rabbit RBCs. By removing these cold autoagglutinins, it is then possible to determine whether any underlying alloantibody reactivity is present. Although this method may also unintentionally adsorb alloantibodies directed towards antigens found on rabbit RBCs, it is still a widely used and convenient method to remove cold autoagglutinins.


Key Words: cold agglutinin, rabbit red blood cells, rabbit erythrocyte stroma, RESt

Principle

Cold-reactive autoagglutinins may mask the presence of underlying clinically significant alloantibodies. Most antigens detected by cold autoagglutinins are often of high prevalence (e.g., I, H, IH); therefore, most reagent red blood cells (RBCs) tested with the patient’s plasma/serum are often reactive, making alloantibody detection and identification difficult. Autoagglutinins may have a wide thermal range, and even if the cold agglutinin itself is not reactive at warmer temperatures, it may still interfere with laboratory tests performed at 37°C or at the antiglobulin phase of testing. Agglutinates that form at lower temperatures may not dissociate, although the testing is performed at warmer temperatures. These agglutinates are said to “carry over” into the testing performed at 37°C, which may mask any true reactivity resulting from IgG class alloantibodies. It has been demonstrated that rabbit RBCs and rabbit RBC stroma will readily adsorb autoagglutinins directed at carbohydrate antigens such as I and IH.1 Adsorption with rabbit RBCs or stroma can remove cold autoagglutinins found in the patient’s plasma or serum that are directed towards antigens expressed on the surface of rabbit RBCs. By removing these cold autoagglutinins, it is then possible to determine whether any underlying alloantibody reactivity is present.

Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies and Equipment</th>
</tr>
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<tbody>
<tr>
<td>Formalin-fixed rabbit RBCs</td>
<td>Pipettes</td>
</tr>
<tr>
<td>RESt</td>
<td>Test tubes (10 × 75 and 16 × 100 mm)</td>
</tr>
<tr>
<td></td>
<td>Calibrated centrifuge</td>
</tr>
<tr>
<td></td>
<td>Ice-slush bath</td>
</tr>
<tr>
<td></td>
<td>Test serum or plasma</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; RESt = rabbit erythrocyte stroma.

Procedural Steps

Adsorption with rabbit RBCs

1. Mix patient plasma/serum with formalin-fixed rabbit RBCs.
2. Incubate the mixture.
3. Centrifuge the mixture.
4. Remove plasma/serum.
5. Test adsorbed plasma/serum.
6. Repeat if needed.

Adsorption with RESt

1. Centrifuge the vial of stroma.
2. Aspirate the supernatant.
3. Add plasma/serum to the stroma.
4. Agitate the mixture.
5. Centrifuge the mixture.
6. Remove plasma/serum.
7. Test adsorbed plasma/serum.
8. Repeat one time if needed, as per the manufacturer’s directions.

RBCs = red blood cells; RESt = rabbit erythrocyte stroma.

Indications

Cold autoagglutinin reactivity may carry over into testing performed at 37°C, where it has the potential to mask underlying alloantibody reactivity. There are multiple methods that can remove this interfering reactivity. The most reliable method would be cold autologous adsorption. This method

1. Cobaugh A. Cold-reactive autoagglutinins may mask the presence of underlying clinically significant alloantibodies. Adsorption with rabbit red blood cells (RBCs) or stroma can remove cold autoagglutinins found in the patient’s plasma/serum that are directed towards antigens expressed on the surface of rabbit RBCs. By removing these cold autoagglutinins, it is then possible to determine whether any underlying alloantibody reactivity is present. Although this method may also unintentionally adsorb alloantibodies directed towards antigens found on rabbit RBCs, it is still a widely used and convenient method to remove cold autoagglutinins.

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Key Words: cold agglutinin, rabbit red blood cells, rabbit erythrocyte stroma, RESt

Principle

Cold-reactive autoagglutinins may mask the presence of underlying clinically significant alloantibodies. Most antigens detected by cold autoagglutinins are often of high prevalence (e.g., I, H, IH); therefore, most reagent red blood cells (RBCs) tested with the patient’s plasma/serum are often reactive, making alloantibody detection and identification difficult. Autoagglutinins may have a wide thermal range, and even if the cold agglutinin itself is not reactive at warmer temperatures, it may still interfere with laboratory tests performed at 37°C or at the antiglobulin phase of testing. Agglutinates that form at lower temperatures may not dissociate, although the testing is performed at warmer temperatures. These agglutinates are said to “carry over” into the testing performed at 37°C, which may mask any true reactivity resulting from IgG class alloantibodies. It has been demonstrated that rabbit RBCs and rabbit RBC stroma will readily adsorb autoagglutinins directed at carbohydrate antigens such as I and IH.1 Adsorption with rabbit RBCs or stroma can remove cold autoagglutinins found in the patient’s plasma or serum that are directed towards antigens expressed on the surface of rabbit RBCs. By removing these cold autoagglutinins, it is then possible to determine whether any underlying alloantibody reactivity is present.

Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies and Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin-fixed rabbit RBCs</td>
<td>Pipettes</td>
</tr>
<tr>
<td>RESt</td>
<td>Test tubes (10 × 75 and 16 × 100 mm)</td>
</tr>
<tr>
<td></td>
<td>Calibrated centrifuge</td>
</tr>
<tr>
<td></td>
<td>Ice-slush bath</td>
</tr>
<tr>
<td></td>
<td>Test serum or plasma</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; RESt = rabbit erythrocyte stroma.

Procedural Steps

Adsorption with rabbit RBCs

1. Mix patient plasma/serum with formalin-fixed rabbit RBCs.
2. Incubate the mixture.
3. Centrifuge the mixture.
4. Remove plasma/serum.
5. Test adsorbed plasma/serum.
6. Repeat if needed.

Adsorption with RESt

1. Centrifuge the vial of stroma.
2. Aspirate the supernatant.
3. Add plasma/serum to the stroma.
4. Agitate the mixture.
5. Centrifuge the mixture.
6. Remove plasma/serum.
7. Test adsorbed plasma/serum.
8. Repeat one time if needed, as per the manufacturer’s directions.

RBCs = red blood cells; RESt = rabbit erythrocyte stroma.

Indications

Cold autoagglutinin reactivity may carry over into testing performed at 37°C, where it has the potential to mask underlying alloantibody reactivity. There are multiple methods that can remove this interfering reactivity. The most reliable method would be cold autologous adsorption. This method
would carry the least amount of risk of missing an underlying alloantibody. When the patient has been recently transfused (within 90 days), however, or if there is not a large enough quantity of the patient’s autologous RBCs, cold autoadsorption cannot be used. Allogeneic adsorptions would be an option in that instance. Both autologous and allogeneic adsorptions may be time-consuming processes, since adsorbing cells need to be washed and enzyme-treated for maximum effectiveness, and, if the cold autoagglutinin is strongly reactive, multiple adsorptions may be necessary. It should be noted that allogeneic reagents have the potential to adsorb clinically significant alloantibodies directed toward high-prevalence antigens, which adds risk to the patient.

The prewarm method may also be used to address interfering cold agglutinin reactivity. This method should be used with caution, however, since it may weaken or completely diminish reactivity of potentially clinically significant antibody specificities that are reactive at 37°C.3

Rabbit RBCs and RBC stroma have demonstrated the ability to adsorb cold agglutinins (anti-I, anti-H, and anti-IH), generally without reducing the reactivity of underlying alloantibodies.5 Although this method may also unintentionally adsorb alloantibodies directed towards antigens found on rabbit RBCs, it is still a widely used and convenient method to remove cold autoagglutinins.5

Procedure

Using formalin-fixed rabbit RBCs, mix 2 mL of the packed rabbit RBCs with 1 mL plasma/serum in a 16 × 100 mm test tube. Incubate the mixture in an ice-slush bath with a temperature of 1–4°C for 30–60 minutes, mixing occasionally. After incubation, centrifuge at 1000g (Serofuge at 3400 rpm) for 5 minutes, and then transfer the serum to a clean test tube. The adsorbed serum should be tested at room temperature (18–30°C) or 36–38°C (depending on the original phase of testing with observed reactivity) to determine whether all the carry-over reactivity of the cold autoagglutinin has been removed. Negative reactions would indicate successful adsorption. If the adsorption was unsuccessful and the cold autoagglutinin persists, the adsorption process can be repeated. If the adsorption is complete, the plasma/serum is now ready for use in antibody detection or identification tests.5

Cold agglutinin reactivity may also be adsorbed using rabbit erythrocyte stroma (REST, Immucor, Norcross, GA). Begin by labeling an adsorption vial containing the rabbit RBC stroma for each test sample to be adsorbed. Centrifuge the adsorption vial(s) for a minimum of 2 minutes or until the stroma is well packed. It is important to ensure that the vial is properly balanced during centrifugation, and the centrifuge brake should not be used during deceleration to prevent the disruption of the packed stroma. Carefully and completely aspirate the supernatant from the vial, and add 1 mL of the plasma/serum to be adsorbed to the packed stroma. Failure to remove all the supernatant will result in the dilution of the adsorbed serum and the consequent dilution of the underlying antibody. Place a stopper in the vial and mix well; mixing can be done using a vortex mixer or by vigorous manual agitation. Incubate the vial in an ice-slush bath with a temperature of 1–4°C for 15–60 minutes, mixing occasionally. After incubation, centrifuge the adsorption vial for a minimum of 2 minutes or until the stroma is well packed. Again, it is important to ensure the vial is properly balanced during centrifugation, and the centrifuge brake should not be used during deceleration to prevent the disruption of the packed stroma. Transfer the adsorbed plasma/serum to a clean test tube. The adsorbed plasma/serum should be tested at room temperature (18–30°C) or 36–38°C (depending on the original phase of testing with observed reactivity) to determine if all the carry-over reactivity of the cold autoagglutinin has been removed. Negative reactions would indicate successful adsorption. If the adsorption was unsuccessful and the cold autoagglutinin persists, the adsorption process can be repeated one additional time using a new vial of stroma. If the adsorption is complete, the plasma/serum is now ready for use in antibody detection or identification tests.5

Limitations

It is possible that a strong cold agglutinin will not be completely adsorbed by this process. According to the manufacturer’s directions, adsorptions using rabbit RBC stroma should be limited to a maximum of two so as to not dilute weakly reactive antibodies. In this instance, other methods, such as cold adsorptions, may be preferred.5 Adsorptions may carry the same risk of dilution. For this reason, it is recommended that each facility validate the maximum number of adsorptions allowed.

Rabbit RBCs/stroma share antigenic determinants with the B antigen, H-related antigens, and P system antigens.5,6 It is likely that antibodies directed toward these antigens would be adsorbed by the rabbit RBCs/stroma. Studies have also shown that these reagents may variably adsorb IgM alloantibodies.7 For these reasons, the rabbit RBC/stroma-
adsorbed plasma/serum should not be used for testing performed at immediate spin, for ABO reverse typing, or for compatibility testing. Moreover, some alloantibodies, such as anti-D, anti-E, and anti-Le\textsuperscript{b} have been shown to be reduced in titer strength or even completely adsorbed by these methods.\textsuperscript{8}

Rabbit erythrocytes readily activate the alternative pathway of complement in human serum; therefore, the complement present in a serum sample may be inactivated when adsorbed with the reagent.\textsuperscript{9,10} Any antibodies that only demonstrate indirectly via the complement they activate may be missed when the adsorbed serum is used in serologic testing. Because most laboratories use plasma and/or anti-IgG exclusively for indirect antiglobulin testing, these antibodies that demonstrate indirectly via the complement they activate would be missed in any routine testing.

**Acknowledgment**

The author would like to thank Dr. John Nobiletti and Joseph Adamy for their assistance and expertise, as well as for their critical reading and editing of the manuscript.

**References**


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Mixed-field agglutination observed in column agglutination testing is not always associated with the A\textsubscript{3} subgroup

N. Anukul, N. Leetrakool, P. Tanan, P. Palacajornsuk, and P. Klangsinsirikul

Mixed-field agglutination (MFA) can be observed in forward typing of samples from A\textsubscript{3} individuals with serologic ABO typing methods. The results of column agglutination testing (CAT) and tube agglutination testing using different antibody clones can be discordant. In this report, we reveal our experience using polymerase chain reaction—sequence-based typing (PCR-SBT) of ABO exon 7 to clarify serologic method discordance of A subgroup blood typing in Northern Thai donors. A total of 21 group A blood donors with either MFA or weak agglutination on routine ABO CAT were recalled. CAT was repeated with human and monoclonal anti-A, and tube agglutination testing with monoclonal anti-A and PCR-SBT of ABO exon 7 was performed. A total of 13 of the 21 donors returned, and ABO CAT with human anti-A was repeated. Eleven samples showed MFA suspected to be the A\textsubscript{3} subgroup, and two samples showed 2+ strength suspected to be the A\textsubscript{weak} subgroup. When tube agglutination testing using monoclonal antibody was performed, MFA was not observed in 9 of 11 samples with previously observed MFA from routine CAT, which were then interpreted as A\textsubscript{3}. From PCR-SBT performed in only exon 7 of the ABO gene, 7 of 13 sample results were consistent with ABO*A2 or ABO*A3 alleles. Two samples suspected to be A\textsubscript{3} or A\textsubscript{weak} had an ABO*A3W allele. In two samples suspected to be A\textsubscript{weak}, no mutation was detected in ABO exon 7, suggesting genetic variation elsewhere in the gene. Although other coding exons were not examined, in the alleles that could be assigned, ABO*A3 alleles were found less frequently than would be predicted from the serologic findings. These findings suggest that when MFA in routine CAT is observed, an A\textsubscript{3} subgroup cannot be presumed. Caution should be exercised when MFA is noted in routine CAT. 

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**Key Words:** ABO gene sequencing, mixed-field agglutination, column agglutination test, discordancy, subgroup of A

ABO testing, required in blood donor processing and pre-transfusion testing, must be accurate to provide compatible blood components for transfusion to patients. If ABO discrepancy—when the red blood cell (RBC) testing result using commercial antisera (forward type) does not agree with the serum/plasma testing result using reagent RBCs (reverse type)—occurs either in donors or recipients, additional testing is needed to resolve the typing.\textsuperscript{1,2} ABO discrepancies can be caused by technical errors or sample-specific characteristics, including weak or variant antigen expression. Technical errors should be ruled out initially. If no problems with technique (including reagents and equipment) are identified, the samples should be examined. Weak or missing reactivity, extra reactivity, and mixed-field agglutination (MFA)—characterized by distinct agglutinated cells mixed with many unagglutinated cells—can occur in both RBC and serum/plasma testing and can result in an ABO typing discrepancy. This report focuses on weak reactivity and MFA in blood group A individuals.

Possible causes of ABO discrepancies associated with weakly expressed antigens observed when the RBCs were typed with anti-A include a subgroup of A, recent transfusion, stem cell transplantation, or malignancy.\textsuperscript{2} We investigated the expression of subgroups of A because our healthy donors had no history of transfusion, transplantation, or serious medical condition. Within the A group, A\textsubscript{1} and A\textsubscript{2} subgroups are the most common. A\textsubscript{1} represents about 80 percent and A\textsubscript{2} represents about 20 percent of group A donors.\textsuperscript{1,2} Subgroups such as A\textsubscript{3}, A\textsubscript{m}, A\textsubscript{v}, and A\textsubscript{e} are found infrequently. These subgroups show weaker reactivity with anti-A reagents, mostly with human polyclonal antibody but also with monoclonal antibody, and can cause ABO discrepancies.\textsuperscript{1} These variants result from genetic variation within the ABO gene producing amino acid substitutions in the glycosyltransferases.\textsuperscript{1,3–5}

In Thailand, most blood bank laboratories perform routine ABO serologic testing using either tube agglutination testing or automated column agglutination testing (CAT). Although CAT is widely used in heavy workload laboratories such as the Thai Red Cross Society, in tertiary hospitals and university hospitals, tube agglutination testing is still the gold standard of serologic testing. Because there is no regulatory requirement regarding ABO discrepancy resolution in Thailand, the Thai Red Cross Society suggests that hospital-based transfusion service laboratories follow AABB guidelines in the interpretation of serologic reactivity and the initial investigation of the discrepancy.\textsuperscript{1} When the discrepancy is resolved, the final ABO group, the reasons for the discrepancy, and the methods of resolution are recorded in the blood bank section of the donor record, and the donor is informed. If the
donor is determined to be a subgroup of A without any clinically significant antibodies, the blood units would be labeled with the name of the subgroup and could be transfused to patients of group A or AB or the identical subgroup. If, however, the discrepancy cannot be resolved by the hospital’s blood bank by any method, then a blood sample can be sent to the Thai Red Cross Society for confirmation. Testing at the Thai Red Cross Society includes conventional tube testing for cell and serum grouping; RBC testing with anti-A, B, anti-A1, and anti-H; testing with lectins; adsorption-elution testing; and saliva testing. In some cases, samples for molecular testing might be sent out (personal communication from Department of Histocompatibility and Immunogenetics Laboratory, National Blood Center, Thai Red Cross Society). This additional testing often will delay the interpretation of the ABO group such that the blood unit will not be released for transfusion.

Serologic-based reagents should be expected to differentiate between A subgroups because weak or no agglutination can lead to wrong ABO group interpretation and the possible transfusion of a weak A subgroup to a group O recipient. This ABO-incompatible transfusion may lead to shortened survival of transfused RBCs in these recipients. Correct differentiation is important for selection of blood products for routine transfusion; thus, accurate ABO typing provides transfusion safety. Moreover, differentiation of A subgroup donors is beneficial in solid organ transplant candidates. Placing a kidney from group A2 living donors into group O or B recipients provides an excellent outcome with immediate function of the allograft and less graft rejection because of the small amount of A antigens in the donor graft. The use of A2 donors can also expand the donor pool. It has been suggested that the use of molecular testing for A1 and A2 subgroups be performed before elective transplantation.

In our university teaching hospital, ABO typing is routinely performed by CAT and follows AABB guidelines for ABO discrepancy resolution. When weak agglutination or MFA is observed, the donor's medical history is reviewed, and the sample is retested by the same CAT card type to rule out technical error. Because the degree of reactivity with monoclonal reagents can vary, retesting with anti-A from a different monoclonal clone, namely one from Thai Red Cross Society with anti-A1 specificity, and with anti-H lectin is performed by tube agglutination testing. If the RBCs do not agglutinate with this monoclonal anti-A1, the A2 subgroup might be suspected. If MFA is again seen and this anti-A1 result is positive, then we presume this sample is of the A3 subgroup. When discordancy between CAT and the tube agglutination method is observed, however, the result is inconclusive. In the present study, polymerase chain reaction–sequence-based typing (PCR-SBT) of ABO exon 7 was used to help clarify the serologic discrepancies in those samples. Because molecular testing is not implemented in either our facility or the Thai Red Cross Society's protocol, this testing is an academic exercise attempted to resolve inconclusive serologic typing.

### Materials and Methods

#### Sample Collection

From 1 January through 31 December 2015, we identified 21 donors with either the rare MFA pattern or only a 2+ or less reaction on ABO CAT with human antisera among all 23,918 donor blood units routinely tested at the blood bank of the Maharaj Nakorn Chiang Mai Hospital (study approval by the ethics committee at the Faculty of Medicine, Chiang Mai University; study code NONE-2558-02885). This 1400-bed,

<table>
<thead>
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<th>Primer name</th>
<th>Sequences (5’ → 3’)</th>
<th>Types</th>
<th>Product size (bp)</th>
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<tr>
<td>ABO-3’ UTR</td>
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<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>ABO-Seq1</td>
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<td></td>
</tr>
<tr>
<td>ABO-Seq2</td>
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<td>Forward</td>
<td></td>
</tr>
<tr>
<td>ABO-Seq3</td>
<td>TCCACCTCGTGAGGAGCGCG</td>
<td>Reverse</td>
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</tr>
<tr>
<td>ABO-Seq4</td>
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</tr>
<tr>
<td>ABO-3’ UTR</td>
<td>See sequence above</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction; bp = base pair.
medical-teaching, tertiary-care hospital serves the northern region of Thailand. These 21 donors were contacted and requested to return to provide an additional 20-mL sample of blood collected in EDTA tubes to repeat the CAT and perform additional ABO tests including tube agglutination testing and PCR-SBT.

**ABO Phenotyping by CAT**

Forward typing and reverse typing were performed using two types of gel cards: the ABO/D + Reverse Grouping card and the DiaClon ABO/D + Reverse Grouping card (Bio-Rad Laboratories, Cressier, Switzerland). The ABO/D + Reverse Grouping card contains a mixture of human polyclonal and monoclonal anti-A (cell line: A5) (referred to as “CAT with human antisera” in this report), and the DiaClon ABO/D+ Reverse Grouping card contains monoclonal anti-A (cell line: A5) (referred to as “CAT with monoclonal antisera” in this report). Testing procedures were performed following manufacturer instructions.

**ABO Phenotyping by Tube Agglutination Test**

Forward typing was performed by testing 5 percent RBC suspensions from each donor with monoclonal anti-A (cell line: combination of 3C4, 6G4, 16G4, and LM103), anti-B, anti-A,B, anti-A1 (cell line: 6C10), and anti-H lectin (extract of *Ulex europaeus* (Thai Red Cross Society). RBCs were washed three times with normal saline to avoid spontaneous agglutination and nonspecific aggregation. Reverse typing was also performed by testing two drops of plasma with 5 percent group A, B, and O cells (prepared in-house). After centrifugation, the agglutination reaction was graded, interpreted, and classified for the resulting ABO subgroups according to the AABB Technical Manual.

**ABO Gene Sequencing by PCR-SBT**

Genomic DNA samples were prepared from EDTA-anticoagulated blood using the Purelink Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA). PCR was performed using primers specific for exon 7 of the ABO gene: forward primer ABO-Ex7 and reverse primer ABO-3’UTR, both providing 725 bp PCR product (Table 1). The PCR mixture included 150 ng genomic DNA, 0.3 μmol/L gene-specific primers, 1x buffer for KOD-Plus-Neo, 0.2 mmol/L dNTP each, 1.5 mmol/L MgSO4, and 1 U KOD-Plus-Neo (Toyobo, Osaka, Japan) in 50 μL reaction volume. The cycling condition was the step-down cycle according to the KOD-Plus-Neo kit’s instructions: 2 minutes at 94°C for 1 cycle, 10 seconds at 98°C and 30 seconds at 74°C for 5 cycles, 10 seconds at 98°C and 30 seconds at 72°C for 5 cycles, 10 seconds at 98°C and 30 seconds at 70°C for 5 cycles, 10 seconds at 98°C and 30 seconds at 68°C for 30 cycles, and 7 minutes at 68°C for the final extension. The PCR products were fractioned on a 1 percent agarose gel containing 0.4 μg/mL ethidium bromide and then purified using the Purelink Quick Gel Extraction Kit (Invitrogen). Nucleotide sequencing was carried out via Sanger sequencing on ABI3730XL (Bio Basic Canada, Markham, Canada) using six primers (Table 1). The Sanger contig overlapping nucleotide segments representing consensus sequences were analyzed based on information from the Blood Group Antigen Gene Mutation Database and the alleles table constructed by the International Society of Blood Transfusion Red Cell Immunogenetics and Blood Group Terminology.

**Family Studies**

Returning donors were interviewed for medical and family history. The donation history of all donors in our laboratory’s records were reviewed. Their family members were encouraged to also participate in this study. After signing the consent form, a 20-mL blood sample was collected in EDTA tubes to perform ABO testing by CAT, tube agglutination testing, and PCR-SBT.

**Results**

**Subjects Studied**

During the 2015 study year, among the 23,918 routine CATs performed in blood donors at our hospital, group A donors constituted 4847 (20.3%) and group AB donors 1459 (6.1%), group B donors numbered 7343 (30.7%), and group O 10,269 (42.9%). Among the 6306 group A and AB donors, we identified 19 (0.30%) with MFA patterns, and 2 (0.03%) donors demonstrated weak (2+) expression by routine CAT with human antisera. The prevalence of ABO phenotypes in our study of Thai donors was similar to that reported in previous studies in which blood group O was approximately 40 percent, group B was 30 percent, group A was 20 percent, and group AB was 10 percent. Among group A, the Thai Red Cross reported in 2017 that the frequency of A3 and A3B classified by MFA detection from tube agglutination testing was 0.04 percent and 0.46 percent, respectively. Of 21 donors who were contacted, a total of 13 (62%) returned, consented to participate in this study, and provided additional blood samples (11 with MFA patterns and 2 with 2+ agglutination). From the review of medical and donation histories, none of the donors had previous non-ABO group-
specific RBC component transfusion, ABO-mismatched stem cells or bone marrow transplants, or history of malignancy. Therefore, weak antigen expression or MFA is likely due to a genetic factor. Five of the 13 donors (donors 3, 9, and 11–13) had previously donated more than three times and had the alert message in the hospital’s donation record as “suspected subgroup.” The other eight donors were first-time blood donors to the center.

Discordant Results of Blood Group Serologic Tests

Table 2 shows the agglutination reaction strength of the returning 13 donors from CAT and tube agglutination testing. From routine CAT with human antisera, the 11 donors with MFA in forward typing were interpreted to be in the A
\(_3\) subgroup (donors 1–11). The two donors with 2+ agglutination strength were suspected to be of the A\(_{weak}\) subgroup (donors 12 and 13). Reverse typing of all 13 studied donors showed the typical agglutination pattern of blood group A.

The results of CAT revealed differences in the reaction patterns of forward typing between CAT with human antisera

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Test method*</th>
<th>Forward typing</th>
<th>Reverse typing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>anti-A</td>
<td>anti-B</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td></td>
<td>Tube</td>
<td>2+mf</td>
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<tr>
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<td>CAT human antisera</td>
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<td>Tube</td>
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<tr>
<td>21</td>
<td>CAT human antisera</td>
<td>mf</td>
<td>4+</td>
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</table>

* Mixed-field agglutination in the tube agglutination test is distinct agglutinated cells with 2+ reaction strength mixed with many unagglutinated cells that can be confirmed under light microscope. Mixed-field agglutination in CAT is agglutinated RBCs in the upper half of the gel column with no agglutinated cells at the bottom.

† Samples from donors 14–21 were tested with only routine CAT human antisera, since they did not return to our laboratory for additional testing.

ID = identification; CAT = column agglutination testing; mf = mixed-field agglutination; neg = negative; ND = not done; wk = weak agglutination.
and CAT with monoclonal antisera. When using CAT with monoclonal antisera, only 9 of the 11 donors showed MFA (donors 1–9) and the other two showed 4+ strength (donors 10 and 11). For donors 12 and 13, weak A expression without MFA was observed by using CAT with human antisera, and MFA could be observed by using CAT with monoclonal antisera.

The tube agglutination test found MFA only in donors 1 and 2 and weak A expression only in donor 13. The other donor samples showed the expected results of the typical A blood group with strong reaction strength (Table 2). When testing RBCs with anti-A1, however, the RBCs of 11 of the 13 donors were not agglutinated by the anti-A1 and thus were re-typed by our study as the A₂ subgroup. Also, the RBCs of donors 1 and 2 were agglutinated by anti-A1 and thus re-typed as the A₁ subgroup⁹ (Table 3).

Because the agglutination patterns between CAT and tube agglutination testing were significantly different, the ABO phenotypes of these 13 donors could not be concluded. We thus performed ABO exon 7 sequencing to aid in the prediction of the subgroup.

### A Allele Sequencing Results

The ABO exon 7 sequencing results of each donor were compared with the Blood Group Antigen Gene Mutation Database, and nucleotide variants were identified by comparing the observed sequence in the coding sequence of A allele located at nucleotide 375–1065 (Table 3). Probable alleles were assigned based on the nomenclature of ABO alleles (Table 3). The variant sequences of all samples were found in heterozygosity. Seven samples (3–9) contained two variants, c.467C>T and c.1061delC, known to be found in multiple alleles of ABO*A₂ and ABO*AW. These findings rule out the A₃ subgroup. Three samples (1, 2, and 10) carry ABO*AW31.01 or ABO*AW31.02-05 alleles, which would be the A₉ subgroup. The remaining samples (11–13) had no changes in exon 7, which suggests that changes are present in other regions of the gene. Additional sequence-based typing would be necessary to assign alleles in these cases.

#### Discussion

In this study, we report our experience with A subgroup typing of Northern Thai blood donors in an attempt to clarify the discordant results between routine CAT and tube agglutination testing by PCR-SBT of ABO exon 7. Interestingly, the results from tube agglutination tests were found to correlate more with PCR-SBT. Only two samples (of 13) showed correlation between tube agglutination testing and CAT in MFA detection, whereas the remainder (11 of 13) were discordant. These 11 samples were initially identified as the A₂ subgroup from CAT with human antisera, but later were identified as the A₉ subgroup from tube agglutination testing with monoclonal antibody. Using different sources and types of antisera may have led to these discordant results. Langston et al.¹⁵ showed that when weak A and weak B are tested by

---

### Table 3. ABO interpretation for 13 donors by CAT, tube agglutination testing, and PCR-SBT

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Human antisera</th>
<th>Monoclonal antisera</th>
<th>Without anti-A1</th>
<th>With anti-A1</th>
<th>Nucleotides in exon 7 that differ from consensus</th>
<th>PCR-SBT</th>
<th>Probable allele (name based on ISBT allele terminology)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>A₁</td>
<td>A₁</td>
<td>A₁</td>
<td>A₁</td>
<td>c.646T/A; c.681G/A; c.771C/T; c.829G/A</td>
<td>ABO<em>AW31.01, ABO</em>AW31.02-05</td>
<td></td>
</tr>
<tr>
<td>3–8</td>
<td>A₂</td>
<td>A₂</td>
<td>A₂</td>
<td>A₂</td>
<td>c.467C/T; c.1061delC/C</td>
<td>ABO<em>A₂.01, ABO</em>A₂.14, ABO<em>A₂.15, ABO</em>A₂.16, ABO<em>A₂.17, ABO</em>A₂.18, ABO*A₂.45</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>A₁B</td>
<td>A₁B</td>
<td>AB</td>
<td>A₁B</td>
<td>c.467C/T; c.1061delC/C</td>
<td>ABO<em>A₂.16, ABO</em>AW.02, ABO<em>AW.03, ABO</em>AW.09, ABO<em>AW.16, ABO</em>AW.17, ABO<em>AW.18, ABO</em>AW.45</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>A₁B</td>
<td>AB</td>
<td>AB</td>
<td>A₁B</td>
<td>c.646T/A; c.681G/A; c.771C/T</td>
<td>ABO<em>A₁.05, ABO</em>A₁.06, ABO<em>A₁.07, ABO</em>A₂.10, ABO*A₂.11, A₂15, A₃43, A₃12, Aₓ26</td>
<td></td>
</tr>
<tr>
<td>11, 12</td>
<td>Aₙ₁B</td>
<td>Aₙ₁B</td>
<td>AB</td>
<td>Aₙ₁B</td>
<td>c.467C/T</td>
<td>ABO<em>IVS2+3G(A₁), ABO</em>248G(Aₙ₁), ABO<em>IVS1+5859C(Aₙ₂), ABO</em>527(A₂), ABO<em>119A(A₃), and ABO</em>188A,189T(Aₓ)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Aₙ₁B</td>
<td>Aₙ₁B</td>
<td>Aₙ₃B</td>
<td>Aₙ₁B</td>
<td>None</td>
<td>ABO<em>IVS2+3G(A₁), ABO</em>248G(Aₙ₁), ABO<em>IVS1+5859C(Aₙ₂), ABO</em>527(A₂), ABO<em>119A(A₃), and ABO</em>188A,189T(Aₓ)</td>
<td></td>
</tr>
</tbody>
</table>

*Because other exons of the ABO gene were not analyzed, the variant sequences found in all donors may be consistent with more than one allele.

‡Full-gene sequencing is further required to confirm the actual allele.

CAT = column agglutination testing; PCR-SBT = polymerase chain reaction–sequence-based typing; ID = identification; ISBT = International Society of Blood Transfusion.
CAT with both monoclonal and polyclonal antisera, mistyping can occur. Even when the same type of antisera (monoclonal) were used, the results were discordant between methods. This finding was most likely because the monoclonal antibodies produced by different cell lines recognize different epitopes of the A or B antigen. Such discordance has not been observed in typical ABO testing, however, and several previous studies have reported the correlation between the tube agglutination tests and CAT in ABO typing. From our results, CAT seems to be more sensitive than the tube agglutination method in terms of detecting MFA and weak agglutination correlated to the previous reports from other CAT systems such as AutoVue Innova and WaDiana Compact. Through the use of these systems, two-cell populations were easily observed. From this point of view, there should be awareness when selecting the ABO typing method and the source of commercial antibodies to be used in a routine laboratory. Some antibody clones may not detect weak antigen expression, resulting in a different interpretation.

The importance of subgroup identification in donors is distinct from that in patients. Although the blood units from the subgroup of A blood donors without clinically significant anti-A1 or other antibodies can be transfused to typical blood group A or AB individuals, once ABO discrepancy occurs, resolution is required to identify the true blood group of such blood units before they are released for transfusion to avoid adverse reaction. Moreover, all the donor’s data will then be recorded in the laboratory information system, and the true blood group will be recorded in the hospital information system. Such data will be useful for transfusion purposes if this donor returns to the hospital as a patient in the future. Note, however, that sequencing of ABO is not a licensed method and should be interpreted with caution, especially when incomplete, as in our cases.

ABO genotyping has not been implemented for routine use in donor centers or hospital laboratories. SBT may be useful for predicting ABO variants but is not ideal for routine ABO typing, since it is more time-consuming and expensive than serologic testing, which is simpler and more rapid. Moreover, SBT, when it does not cover the entire coding or gene region, can result in an inability to assign alleles or an inaccurate interpretation of alleles. Additionally, predictions may be inaccurate because of alterations of gene expression unrelated to the gene regions interrogated. In our study, we used SBT of ABO exon 7 as an additional test, along with additional serologic testing, to explore the genetic cause of subgroup variation and resolve ABO discrepancy.

Because the majority of single nucleotide polymorphisms in the ABO coding region are located in exon 7 of the ABO gene and because of limited resources, we selected to examine exon 7 to identify the types of A allele. The cost of ABO exon 7 genotyping by PCR-SBT is approximately $150 per sample. In our study, PCR-SBT was performed only on samples with inconclusive results from the two accepted methods of serologic testing. Among our A subgroup donors, mutations at c.467C>T and c.1061delC of the ABO*A2.01 allele were common, as reported in other populations. Two AB donors with the A subweak subgroup exhibited the ABO*A1.02 allele with c.467C>T, which is common in individuals of Asian descent, and one group AB donor exhibited no changes in exon 7 of the A allele. This finding suggests that other variants may be present in the other exons and/or introns of the ABO gene.

Because this study sequenced only ABO exon 7, we recognize that there is a limitation that genetic variation may be missed and allele assignments may be incorrect. Table 3 lists the probable allele assignment as well as other possible alleles for each sample. In samples 3–9, c.467C>T and c.1061delC were also consistent with alleles ABO*A2.14 and ABO*A2.15, which share coding sequence with ABO*A2.01, and with alleles ABO*A2.16, ABO*AW.02, ABO*AW.03, ABO*AW.09, ABO*AW.16, ABO*AW.17, ABO*AW.18, and ABO*AW.45, which do not share coding or splice site sequence with ABO*A2.01. In donors 1 and 2, ABO*AW31.02-05 allele was detected, and if exon 6 had been analyzed and c.297A>G had been found, ABO*AW31.01 allele would be concluded. In donor 10, a novel allele was identified that is similar to ABO*AW31.01 but without c.829G>A. No genetic variants were found in ABO exon 7 in donor 13. Based on the serology, it is possible that this donor carries one or more variants in ABO gene regions not interrogated by this study. Therefore, extending the sequencing to the other exons would be useful to identify the specific allele.

In conclusion, although CAT can perform blood grouping of typical ABO samples with accuracy that is comparable to tube agglutination testing, for A subgroups, CAT is more sensitive in MFA detection, but interpretation may differ from tube agglutination testing and PCR-SBT. From this study with only ABO exon 7 sequencing, ABO*A43 alleles were less frequent than expected. Hence, observing MFA from routine CAT does not imply an A4 subgroup and requires additional testing. We explored the use of ABO exon 7 sequencing to help clarify inconclusive results from the serologic methods;
this molecular testing can be an option in these inconclusive cases.21,22

Acknowledgments

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NA analyzed the data, and drafted and revised the manuscript; NA and NL designed and performed the research study; NL and PT contributed essential reagents, tools, and blood donor data; and PP and PK critically reviewed the results and revised the manuscript.

References


Manuscripts

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

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The use of low-ionic-strength saline (LISS) solution as an enhancement for antibody screening and crossmatching was first described by Löw and Messeter in 1974. This method allowed for a reduced incubation time while maintaining adequate specificity and sensitivity of the antiglobulin test (AGT). Since then, the LISS-AGT tube method has been widely used in antibody detection and identification, as well as compatibility testing. As initially described, the method used red blood cells suspended in LISS. Modifications of the method led to development of the commercially prepared LISS additive solutions in use today. The LISS-AGT can be used effectively to detect alloantibodies of all major blood groups in antibody detection, antibody identification, and crossmatching procedures. *Immunohematology* 2018; 34:57–60.

**Key Words:** low-ionic-strength saline, LISS, antiglobulin test, LISS-AGT, antibody detection, enhancement

### Principle

Prior to the development and implementation of low-ionic-strength saline (LISS) solutions for enhancement of the indirect antiglobulin test (IAT) in blood bank serologic testing, the standard tube procedures for antibody detection/identification and crossmatching involved testing patient serum or plasma with commercially prepared screening or donor red blood cells (RBCs) using either a saline method with no enhancement media or 22–30 percent bovine serum albumin (BSA) additive. These methods required a minimum incubation period of 30–60 minutes before completing the antiglobulin test (AGT). Introducing LISS into the test system aids in reducing the electrostatic charge surrounding the RBC, allowing for more rapid antibody uptake, thus enhancing the first stage of the agglutination reaction. Wicker and Wallas identified that the use of an LISS RBC suspension media combined with an AGT reduces the incubation time to 10–15 minutes while increasing sensitivity to detect antibodies previously undetectable by the BSA-AGT method. Additionally, the LISS method allows detection of directly agglutinating antibodies after the 37°C incubation and before the AGT, thus helping to distinguish multiple specificities or to detect potentially clinically significant IgM reactivity. Current commercially available LISS additive solutions contain sodium chloride, glycine, bovine albumin, and other proprietary substances.

### Background

The use of LISS for the resuspension of RBCs used in serologic testing was described as early as 1964. Antibody uptake is enhanced in low-ionic media that contain significantly fewer Na+ ions than normal saline solutions. Hughes-Jones et al. found that the rate of antibody uptake increased almost 1000 times when the ionic strength of the solution was

### Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>LISS additive solution (commercially prepared)</td>
<td>• 10 × 75 or 12 × 75 mm test tubes</td>
</tr>
<tr>
<td>Anti-human globulin (polyspecific or anti-IgG)</td>
<td>• Transfer pipettes or serologic pipettes</td>
</tr>
<tr>
<td>IgG-coated control RBCs</td>
<td>• Calibrated serologic centrifuge</td>
</tr>
<tr>
<td>0.9% saline or PBS (pH 6.5–7.5)</td>
<td>• 37°C heat block or water bath</td>
</tr>
<tr>
<td><a href="https://www.example.com">10 × 75 or 12 × 75 mm test tubes</a></td>
<td>• Timer</td>
</tr>
<tr>
<td><a href="https://www.example.com">Transfer pipettes or serologic pipettes</a></td>
<td>• Optical viewing aid</td>
</tr>
</tbody>
</table>

LISS = low-ionic-strength saline; RBCs = red blood cells; PBS = phosphate-buffered saline.

### Procedural Steps

- Mix test serum or plasma with RBCs to be tested.
- Add LISS reagent according to manufacturer’s instructions.
- Incubate at 36–38°C for the time specified in the manufacturer’s instructions.
- Centrifuge, examine for hemolysis, and resuspend RBCs to examine for agglutination. Record results.
- Wash the test reaction three to four times with saline. Decant completely after the last wash.
- Add anti-IgG, centrifuge, and resuspend RBCs to examine for agglutination. Record results.
- Add IgG-coated control cells to all negative reactions. Centrifuge and resuspend RBCs to examine for agglutination. Record results.

RBCs = red blood cells; LISS = low-ionic-strength saline.
LISS additive solutions became popular in the early 1980s when commercial reagent manufacturers began to provide these reagents. Because of increased sensitivity and reduced incubation times, these methods rapidly became the “standard of practice” for antibody detection/identification and compatibility testing. Lown et al. described their dilemma in selecting the “best” antibody detection method. They outlined the evolution from albumin enhancement to LISS to PeG and finally to gel testing, assessing the balance of sensitivity, specificity, and patient safety for each. An abstract presented at the 1997 AABB annual meeting reported comparison of LISS and PeG tube methods with gel and solid-phase methods. In that study, the LISS-AGT was comparable in specificity but showed a sensitivity of 91.2 percent when compared with PeG (96.8%), gel (95.9%), and solid phase (99.1%). Dinardo et al. provided evidence for use of the LISS-AGT crossmatch method with a 5-minute incubation for emergency situations. Whereas other methods require 15–60 minutes for incubation, they showed that a 5-minute LISS-AGT method could provide adequate sensitivity and specificity for providing crossmatch-compatible RBCs for patients requiring an antihuman globulin (AHG) crossmatch.

**Indications**

LISS-AGT or LISS-IgG is used in tube methods to detect and identify clinically significant alloantibodies in patient or donor serum or plasma and for compatibility testing at the AHG phase. An LISS tube method allows reading the agglutination reaction after the 37°C incubation and again at the AHG phase of testing. These methods can be useful when resolving antibody detection tests in patients with warm autoantibodies. Routine use of a more sensitive method (PeG, solid-phase, or gel testing) may detect weak autoantibodies. Use of an LISS-AGT method may reduce or eliminate reactivity of the weak autoantibody while allowing detection and identification of clinically significant alloantibodies.

**Procedure**

Label test tubes for the reagent RBC samples to be used in the testing. Confirm with the manufacturer’s instructions for use (IFU) if glass tubes are required. Dispense two drops (~100 µL) of the serum or plasma to be tested into each labeled tube. Add 1 drop (~50 µL) of the 3–5 percent RBC suspension to be tested (washed and resuspended in 0.9% saline or commercially prepared reagent RBCs) and mix. Add the appropriate number of drops of the LISS additive.
reagent, according to the manufacturer’s IFU. Mix. Incubate at 36–38°C for the time specified by the manufacturer’s IFU. Centrifuge for a time appropriate to the calibrated centrifuge in use. Resuspend the RBCs, and examine for hemolysis and agglutination. Record results. Wash the RBCs in each tube three to four times with an adequate amount of saline, decanting carefully between each wash. Resuspend the RBCs before adding the next wash saline. Decant completely after the last wash. Add one to two drops of polyspecific AHG or anti-IgG, according to the manufacturer’s IFU. Centrifuge for a time appropriate to the calibrated centrifuge in use. Resuspend the RBCs and examine for agglutination. Negative reactions may be read using an optical aid. Record results. Add IgG-coated control cells to all negative reactions. All reactions should be positive after addition of the IgG-coated control cells. If not, the result is invalid, and the test must be repeated.

Limitations

The LISS-AGT tube test should always be performed and interpreted according to the IFU provided by the specific manufacturer of the LISS additive. Proportions of serum/plasma:cells:LISS should be strictly followed to achieve the desired ionic strength of the final reaction medium. Cold-reactive antibodies may not react optimally without a room temperature incubation step. The LISS-AGT method is not recommended as the sole verification of ABO compatibility. If RBCs used in the test system have a positive direct antiglobulin test (DAT), reactivity will be present in the IAT, regardless of the presence of specific antibody in the serum or plasma directed against RBC antigens. Incubation for less than 10 minutes or for more than 30 minutes may result in weaker reactions.

As with all serologic testing, false-positive or false-negative results may be obtained because of contaminated reagents or other test materials; improper test performance, including incubation, centrifugation, and washing; improper storage of reagents; omission of test reagents; and prolonged storage of test RBCs. No test methods will detect all RBC antibodies. If plasma samples are used for testing, complement-dependent antibodies may not be detected.

Quality Control

LISS additives should be tested as part of day-of-use quality control procedures using a weakly reactive antibody sample to ensure adequate antibody detection enhancement. Additionally, an autocontrol control or a DAT on the patient’s RBCs should be included in antibody identification procedures to assist in the differentiation of alloantibodies from auto-antibodies.

During performance of the LISS-AGT, if IgG-coated control cells (added after reading the final reaction) are non-reactive, the result is invalid, and the test must be repeated.

References


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Antigens belonging to the Rh and Kell blood group systems are of major clinical significance because of their immunogenicity and the potential of their consequent antibodies to cause in vivo destruction of exogenous red blood cells (RBCs). Despite the widespread use of transfusion, there are sparse data on the prevalence of Rh and Kell system antigens and their ethnic variability in Nigeria. The objective of this study was to determine the prevalence of the five major Rh (D, C, c, E, e) and Kell (K) system antigens in Nigeria with the goal of understanding alloimmunization risk in transfusion recipients and improving transfusion safety through the availability of resources, such as antisera for extended RBC typing and antigen panels for alloantibody detection. A multi-ethnic cohort of 302 healthy Nigerian individuals was created to study RBC antigen prevalence. The antigen status of these individuals for Rh and K antigens was determined using commercially prepared antisera and conventional tube agglutination methods. The prevalence of the Rh antigens in the study cohort was found to be: D (92.7%), C (20.5%), c (97.7%), E (19.5%), and e (97.4%). Dce was the most common Rh phenotype (53.3%). The prevalence of K was 0 percent. For all antigens, there was no association between ethnicity and antigen prevalence. This study is the first to document the prevalence of the major Rh and K antigens in the Nigerian population, using a multi-ethnic cohort. Serologic testing demonstrates a zero prevalence of K antigen, which has never been described. C and E pose the higher risks of alloimmunization, hence showing a need for extended RBC typing and matching in at-risk blood recipients. This study demonstrates that phenotyping for major Rh and K antigens within the Nigerian population can potentially improve transfusion safety and prevent alloimmunization. Immunohematology 2018;34:61–65.

Key Words: blood group, blood group antigens, antigen phenotypes, Rh, Kell, Nigeria

The clinical importance of any blood group antigen is determined by its ability to induce antibody formation and the ability of these antibodies to cause in vivo destruction of red blood cells (RBCs). Of the blood group antigens, the Rh and Kell antigens are particularly clinically significant because of their immunogenicity, high risk of alloantibody formation, and ability to cause severe hemolytic transfusion reactions. In many well-developed countries, such as the United States, the prevalence of RBC antigens and their ethnic variation are well established. In such countries, high-risk patients (such as those requiring frequent transfusion) are often preemptively matched for Rh and Kell system antigens in an effort to decrease the risk of alloimmunization. Unfortunately, this practice is impossible in developing countries such as Nigeria. In these areas with limited resources, antibody screening and RBC phenotyping are not routinely available. Because of the unavailability of testing, data on the prevalence and ethnic variation of the Rh and Kell antigen systems in Nigeria are very limited. This lack of data is especially problematic in sub-Saharan African countries, where sickle cell disease is highly prevalent and RBC transfusion is a mainstay of therapy. In an effort to improve transfusion safety both in Nigeria and for black individuals worldwide with shared African ancestry, we conducted a study to determine the phenotypic distribution of the major Rh and K antigens in Nigeria.
(20–25°C) or within 14 days of sample collection if stored at a temperature of 1–6°C. Rh and K antigen typing was carried out using the conventional tube agglutination method and commercially sourced antisera according to the manufacturer’s instructions (Lorne Laboratories, Reading, UK). Each batch of tests was accompanied with control samples. Positive control cells were RBCs with single-dose antigen expression (i.e., C+c+ or E+e+). Negative control cells were RBCs that lacked the target antigens. All serologic reactions were carried out at optimal temperature (the temperature of the water bath was quality controlled with an external, calibrated thermometer). All centrifugations were performed at 1000 relative centrifugal force (RCF) for 20 seconds or suitable alternate force and time. Before reading each test after centrifugation, the tube was shaken gently to dislodge the RBC button from the bottom of the tube. All antisera were stored at 2–8°C when not in use.

**Statistical Analysis**

Of the Rh and Kell antigens studied, the antigen with the lowest prevalence is K, with a reported prevalence of 2 percent for black individuals in the United States. Assuming the percentage of each antigen in Nigeria is 2 percent, sample size was calculated. For 80 percent power, two-sided alpha of 5 percent, to detect a difference of 0.05 (P1 – P0), with population proportion of 2 percent under null, a sample of 96 was required. Data were inputted and analyzed using Statistical Package for Social Sciences (SPSS) version 16 (IBM, Armonk, NY). Variations in distributions of the blood group antigens were tested with the $\chi^2$ test or Fisher’s exact test, as appropriate. Probability value of less than 5 percent ($p = 0.05$) was considered statistically significant.

**Results**

**Patient Demographics**

Between January and April 2015, 302 healthy individuals were recruited from the University of Benin for the study of RBC antigens in the Nigerian population. Demographics of study participants ($N = 302$) are shown in Table 1. Mean age was 22.8 ± 3.3 years, and participants were primarily male (63.3%). The majority of the study cohort (77.2%) was composed of individuals from five major ethnic groups in Southern Nigeria, with Bini being the predominant ethnic group in the study population (27.5%). Representatives from 26 other Nigerian ethnic groups constituted 22.8 percent of the study cohorts, with less than 10 individuals from each group.

**Table 1. Demographics of study participants, $N = 302$**

<table>
<thead>
<tr>
<th></th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>16–25</td>
<td>256 (84.8)</td>
</tr>
<tr>
<td>26–35</td>
<td>44 (14.6)</td>
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<tr>
<td>36–45</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>191 (63.3)</td>
</tr>
<tr>
<td>Female</td>
<td>111 (36.8)</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
</tr>
<tr>
<td>Bini</td>
<td>83 (27.5)</td>
</tr>
<tr>
<td>Ibo</td>
<td>70 (23.2)</td>
</tr>
<tr>
<td>Esan</td>
<td>37 (12.3)</td>
</tr>
<tr>
<td>Yoruba</td>
<td>25 (8.3)</td>
</tr>
<tr>
<td>Urhobo</td>
<td>18 (6)</td>
</tr>
<tr>
<td>Other*</td>
<td>69 (22.8)</td>
</tr>
</tbody>
</table>


**Fig. 1 Rh and Kell antigen prevalence.** Rh and Kell (K) antigen typings were completed using specific antisera and tube agglutination method for all participants in the study cohort ($N = 302$). Number of participants typing serologically positive is shown on the y-axis.
were identified in our study cohort. The most common Rh phenotype was Dccee (53.3%).

No statistically significant difference (p > 0.05) was observed in antigen prevalence among the studied ethnic groups in Nigeria (Table 3). Among the studied antigens, the probabilities of antigen-mismatched transfusions are highest for C and E (Table 4).

**Discussion**

Our study is the first to systematically determine the prevalence of the five major Rh and Kell (K) antigens in a multi-ethnic cohort from Nigeria. The prevalence of the D antigen in discrete parts of Nigeria has been well described, ranging from 93.9 percent to 98.8 percent, whereas the prevalence of the other four major Rh antigens in Nigeria is not well known. Prior studies on Rh antigen prevalence in Nigeria have been limited because they never effectively reflected the multi-ethnic makeup of Nigeria, which is a critical factor in determining the distribution of the Rh antigens. In contrast, our study benefited from the diverse ethnic background of our cohort, which included individuals from more than 30 ethnic groups (Table 1). In our study, the distribution of Rh antigens was consistent with what is reported for black individuals in the United States (Fig. 1).

Rh phenotyping was also in agreement with what is reported for the black population in the United States (Table 2). Table 5 highlights data from previous studies on Rh antigen distribution in Port Harcourt and Calabar, Nigeria. Significant differences are observed in the distribution of the Rh antigens from Calabar and Port Harcourt, when compared with the index study and U.S. black individuals. This finding is somewhat surprising, since there was no multi-ethnic variations observed in the index study. A wider and larger sample size would be more representative and conclusive.

### Table 2. Rh and Kell antigen phenotypes possible and most probable Rh genotypes, N = 302

<table>
<thead>
<tr>
<th>Rh phenotypes</th>
<th>Number (%)</th>
<th>Possible genotypes</th>
<th>Most probable genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+,–,–,E–e+ (D–,–ee)</td>
<td>3 (1)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>D+,–,–,E+e+ (D–,–Ee)</td>
<td>1 (0.3)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>D+C–c+,–,– (Dcc–,–)</td>
<td>3 (1)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Dccee</td>
<td>161 (53.3)</td>
<td>R,R,R,Rr</td>
<td></td>
</tr>
<tr>
<td>DcEe</td>
<td>46 (15.2)</td>
<td>R,R,R,Rr</td>
<td></td>
</tr>
<tr>
<td>DcE</td>
<td>5 (1.7)</td>
<td>R,R,R,Rr</td>
<td></td>
</tr>
<tr>
<td>DCce</td>
<td>51 (16.9)</td>
<td>R,R,R,Rr</td>
<td></td>
</tr>
<tr>
<td>DCcEe</td>
<td>7 (2.3)</td>
<td>R,R,R,Rr,Rr; R,R; R,R</td>
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</tr>
<tr>
<td>DcE</td>
<td>3 (1)</td>
<td>R,R,R,Rr</td>
<td></td>
</tr>
<tr>
<td>Dce</td>
<td>20 (6.6)</td>
<td>R,R</td>
<td></td>
</tr>
<tr>
<td>dcEe</td>
<td>1 (0.3)</td>
<td>r,r</td>
<td></td>
</tr>
<tr>
<td>dCcEe</td>
<td>1 (0.3)</td>
<td>r,r; r,r; r,r</td>
<td></td>
</tr>
<tr>
<td>dce</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Antithetical deletion of antigens present.

### Table 3. Antigen distribution and ethnic variability, N = 302

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Ethnic group</th>
<th>Bini (n = 83)</th>
<th>Urhobo (n = 70)</th>
<th>Ibo (n = 70)</th>
<th>Yoruba (n = 25)</th>
<th>Esan (n = 37)</th>
<th>Other Edo (n = 21)</th>
<th>All Others (n = 48)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>POS</td>
<td>75</td>
<td>16</td>
<td>68</td>
<td>22</td>
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<td>18</td>
<td>46</td>
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<tr>
<td></td>
<td>NEG</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>POS</td>
<td>16</td>
<td>3</td>
<td>17</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>9</td>
<td>0.807</td>
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<td>53</td>
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<td>28</td>
<td>19</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>POS</td>
<td>79</td>
<td>17</td>
<td>70</td>
<td>24</td>
<td>37</td>
<td>21</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>15</td>
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<td>16</td>
<td>38</td>
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<tr>
<td>e</td>
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<td>82</td>
<td>18</td>
<td>67</td>
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<td>21</td>
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<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>POS</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NEG</td>
<td>83</td>
<td>18</td>
<td>70</td>
<td>25</td>
<td>37</td>
<td>21</td>
<td>48</td>
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</table>

POS = positive; NEG = negative.
Table 4. Probabilities of antigen-mismatched transfusions in the study population, N = 302

<table>
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<tr>
<th>Antigen System</th>
<th>Antigen</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Probability (%)</th>
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<td>Rh system</td>
<td>D</td>
<td>92.7</td>
<td>7.3</td>
<td>8.6</td>
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<tr>
<td></td>
<td>C</td>
<td>20.5</td>
<td>79.5</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>97.4</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>97.7</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>19.5</td>
<td>80.5</td>
<td>15.7</td>
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</table>

Kell system

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Positive (%)</th>
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<th>Probability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>—</td>
<td>100</td>
<td>0</td>
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Table 5. Comparison of prevalence of Rh and Kell antigens from other studies

<table>
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<th>Reference</th>
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<tr>
<td>Index study</td>
<td>D 92.7</td>
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<tr>
<td>U.S. black population</td>
<td>C 20.5</td>
</tr>
<tr>
<td>Port Harcourt, Nigeria</td>
<td>e 97.4</td>
</tr>
<tr>
<td>Calabar, Nigeria</td>
<td>c 97.7</td>
</tr>
<tr>
<td>Sokoto, Nigeria</td>
<td>K 100</td>
</tr>
<tr>
<td>Port Harcourt, Nigeria</td>
<td>E 82.0</td>
</tr>
<tr>
<td>Sokoto, Nigeria</td>
<td>K 95.56</td>
</tr>
<tr>
<td></td>
<td>K 98.5</td>
</tr>
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</table>

Possible variations resulting from differences in study populations and ethnicity cannot be completely excluded. It is worth noting that the RhNull phenotype was not observed in this study. It is also worthy to note that no rr individuals were found in this study despite its ample sample size. Dccee is the most common Rh phenotype (53.3%) in our cohort (Table 2). In white individuals, the prevalence of the haplotypes from the most common to the least common is R, r, R, R, r, r, r, r, r, and r. In black individuals, the most common to the least common is r, r, r, r, r, r, and r. Determining the most probable genotype depends on the knowledge of the frequency at which a particular antigenic combination derives from a single gene complex within a particular population. We observed R,r,r (2.3%) to be slightly more common than R,R (1%) based on deductions of the most probable genotype, which appears inconsistent with the U.S. black population. Furthermore, the full Rh phenotype could not be characterized in seven (2.3%) participants (Table 2), most likely because of antithetical deletion of these antigens. Future efforts at genotyping would help in elucidating the gray areas and their underlying mechanism(s).

Unlike the Rh antigens, there are minimal reports on the distribution of K in Nigeria and no reports on the prevalence of other clinically significant Kell antigens. In our study cohort, no participants were positive for K. This finding is in agreement with two existing studies of K in Nigeria (reported prevalence of 1% to 2%) and what is reported for black populations in the United States. Notably, in our study cohort, the prevalence of all RBC antigens was not statistically correlated with ethnicity. Perhaps a large multicenter-based study will help to establish a better ethnic homogeneity and a sizeable study size to confirm or refute ethnic variability of these antigens.

Current practice in Nigeria does not include alloantibody screening or RBC phenotyping beyond D, even in at-risk blood recipients who require frequent transfusion. Such individuals include patients with sickle cell disease; multiparous women; and individuals with refractory anemia, myelodysplastic syndrome, or myelofibrosis and other clinical conditions associated with some form of transfusion dependence. In particular, sickle cell disease presents a huge burden, affecting at least 2 percent of the over 180 million Nigerian population. Extended RBC typing will ensure provision of high-quality blood components and reduce the rate of alloimmunization among at-risk individuals. The national transfusion service in Nigeria lacks cohesiveness over the regional and state transfusion mechanisms. Uncommon antisera for extended RBC typing and cell panels for antibody identification are not routinely available. There is no reference transfusion laboratory. There are no data on hemovigilance reporting.

Theoretically, the risk of Rh alloimmunization in Nigeria was highest for C and E antigens. Available literature also suggests these Rh antigens are implicated in recipient alloimmunization in Nigeria. At a minimum, these authors recommend extended RBC typing and matching in at-risk blood recipients for at least C and E, particularly for patients with sickle cell disease. Optimal transfusion service delivery would require more robust data on the prevalence of other blood antigens. Uncommon antisera for extended RBC typing and cell panels for antibody identification are not routinely available. There is no reference transfusion laboratory. There are no data on hemovigilance reporting.

We acknowledge that future studies with an expanded cohort are needed to confirm our findings. This expanded cohort could serve as a local donor data bank, a resource for providing compatible blood and for preparing indigenous RBC panels. Ultimately, our study constitutes the first description...
of Rh and K antigens in Nigeria, using a multi-ethnic cohort to reflect the heterogeneous population of Nigeria. These findings are applicable to black populations worldwide with shared African ancestry.

Acknowledgments

The authors thank James Adewuyi (Hematology, University of Ilorin, Nigeria), Gowthami Arepally (Duke Hematology, Durham, NC), and Marilyn Telen (Duke Hematology/Transfusion Service, Durham, NC) for their role as scientific advisors and for providing critical review of the manuscript. The authors acknowledge Maragatha Kuthichibhatla for her role as statistical advisor.

References


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Clinically significant naturally occurring anti-N and anti-S in a blood donor: a rare finding

Alloimmunization is triggered when an individual whose red blood cells (RBCs) are lacking particular antigens is exposed to these antigens through transfusion or pregnancy, causing the formation of immune antibodies. In addition to these exogenous exposures, underlying inflammatory or autoimmune conditions may lead to formation of unexpected antibodies. Individual factors also play a role, since some people are responders and others are non-responders. We report a case of naturally occurring alloanti-N and alloanti-S in a healthy D+ blood donor. Both antibodies were reactive over a wide thermal amplitude and hence were potentially clinically significant. This case highlights the importance of incorporating the indirect antiglobulin test (IAT) to test for unexpected RBC antibodies for all blood units as a routine protocol.

In our blood bank, a tertiary care referral and teaching institute, immunohematology workup of donor samples includes ABO and D blood typing as well as testing with A1 lectin (for A and AB blood groups) and H lectin (for O blood group) to identify A2, A2B, and Bombay blood groups. In addition, all D- samples are subjected to weak D testing for confirmation of D- status. An IAT for unexpected RBC antibodies is performed by tube method with pooled O cells for all donor units irrespective of their D status. The inclusion of the IAT was recently started in our facility, about 10 months before the case presented here.

Primarily because of financial constraints, many small centers (especially in developing countries) only perform the IAT for D- blood donors to rule out the presence of alloanti-D. To date, we have detected six donors whose samples were IAT positive (five alloantibodies and one autoantibody) in 2300 donors tested, both D+ and D- donors.

A 19-year-old, first-time, voluntary (family) male donor without any significant transfusion, medical, or drug history donated whole blood in the department of Transfusion Medicine and Blood Bank at the All India Institute of Medical Sciences. His blood sample typed as group O, D+. The Immunohematology Laboratory reported a positive IAT identified during processing of the donation. The autocontrol was negative. Antibody identification with an 11-cell panel (Ortho Clinical Diagnostics, Raritan, NJ) demonstrated the presence of anti-N and anti-S in the plasma, reactive at a thermal amplitude of 22–37°C. A direct antiglobulin test on the donor’s RBCs using polyspecific antihuman globulin (anti-IgG and C3d) was negative. Alloabsorptions were performed with RBCs of appropriate phenotypes, confirming the presence of the alloantibodies. Dithiothreitol (DTT) treatment of the plasma demonstrated the IgM and IgG nature of the antibodies. The antibodies showed dosage phenomena, and the reactivity decreased after enzyme (papain) treatment. The donor’s RBC phenotype for common MNS system antigens was M+N−S−s+.

IgM antibody titers of anti-N and anti-S were 16 and 4, respectively, by gel card method. After DTT treatment, IgG antibody titers for both anti-N and anti-S were 2.

There was no significant medical and drug history for the donor except that he contracted typhoid about 2 years before this donation. All immunohematology testing was done on the donor’s pilot tube and bag sample. No additional sample was collected from the donor for testing. The units were discarded because of the positive IAT.

In the presented case, potentially clinically significant, naturally occurring anti-N and anti-S, reacting at wide thermal amplitude, were detected in the donor’s plasma. There are a few published reports of naturally occurring anti-N and anti-S in a healthy donor population. In the MNS system, anti-M is known to be the most common naturally occurring antibody, usually IgM in nature and reacting in saline tests performed below 37°C; anti-N is relatively less common. Anti-S is commonly an IgG immune antibody reactive at 37°C. Pepkowitz et al. reported a case of a naturally occurring anti-S in a patient with mixed connective tissue disease.

Among MNS phenotypes, M+N+S+s+ (19.55%) was the most common, and M−N+S+s− (1.26%) was the least common phenotype in a study done on 1240 north Indian blood donors. A similar study reported M+N+S−s+ as the most common and M−N+S−s− as the least common phenotype. The MNSs phenotype of our donor was M+N−S−s+, which was seen in 15.8 percent and 22.61 percent of the donor populations in these studies. Both anti-N and anti-S have been implicated in hemolytic transfusion reactions (HTRs). One of us has published a case report describing a patient who developed autoimmune hemolytic anemia due to autoanti-N, which was IgM in nature but reacted at 37°C. Few case reports describe hemolytic reactions due to both naturally occurring and immune anti-N. There are two case reports in the literature in which anti-S caused a delayed HTR in patients with multiple alloantibodies.
The donor in the presented case was identified as IAT positive during routine donor processing. In a study by Keokhamphou et al.\textsuperscript{11} of 1181 Lao blood donors, the prevalence of RBC alloantibodies was found to be 3.90 percent. A study by Pahuja et al.\textsuperscript{12} reporting on antibody detection testing of 7756 Indian donors showed that 0.05 percent had alloantibodies in their serum. In another study from India, 0.09 percent of donors were found to be alloimmunized, with the majority of antibodies being in the MNS blood group system.\textsuperscript{13} One study quoted the incidence of irregular antibodies among 31,367 Indian donors as 0.009 percent.\textsuperscript{14} Both antibodies in our case were potentially clinically significant based on reactivity characteristics. Transfusion of blood components—especially plasma—from this donor could have resulted in an HTR.

Through this case, we wish to emphasize that the protocol of performing an IAT for antibody screening of blood donors, irrespective of their D status, should be incorporated in all blood banks, since this testing will improve the safety of blood transfusion for patients. Instead of initial testing with a three-cell panel, performing an IAT with pooled group O cells selected to possess the appropriate antigens can be used for all donors as the initial screening procedure. Antibody screening with the three-cell panel can be applied only for IAT-positive donors, thus paving the way for better transfusion practices and reducing the risk of adverse reactions, while maintaining financial responsibility. This situation is more relevant in plasma transfusions since the presence of irregular RBC alloantibodies in the donor plasma can lead to adverse transfusion reactions in cases where a large amount of plasma is transfused (as in massive transfusions) and in pediatric patients.

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Director
Department of Transfusion Medicine
Medanta—The Medicity
Gurugram, India
References


September 11, 2018

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

September 12, 2018

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

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The Grifols Academy of Immunohematology is approved as a P.A.C.E® provider by the American Society for Clinical Laboratory Science (ASCLS). All our programs offer C.E. credit.

For more information, please contact the Grifols Academy of Immunohematology at:
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: 10.5-11.5 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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*All dates subject to change.

2018 Hands-On Schedule*

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2018 Webinar Schedule*

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<tr>
<td>December 19</td>
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*All dates subject to change.
The Department of Transfusion on Medicine Specialist in Blood Bank Technology Program

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

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

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

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

Interested applicants should contact:

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

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

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

The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is an onsite work-study, graduate-level training program for certified Medical Technologists, Medical Laboratory Scientists, and Technologists in Blood Banking with at least 2 years of full-time Blood Bank experience.

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

Contact: Lorraine N. Blagg, MA, MLS(ASCP)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
Masters of Science (MSc) in Transfusion and Transplantation Sciences at the University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2018 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following the MSc. The syllabus is organized jointly by the Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

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

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

The course is accredited by the Institute of Biomedical Sciences.

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

For further details and application forms, please contact:

Dr. Patricia Denning-Kendall
University of Bristol
Paul O’Gorman Lifeline Centre
Department of Pathology and Microbiology
Southmead Hospital
Westbury-on-Trym, Bristol BS10 5NB, England
Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: p.a.denning-kendall@bristol.ac.uk
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Certificate and Masters in Clinical Laboratory 
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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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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
Quality Assurance Officers

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

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

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

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

Facilities with CAAHEP-accredited programs, onsite or online, are listed below.
Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org.

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

Revised October 2017
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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

American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
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- Autoimmune neutropenia (AIN)
- Transfusion-related acute lung injury (TRALI)

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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
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Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Avenue
Farmington, CT 06032

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Instructions for Authors

I. GENERAL INSTRUCTIONS
Before submitting a manuscript, consult current issues of Immunohematology for style. Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. Each component of the manuscript must start on a new page in the following order:
   1. Title page
   2. Abstract
   3. Text
   4. Acknowledgments
   5. References
   6. Author information
   7. Tables
   8. Figures

B. Preparation of manuscript
   1. Title page
      a. Full title of manuscript with only first letter of first word capitalized (bold title)
      b. Initials and last name of each author (no degrees; ALL CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
      c. Running title of ≤ 40 characters, including spaces
      d. Three to ten key words
   2. Abstract
      a. One paragraph, no longer than 300 words
      b. Purpose, methods, findings, and conclusion of study
   3. Key words
   4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
      a. Introduction — Purpose and rationale for study, including pertinent background references
      b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
      c. Materials and Methods — Selection and number of subjects, samples, items, etc., studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patients’ names or hospital numbers.
      d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
      e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions of 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.
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      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:

      | Phenotype | Allele Name | Nucleotide(s) | Exon(s) | Amino Acid(s) | Allele Detail | References |
      |-----------|-------------|---------------|---------|---------------|---------------|------------|
      | e weak    | RHCE*01.01  | 48G>C         | 1       | Trp16Cys      | RHCE*ce48C    | 1          |

      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*DIVα-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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