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*Dedicated to advancement and education in molecular and serologic immunohematology*
Blood Group Review
An update on the Augustine blood group system
G. Daniels

SeroLogic Method Review
Donath-Landsteiner Test
M. Kilty and T.S. Ipe

Blood Group Review
An update on the CD59 blood group system
C. Weinstock, M. Anliker, and I. von Zabern

SeroLogic Method Review
ZZAP treatment of red blood cells
S.I. Marckwardt

Blood Group Review
An update on the Duffy blood group system
G.M. Meny

Review
SeroLogic problems associated with administration of intravenous immune globulin (IVIg)
D.R. Branch

Blood Group Review
An update on the Knops blood group system
J.M. Moulds

SeroLogic Method Review
Inhibition of blood group antibodies by soluble substances
K.M. Byrne, C.M.C. Mercado, T.N. Nnabue, T.D. Paige, and W.A. Flegel

Blood Group Review
An update on the Lutheran blood group system
G. Daniels
On Our Cover

Eugene Van Mieghem grew up in Antwerp, Belgium, and in mood and subject his art never strayed far from the busy port’s austere setting. His laudatory yet realistic portrayals of the laborers and emigrant poor on the wharves and piers reflect his socially aware empathy and insight. At the start of his career, he used his wife Augustine Pautre as his primary model and first muse, and she predominates his early work. Tragically Augustine succumbed to tuberculosis after only 3 years of marriage, but Van Mieghem’s charged depictions of her during her illness have been compared with similar efforts by Ferdinand Hodler and Rembrandt. Van Mieghem completed De bloem van de voorstad (translation, Flower of the City) in 1901. This issue of Immunohematology includes an update of the Augustine blood group system.

David Moolten, MD
An update on the Augustine blood group system

G. Daniels

This update of the Augustine (AUG) blood group system (Daniels G. The Augustine blood group system, 48 years in the making. Immunohematology 2016;32:100–3) describes two antigens that have been added to the Augustine system (International Society of Blood Transfusion system 36), bringing the number of antigens in the system to four. Further information on the clinical significance of Augustine system antibodies and the function of the Augustine glycoprotein, equilibrative nucleoside transporter 1, is presented. Immunohematology 2019;35:1–2.

Key Words: Augustine, SLC29A1, adenosine transport, erythropoiesis, ENT1

New Augustine Antigens

Since the publication of the original review,¹ two new antigens have been added to the Augustine (AUG) blood group system: AUG3 and AUG4 (Table 1).

An antibody to a low-prevalence antigen (AUG3) caused severe hemolytic disease of the fetus and newborn (HDFN) requiring two exchange and four top-up transfusions. Targeted exome sequencing for blood group genes revealed that the baby was heterozygous for a novel variant in the Augustine gene, SLC29A1, encoding p.Thr387Pro in the fifth extracellular loop of equilibrative nucleoside transporter 1 (ENT1). The baby's brother, father, two paternal aunts, and grandmother were all heterozygous for the variant allele, and their red blood cells (RBCs) were all AUG:3.²

An antibody to a high-prevalence antigen (AUG4) in a white woman, who had been pregnant and transfused, was first found in 1995. Recent whole exome sequencing revealed homozygosity for a missense mutation in SLC29A1 encoding p.Asn81Ser. The variant was present in 0.1 percent of individuals in the Exome Aggregation Consortium but was never found in a homozygous state. The AUG:–4 RBCs of the propositus were AUG:1,2, confirming that ENT1 is present, but flow cytometric analysis revealed an approximately 30 percent reduction in surface ENT1 relative to that on AUG:1,2,4 (common phenotype) RBCs. The antibody of the propositus reacted with AUG:1,2 and AUG:1,–2 RBCs, but not with AUG:–1,–2 (null phenotype) RBCs.³

Clinical Significance of Augustine System Antibodies

An acute hemolytic transfusion reaction caused by anti-AUG2 (anti-At²)⁴ and severe HDFN caused by anti-AUG3² confirm previous reports that Augustine system antibodies have the potential to be dangerously hemolytic.

Functional Aspects of the Augustine Glycoprotein

Analysis of mature RBCs and developing erythroid cells from the three previously reported AUG:–1,–2 (null phenotype) siblings revealed macrocytosis and abnormal morphologies together with a decreased level of proliferation during erythropoiesis and delayed erythroblast maturation. This finding suggests a role of ENT1-mediated adenosine transport in erythroid differentiation.⁵

Table 1. Antigens of the Augustine system

<table>
<thead>
<tr>
<th>Number</th>
<th>Antigen</th>
<th>Prevalence</th>
<th>Nucleotides</th>
<th>Exon</th>
<th>Amino acids</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUG1</td>
<td>High</td>
<td>c.589+1G&gt;C</td>
<td>6</td>
<td>Slice site</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AUG2</td>
<td>At²</td>
<td>High</td>
<td>c.1171G&gt;A</td>
<td>12</td>
<td>Glu391Lys</td>
<td>1</td>
</tr>
<tr>
<td>AUG3</td>
<td>Low</td>
<td>c.1159A&gt;C</td>
<td>12</td>
<td>p.Thr387Pro</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>AUG4</td>
<td>High</td>
<td>c.242A&gt;G</td>
<td>3</td>
<td>p.Asn81Ser</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
References


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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.
The Donath-Landsteiner (DL) test is a serologic test used to detect the presence of a biphasic hemolysin. This autoantibody is seen in patients with paroxysmal cold hemoglobinuria. The test relies on the characteristic cold binding of an IgG autoantibody with specificity to the P blood group antigen. This autoantibody causes complement-mediated red blood cell (RBC) lysis when warmed to body temperature. In this review, we describe the various methods for performing the DL test—namely a direct test, an indirect test, an indirect test with modifications such as the use of enzyme-treated RBCs and two stages, and an indirect antiglobulin DL test—and highlight the advantages and disadvantages of each. Our focus is on the indirect testing method as it is most commonly used in blood bank laboratories. *Immunohematology* 2019;35:3–6.

**Key Words:** Donath-Landsteiner test, paroxysmal cold hemoglobinuria, biphasic hemolysin, hemolysis, autoantibody

The Donath-Landsteiner (DL) test is a serologic test used to detect the presence of a biphasic hemolysin, seen in patients with paroxysmal cold hemoglobinuria (PCH). The test relies on the characteristic cold binding of an IgG autoantibody with specificity to the P blood group antigen, which causes complement-mediated red blood cell (RBC) lysis when warmed to body temperature. Julius Donath and Karl Landsteiner first described the antibody responsible for this hemolysis in 1904.1 DL antibodies are usually low titer (<32) autoantibodies that have low thermal amplitude (<20°C).1,2 The biphasic hemolysis test was the first noted for the identification of this autoantibody.1,2

PCH is a rare form of direct antiglobulin test (DAT)-positive autoimmune hemolytic anemia. According to Dacie,3 there are three types of PCH: acute, chronic syphilitic, and chronic non-syphilitic. Acute PCH occurs primarily in children. Chronic syphilitic and non-syphilitic types can be more difficult to diagnose and are often seen in adult patients.3,4 The DL test is the diagnostic test for PCH.5

**Principle**

The DL test exploits the biphasic nature of the DL autoantibody, which is usually IgG. Some case reports, however, show these antibodies having IgM and IgA class specificities.6,7 Initially, at cold temperatures, the antibodies bind to RBCs, causing complement (C3) to irreversibly bind to the RBCs at the same time. As this complex is warmed to 37°C, activation of the complement cascade leads to intravascular hemolysis of RBCs when the autoantibody dissociates. This test shows a positive result when hemolysis is visibly seen in the test system (Fig. 1).
Indications

The DL test should be considered when a patient presents with symptoms including recurrent fevers, shaking chills, abdominal pain, and laboratory findings of an intravascular hemolytic anemia with both hemoglobinemia and hemoglobinuria. The onset of the disease is predominantly precipitated by a recent upper respiratory illness, either viral or bacterial. The patient’s RBC sample should also have a positive DAT due to C3 only and no demonstrable autoantibody activity by routine methods.

Procedure

The DL test was developed on the characteristic biphasic hemolysis that was seen in vivo when PCH was highly associated with syphilis, and hemoglobinemia was directly related to exposure to cold. The basic steps of the test are to incubate the patient’s sample at a cold temperature to allow the antibody to attach and then to incubate the sample at body temperature to activate complement and detect the presence of RBC lysis. An important aspect of the test is that the patient’s samples have to be kept at 37°C after collection. Historically, the test consisted of two different methods: a direct DL test and an indirect DL test. Modifications such as the indirect DL test with enzyme-treated RBCs, a two-stage test, and an indirect antiglobulin test (IAT) were developed and used in patients strongly suspected of having the DL antibody.

Direct DL Test

The direct DL test is performed by collecting two whole-blood samples into tubes without anticoagulant at 37°C. One sample is incubated at 37°C for 1.5 hours, and the other sample is incubated in melting ice (approximately 0°C) for 1 hour and then at 37°C for 30 minutes. Both samples are centrifuged, and the supernatants are observed for hemolysis. A positive direct DL test occurs when only the sample incubated at 0°C and then at 37°C shows hemolysis. This test is primarily used as an initial screening test in hospitals. When this test is negative, the indirect DL test is performed.

Indirect DL Test

The indirect DL test was developed to increase the sensitivity and specificity of the test system. This test is performed on a fresh blood sample that is collected and allowed to clot at 37°C. The clotted sample is then centrifuged
at 37°C, and the serum is separated from the RBCs. Freshly collected pooled normal sera are used as a complement source.

Label three sets of three tubes: 1A, 1B, 1C; 2A, 2B, 2C; and 3A, 3B, 3C (Fig. 1). Add 10 drops of the patient’s separated serum to each of the tubes labeled 1A, 1B, 1C and 2A, 2B, 2C. Add 10 drops of normal pooled sera to each of the tubes labeled 2A, 2B, 2C and 3A, 3B, 3C. Add 1 drop of a 50 percent suspension of washed P+ RBCs to all nine tubes. Place the three tubes labeled 1A, 2A, 3A in a bath of melting ice for 30 minutes and then at 37°C for 1 hour. Place the three tubes labeled 1B, 2B, 3B in a bath of melting ice for 90 minutes. The three tubes labeled 1C, 2C, 3C are kept at 37°C for 90 minutes. After the completion of incubation, gently mix the tubes and centrifuge. Examine the supernatant for hemolysis. A positive indirect test occurs when tubes 1A and/or 2A show hemolysis and there is no hemolysis in any of the other tubes. When this test is negative but there is a strong suspicion for PCH, a modified indirect test can be performed. The three modifications include using enzyme-treated reagent RBCs, performing a two-stage test, and testing the DL antibody by the IAT.

After identification of the biphasic antibody, one can further demonstrate the anti-P specificity by repeating the test steps described previously but adding washed ABO-compatible p or Pk RBCs in place of the P+ RBCs. These tubes should have no observable hemolysis, thus confirming the antibody specificity.

**Enzyme-treated indirect DL test**

The use of enzyme-treated reagent O RBCs is one means of increasing the sensitivity of the indirect DL test. Various enzymes can be used, including 1 percent papain. This modified test is performed similarly to the indirect DL test. Treating RBCs with enzymes causes increased exposure of the P antigen on the RBC membrane. This increased sensitivity allowed for detection of low-titer DL antibodies in two patients whose DL tests were only positive when papain-treated P+ RBCs were used.

**Two-stage indirect DL test**

Currently, the two-stage indirect DL test is not often performed in blood bank laboratories. This test is performed by initially incubating the patient’s serum at 0°C with group O RBCs. If hemolysis is present, the tube is centrifuged, and the patient’s serum is removed and replaced with freshly collected pooled normal sera. This replacement increases the sensitivity of the test because of the additional complement that is present for the second half of the assay while decreasing antibody inhibition.

**Indirect Antiglobulin test**

The IAT can also be used for the detection of the DL antibody because the antibody is IgG. The IAT should be performed with anti-IgG reagents after the low temperature incubation.

**Procedure Summary**

The procedural method for the DL test suggested by the AABB is the described indirect DL test. This indirect testing method is widely accepted.

**Limitations**

The DL test has several limitations. The blood bank staff performing the procedure need to be highly skilled and meticulous about the temperature requirements of the DL sample throughout collection, clotting, and testing. Given these restrictions, the procedure requires considerable time and resources and is expensive to perform at institutions with limited resources and expertise. At institutions performing high-complexity testing, this procedure can be simple to perform. The timing of the blood sample collection is also important. The DL antibody is transient, and the in vivo titer rises and falls very quickly. The titer is at its highest concentration during the period of clinical hemolysis.

In addition, there are further limitations that depend on the assay used. The direct DL test is more prone to false-negative results than the indirect DL test. This finding could be due to low antibody titer, low complement level, or C3dg presence on the patient’s RBCs. C3dg is protective by preventing complement-mediated lysis. Low complement levels in the patient due to consumption during the hemolytic process can also cause a false-negative result. Also, additional sample volume is needed when performing the direct DL.

The indirect DL test can be falsely negative when the antibody titer is low. False-negative results can also occur because of autoadsorption of antibody (if serum separation is not carried out strictly at 37°C) or neutralization of anti-P by globoside in fresh serum (added as a complement source). This latter scenario can be avoided by performing a two-stage DL test.

The enzyme-treated indirect DL test makes the treated RBCs more prone to lysis and therefore comparison with controls is important. Use of the IAT can lead to a false-positive
result due to carryover of direct agglutination by a cold IgM antibody.\textsuperscript{1,11}

Another limitation occurs when the patient’s serum is red (due to free hemoglobin) before the test.\textsuperscript{1,2} Preanalytical analysis of hemolysis should be taken into account, and special care should be taken to avoid hemolysis during the collection process. If the patient experiences in vivo hemolysis, the presence of free hemoglobin in the serum may be unavoidable.

Both the direct and indirect DL test can be falsely positive when lysis occurs because of the presence of a cold-reacting IgM autoantibody.\textsuperscript{2} This reactivity is usually seen in patients with cold agglutinin disease.

Quality Control

The tubes labeled 3A, 3B, 3C serve as a negative control for the indirect DL test. Other negative controls include the tubes incubated only at 37°C (for both direct and indirect tests) and those incubated only in melting ice.

Summary

The DL test is the diagnostic assay for detecting the presence of a biphasic hemolysin. A high index of suspicion is required when a patient presents with symptoms and laboratory findings consistent with PCH, given the variable titers of this antibody. The DL test can be performed in several ways: direct, indirect, or indirect with modifications. Each of these assays has benefits and limitations.

References


Morgan Kilty, MHA, MLS(ASCP), SBB, Product Manager, QualTex Laboratories, San Antonio, TX; and Tina S. Ipe, MD, MPH (corresponding author), Medical Director of Donor Services, Associate Medical Director of Transfusion Medicine, Houston Methodist Hospital, Department of Pathology and Genomic Medicine, 6565 Fannin Street, MS 205, Houston, TX 77030, tsipe@houstonmethodist.org.
An update on the CD59 blood group system

C. Weinstock, M. Anliker, and I. von Zabern

This update of the CD59 blood group system (Weinstock C, Anliker M, von Zabern I. CD59: a long-known complement inhibitor has advanced to a blood group system. Immunohematology 2015;31:145–51) increases the number of reported patients with CD59 deficiency from 10 to 14. All of these 14 patients suffered from severe illness. Recently, a new variant allele was found in heterozygosity. Flow cytometry data suggest that this variant was expressed on the red blood cells of the propositus. Although additional alleles have been found, the CD59 system (International Society of Blood Transfusion system 35) continues to have one antigen. Immunohematology 2019;35:7–8.

Key Words: CD59, MIRL, complement regulatory protein, blood group.

Introduction

CD59 is a 20-kDa cell membrane glycoprotein, present on a large number of cells, including red blood cells (RBCs), that binds the complement components C8 and C9 and, thereby, protects the cell from a complement attack. CD59 was assigned a blood group system after a CD59-deficient child presented with anti-CD59, reacting with all CD59-carrying RBCs. In contrast to patients with paroxysmal nocturnal hemoglobinuria, where an acquired mutation in a stem cell clone causes the maturation of CD59-deficient RBCs, in this child, all cell types were CD59-deficient. The cause was homozygosity for a CD59 null allele. Since the first publications in 1990 and 2013, a total of 14 children with CD59 deficiency have been reported (Table 1). Leading symptoms of CD59 deficiency are chronic inflammatory demyelinating neuropathy causing pareses and muscular weakness, ischemic or hemorrhagic lesions in the central nervous system, and hemolytic episodes. Several of these 14 patients had been transfused; only one of them made an anti-CD59.

The CD59 Gene and Its Variations

The CD59 gene spans over 33 kbp. Eight transcript variants have been deposited as reference sequences in the National Center for Biotechnology Information database (Fig. 1). The International Society of Blood Transfusion Red Cell Immunogenetics and Blood Group Terminology working party refers to the genomic sequence NG_008057 and the transcript variant NM_203330.

In 2018, another CD59 allele was found (Table 2) and was carried in heterozygosity together with the wild-type allele. When testing the propositus’ RBCs by flow cytometry using fluorescence-labeled monoclonal anti-CD59, the authors found the density of CD59 comparable to that of controls and concluded that both wild-type CD59 and the variant CD59 were expressed on RBCs. If this finding holds true, the binding site for the reagent anti-CD59 was conserved in this variant. Any further investigation of the variant CD59 protein might be hampered by the presence of the wild-type CD59.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Clinical symptoms</th>
<th>Ethnic origin</th>
<th>Number of patients</th>
<th>Deceased at time of report</th>
<th>Onset of disease</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Val42Serfs*38</td>
<td>• Hemolysis</td>
<td>Japanese</td>
<td>1</td>
<td>1</td>
<td>13 years</td>
<td>Yamashina et al.4</td>
</tr>
<tr>
<td>p.Ala121Glnfs</td>
<td>• Cerebral infarction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Motoyama et al.6</td>
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<tr>
<td>p.Cys89Tyr</td>
<td>• Chronic inflammatory demyelinating neuropathy</td>
<td>North-African Jewish</td>
<td>5</td>
<td>1</td>
<td>3–7 months</td>
<td>Nevo et al.9</td>
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<tr>
<td></td>
<td>• Flaccid pareses/muscular weakness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ben-Zeev et al.7</td>
</tr>
<tr>
<td></td>
<td>• Two out of seven with ischemic CNS lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Acute and chronic hemolysis, transfusions required</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asp49Valfs*31</td>
<td>• Chronic inflammatory demyelinating neuropathy</td>
<td>Turkish</td>
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<td>0</td>
<td>7 months</td>
<td>Höchsmann et al.3</td>
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<tr>
<td></td>
<td>• Flaccid pareses/muscular weakness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haliloglu et al.8</td>
</tr>
<tr>
<td></td>
<td>• Ischemic or hemorrhagic CNS lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Klemann et al.9</td>
</tr>
<tr>
<td></td>
<td>• Acute and chronic hemolysis, four patients required transfusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ardicli et al.10</td>
</tr>
</tbody>
</table>

CNS = central nervous system.
References


Fig. 1 CD59 gene structure and mRNA isoforms. Viewing the figure from top to bottom: NG_008057 is the reference sequence for the CD59 gene, and the black boxes represent the exons. The exons of the eight transcription variants deposited in the National Center for Biotechnology Information database are depicted under the reference gene. The hatched boxes represent the coding sequence. The numbers indicate the first and the last base pair of the exons or of the coding sequence, respectively. Numbering is according to the numbering of the reference gene NG_008057.1. n.a = not applicable.

Table 2. CD59 alleles

<table>
<thead>
<tr>
<th>Allele name</th>
<th>DNA</th>
<th>Exon¹</th>
<th>Protein</th>
<th>Year of first description</th>
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</thead>
<tbody>
<tr>
<td>CD59*01N.01</td>
<td>c.146delA</td>
<td>5</td>
<td>p.Asp49Valfs*31</td>
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</tr>
<tr>
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<td>c.123delC/c.361delG</td>
<td>5/6</td>
<td>p.Val42Serfs*38, not applicable</td>
<td>1990⁴⁶</td>
</tr>
<tr>
<td>CD59*01N.03</td>
<td>c.266G&gt;A</td>
<td>6</td>
<td>p.Cys89Tyr</td>
<td>2012²</td>
</tr>
<tr>
<td>To be named</td>
<td>c.238A&gt;G</td>
<td>6</td>
<td>p.Arg80Gly</td>
<td>2018⁴⁶</td>
</tr>
</tbody>
</table>

¹Exons were counted as in the reference sequence NG_008057.1.

C. Weinstock et al.
ZZAP treatment of red blood cells

S.I. Marckwardt

ZZAP is a mixture of a sulfhydryl reagent (dithiothreitol) and a proteolytic enzyme (papain or ficin). This reagent dissociates IgG and complement from red blood cells, allowing for phenotyping, enhanced adsorption, or denaturing of multiple blood group system antigens to aid in completing complex antibody workups. ZZAP treatment destroys all antigens in the Kell, Landsteiner-Wiener, Cartwright, Dombrock, and Knops blood group systems as well as antigens destroyed by proteases (e.g., M, N, S, Fya, and Fyb). *Immunohematology* 2019;35:9–10.

**Key Words:** ZZAP, ficin, papain, DTT

**Principle**

ZZAP is a mixture of a sulfhydryl reagent (dithiothreitol [DTT]) and a proteolytic enzyme (papain or ficin). It was first described by Branch and Petz in their 1982 article in the *American Journal of Clinical Pathology*. ZZAP is used to dissociate IgG and complement from red blood cells (RBCs), an action that neither reagent can achieve alone. According to Branch and Petz, it is believed that ZZAP “reduces interchain disulfide linkages, increasing exposure of the IgG polypeptides to the surrounding medium, allowing the proteolytic enzyme increased accessibility to the peptide groups. The IgG molecule may lose integrity and dissociate completely from the RBC antigen with which it had reacted.”

**Indications**

ZZAP treatment is useful in several ways. It may eliminate spontaneous RBC agglutination, allowing for antigen typing of RBCs with direct agglutinating antisera, i.e., resolving an ABO discrepancy or performing Kidd typings. Because ZZAP removes IgG from the RBCs, it may also allow for antigen typing of RBCs using indirect antiglobulin test (IAT) methods if the antigen is not destroyed by the reagent. Because ZZAP denatures many antigens determined in a common RBC phenotype (K, M, N, S, s, Fya, Fyb), this application has limited value. As a pretreatment before adsorption, ZZAP-treated RBCs may remove autoantibody more quickly and completely than untreated RBCs.

Lastly, ZZAP treatment can be helpful in complex antibody investigations. It can be used to create RBCs lacking all antigens in blood group systems destroyed by ficin/papain or DTT; for example, it can be used to denature all Kell system antigens, essentially creating Knys RBCs. ZZAP can also be used to treat reagent panel cells for use in serologic investigations, particularly those showing panagglutination. By comparing the untreated panel results with ZZAP-treated panel results, the antibody specificity(ies) may be more rapidly identified.

**Procedure**

Prepare ZZAP by mixing 0.5 mL of 1 percent cysteine-activated papain with 2.5 mL of 0.2 M DTT and 2 mL of pH 7.3 phosphate-buffered saline (PBS) or 1.0 mL of 1 percent ficin, 2.5 mL of 0.2 M DTT, and 1.5 mL of pH 7.3 PBS. Add 2 volumes of ZZAP to 1 volume of packed RBCs. Incubate the ZZAP/RBC mixture at 37°C for 30 minutes, mixing occasionally. Lastly, wash the RBCs at least three times using large volumes of isotonic saline before preparing the RBCs to the concentration needed for testing.
**Limitations**

ZZAP treatment destroys all antigens in the Kell, Landsteiner-Wiener, Cartwright, Dombrock, and Knops blood group systems as well as antigens destroyed by proteases (e.g., M, N, S, Fy$, and Fy$^b$). Therefore, ZZAP-treated RBCs cannot be used to antigen-type for these antigens.

**Quality Control**

The enzyme and DTT reagents used in preparation of ZZAP should be qualified for use during individual preparation. Quality control (QC) of ZZAP-treated RBCs is specific for the intended use.

- **ZZAP treatment for adsorption:** QC is generally not performed before using ZZAP-treated RBCs for adsorption. However, if an antibody is ruled out based on the assumption that ZZAP destroyed the antigen and would leave the antibody in the adsorbed plasma, then the treated adsorbing RBC must be tested for the antigen to prove that it was in fact destroyed [i.e., ruling out anti-Fy$^a$ after using an Fy$(a+)$-adsorbing RBC].
- **ZZAP treatment for antigen typing:** Treated known antigen-positive and -negative control RBCs should be tested in parallel with treated patient RBCs. Before using ZZAP-treated RBCs for antigen typing by IAT, a direct antiglobulin test (DAT) with a saline control should be performed to ensure that all IgG coating on the RBCs has been removed and the RBCs are now DAT-negative.
- **ZZAP treatment to disperse spontaneous agglutination:** Test an inert control (6–8% albumin or antibody-negative ABO-compatible plasma) with direct agglutinating antisera to verify that the spontaneous agglutination is no longer present.
- **ZZAP treatment to destroy a specific antigen:** Known double-dose antigen-positive RBCs should be tested before and after treatment with the corresponding antisera to verify that denaturation occurred.

**Acknowledgments**

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**References**


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**State Blood Bank Meeting Organizers**

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please send a request, 4 months in advance, to immuno@redcross.org.
This update of the Duffy (FY) blood group system (Meny GM. The Duffy blood group system: a review. Immunohematology 2010;26:51–6) includes additional variants to the Duffy system (International Society of Blood Transfusion system 8; five antigens) identified through molecular studies. The most interesting clinical updates, however, include further evaluation of the roles of the Duffy glycoprotein, also known as the atypical chemokine receptor 1, in malaria and hematopoiesis. The transition to understanding the important role of blood group antigens in homeostasis and disease continues. Immunohematology 2019;35:11–12.

**Key Words:** ACKR1, DARC, GATA, Duffy

**Introduction**

A description of Duffy (FY) blood group system antigens and antibodies and how this information is used in transfusion management was featured prominently in the original Duffy blood group review published in 2010. At that time, the Duffy glycoprotein was also known to bind to a variety of chemokines of the CXC and CC classes and was referred to as the Duffy antigen receptor for chemokines (DARC). Ongoing research studies of FY, particularly at the molecular level, continue to expand our understanding of the important role of blood group antigens in homeostasis and disease. Recently, a new nomenclature was adopted and approved; DARC is now known as atypical chemokine receptor 1 (ACKR1).

**Molecular Update**

As with other blood group systems, the number of variants identified in the Duffy blood group system continues to increase as the use of molecular methods for blood typing patients and donors becomes more widespread. The total number of variants remains small (<50) when compared with the hundreds identified to date in other blood group systems such as Rh. The practical implications in clinical situations for blood donors and patients carrying these variants has yet to be described.

**ACKR1 and Malaria**

*Plasmodium vivax* is a major infectious species causing human malaria in Asia and Latin America but is an uncommon cause of malaria in Africa. Individuals with the Fy(a–b–) phenotype may have a selective advantage in that their red blood cells (RBCs) are resistant to *P. vivax* invasion. Liu et al. believe that human *P. vivax* likely arose from within the diverse strain of *Plasmodium* species infecting primates in central Africa. They speculate that this ancestral African *P. vivax* stock infected humans, gorillas, and chimpanzees until the GATA-1 [Fy(a–b–)] mutation appeared, which seemed to eliminate the infection in humans.

*P. vivax* infection was identified in Fy(a–b–) individuals in several countries, including Africa. For example, Menard et al. identified clinical *P. vivax* infection in 4.9 percent of individuals with the Fy(a–b–) phenotype, including the blood stage development of *P. vivax* confirmed by microscopic examination of blood smears. *P. vivax* polymerase chain reaction positivity was noted in 8.8 percent of asymptomatic individuals with the Fy(a–b–) phenotype. Mendes et al. showed two different strains of *P. vivax* infection in 10.9 percent of mosquitos and in 4.6 percent of individuals with

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Allele</th>
<th>Nucleotide change</th>
<th>Predicted amino acid change</th>
<th>Number of other reported variants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fy(a+)</td>
<td>FY*01</td>
<td>125A&gt;G</td>
<td>Asp42Gly</td>
<td>NA</td>
</tr>
<tr>
<td>Fy(b+)</td>
<td>FY*02</td>
<td>125G&gt;A</td>
<td>Gly42Asp</td>
<td>NA</td>
</tr>
<tr>
<td>Fy(a–b–)</td>
<td>FY*01N</td>
<td>–67T&gt;C;125A&gt;G</td>
<td>NA</td>
<td>8</td>
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<tr>
<td>Fy(a–b+)</td>
<td>FY*02N</td>
<td>–67T&gt;C</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td>Fy(a+w)</td>
<td>FY*01W</td>
<td>125A&gt;G;265C&gt;T</td>
<td>Asp42Gly;Arg89Cys</td>
<td>2</td>
</tr>
<tr>
<td>Fy(b+w)</td>
<td>FY*02W</td>
<td>265C&gt;T;298G&gt;A</td>
<td>Arg89Cys; Ala100Thr</td>
<td>3</td>
</tr>
</tbody>
</table>

*Table modified from Moller et al. Only variants approved by the International Society of Blood Transfusion are included. NA = not applicable.
the Fy(a–b–) phenotype. Originally, Miller et al. concluded that *P. vivax* (and *Plasmodium knowlesi*) invades RBCs by interacting with receptors on the RBCs, and Duffy antigens were those receptors. However, *P. vivax* erythrocyte receptors involved in the invasion of Fy(a–b–) RBCs have yet to be identified.\textsuperscript{11}

There is currently no effective vaccine for *P. vivax*, with limited hope on the horizon as vaccine development remains in pre-clinical stages.\textsuperscript{12,13}

**ACKR1 and Hematopoiesis**

Individuals of African ancestry can have neutropenia under physiological conditions, which is linked to a variant of ACKR1 that also results in the Fy(a–b–) phenotype on RBCs. Duchene et al.\textsuperscript{14} examined the role that ACKR1 played in murine hematopoiesis and found that nucleated erythroid cells highly express ACKR1, which facilitates contact with hematopoietic stem cells. The absence of erythroid ACKR1 ultimately gave rise to phenotypically distinct neutrophils, which left the circulation. It is possible that this phenomenon provides a selective advantage to Duffy negative individuals by enhancing innate responses to invading microbial pathogens.

**References**


Geralyn M. Meny, MD, MS, Director, Medical Affairs, Diagnostics USA, Grifols Diagnostics, 201 Carlson Circle, San Marcos, TX 78666, geralyn.meny@grifols.com.
Intravenous immune globulin (IVIg) is manufactured from large pools of donor plasma and contains a high diversity of antibodies, primarily IgG. For this reason, IVIg is routinely used as antibody replacement therapy for patients having primary immunodeficiencies. In 1981, IVIg was also found to be a strong immunomodulator of various inflammatory and autoimmune conditions. This observation has led to the exponential increase in the use of IVIg throughout the world, with the United States and Canada being the biggest users of IVIg. Although relatively rare, adverse events, such as hemolytic anemia and thrombosis, can complicate the administration of IVIg. More frequently, the administration of IVIg can cause serologic challenges for the transfusion service including ABO discrepancies, positive direct antiglobulin tests, positive antibody detection tests, and incompatible crossmatches. This article will review each of the potential transfusion service challenges associated with IVIg administration.

Key Words: IVIg, ABO discrepancies, DAT, isoagglutinins, alloantibodies

Introduction

Preparations of intravenous immune globulin (intravenous gamma globulin; IVIg) are produced from large pools of donor plasma. One lot of IVIg is produced by pooling the plasma of up to 60,000 donors.1,2 IVIg was first produced in the 1970s after the development of the cold alcohol fractionation process (Cohn fractionation) of antibodies from pooled plasma; the final product contains mostly IgG.3–6 The original use for IVIg was as antibody replacement for patients, mostly children, with primary immunodeficiency.3,5,6 In 1981,7 while treating children with primary immunodeficiency and concomitant thrombocytopenia, Paul Imbach observed that platelet counts increased after the administration of IVIg. Imbach suggested that IVIg might be useful for the treatment of immune thrombocytopenia (ITP); this hypothesis was quickly supported by a number of studies.8 The efficacy of high-dose (1 to 2 g/kg) IVIg use in ITP led other investigators to show that IVIg can effectively ameliorate a number of other autoimmune and inflammatory diseases.9,10 IVIg has now been approved by the U.S. Food and Drug Administration for use in ITP, Kawasaki disease (where it is most effective at an even higher dose of 4 g/kg), as well as chronic inflammatory demyelinating polyradiculoneuropathy and other neurologic disorders. There are many other conditions where IVIg has been used “off label.”11

Because IVIg is produced from donor plasma, the final product contains a great diversity of IgG antibodies, some of which may cause clinical complications. Anti-A and/or anti-B antibodies present in IVIg can cause immune-mediated hemolytic anemia in non–group O patients, a finding reported to be about 34 percent of patients receiving IVIg in one prospective study.12 The hemolysis can occasionally be severe, even life-threatening.12–16 Some manufacturers have removed the majority of ABO isoagglutinins from IVIg by a chromatographic step in their processing, which may limit the risk of hemolytic anemia.13,17 Thrombosis can also result from IVIg infusion, but this is quite rare compared with IVIg-associated hemolysis.18 In addition to adverse events, administration of IVIg can create challenges for the transfusion service including ABO discrepancies, positive direct antiglobulin tests (DATs), positive antibody detection tests, and incompatible crossmatches.

Reverse ABO Grouping

Because up to 4 g/kg of IVIg may be given to an individual with blood group A, B, or AB, these patients will often have circulating anti-A and anti-B due to passive transfer. In ABO testing, these antibodies can interfere with an accurate reverse plasma/serum grouping. Because these passively transfused antibodies are IgG, they may not directly agglutinate the reverse typing cells. However, because of the high antigen site density of ABO antigens,13 it is possible that they could cause some direct agglutination.
When trying to resolve an ABO discrepancy between forward and reverse typing where unexpected antibodies are detected, it should be determined whether the patient has recently received high-dose IVIg. If so, the discrepancy is most likely due to the passive transfer of isoagglutinins. Repeating the reverse typing several days after the last IVIg dose should provide accurate reverse grouping results. Additionally, if a group B individual receiving high-dose IVIg has an ABO cell/plasma discrepancy and anti-A cannot be demonstrated by the manufacturers’ recommended direct agglutination method (where the result may suggest a subtype), then do not test the plasma against group A or B red blood cells (RBCs) using the indirect antiglobulin test (IAT). Positive reactions could be seen with both cells due to the circulating IVIg instead of the true ABO antibody. A similar caveat must be heeded with a group A individual.

**Direct Antiglobulin Test**

When group A, B, or AB patients receive high-dose IVIg, the IgG isoagglutinins can bind to the patients’ RBCs in vivo and result in a positive DAT due to IgG sensitization. The DAT strength of reactivity can be as strong as 3+. An eluate would be nonreactive with group O screening cells but positive with A or B reverse typing cells by IAT depending on the patient’s ABO type. When a group A, B, or AB patient is found to have a positive DAT with an eluate that demonstrates isoagglutinin reactivity, determine whether the patient has received high-dose IVIg therapy within the last 3–5 days. The clinical team of such a patient should be alerted to closely watch the patient for development of a hemolytic event.

Rarely, IVIg may contain RBC alloantibodies to antigens other than A or B. If IVIg is infused in a patient having the corresponding antigen, this may result in a positive IgG DAT. An eluate may reveal an antibody that would resemble an autoantibody, but with a common specificity, such as anti-D or anti-C. Rarely, passive antibodies to antigens other than A or B can cause hemolytic anemia. Reports of IVIg containing antibodies to Rh or other RBC antigens are now less common due to more stringent manufacturing standards.

**Unexpected Alloantibodies in Antibody Screening**

As mentioned earlier, some preparations of IVIg may contain antibodies to RBC antigens such as D, C, or K. Such antibodies could be detected in antibody detection tests and/or result in incompatible crossmatches. Indeed, the detection of these antibodies may suggest the presence of an alloantibody when the patient’s RBCs are antigen negative. Passive transfer of antibodies should be suspected if the patient has received high-dose IVIg and has no history of sensitization, transfusion, or pregnancies.

**Summary**

IVIg given at high doses is an effective therapy for assorted autoimmune/inflammatory conditions, and its clinical use will continue to increase. Unfortunately, serologic problems in the blood bank can arise due to passive transfer of antibodies contained in IVIg. This review has addressed these potential problems and proposed ways to resolve them.

**References**


19. Puga Yung G, Seebach JD, Baerenzung N, Pendergrast J, Cserti-Gazdewich C, Branch DR. IgG subclasses determined from eluates of DAT positive patients after high-dose IVIg therapy do not predict hemolysis and are primarily of the IgG2 subclass. Transfusion (in press).


Donald R. Branch, PhD, Professor, Departments of Medicine and Laboratory Medicine and Pathobiology, University of Toronto, Division of Experimental Therapeutics, Toronto General Hospital Research Institute, University Health Network, and the Centre for Innovation, Canadian Blood Services, 67 College Street, Toronto, Ontario M5G 2M1, Canada, don.branch@utoronto.ca.
An update on the Knops blood group system

J.M. Moulds

This update of the Knops (KN) blood group system (Moulds JM. The Knops blood group system. Immunohematology 2010;26:2–7) adds no new antigens to this system (International Society of Blood Transfusion system 22), which currently has nine antigens. However, the molecular basis of York, KN5, or Yk\textsuperscript{a} has been identified as c.4223C>T and designated KN*01.-05. Although not considered clinically significant in the field of transfusion medicine, there has been great interest in the Knops polymorphism by investigators working on malaria documented by numerous studies over the past 8 years. Immunohematology 2019;35:16–18.

Key Words: Knops, blood group antigens, malaria, PfRh4

York Antigen

York (Yk\textsuperscript{a}) was assigned when the Knops (KN) blood group system (system 22) was established.\textsuperscript{1} The number given for the Yk(a–) phenotype was KN:–5, and the allele was designated as KN*01.-05. Although the molecular mechanism for the more well-known high-prevalence antigens (e.g., Kn\textsuperscript{a}, McC\textsuperscript{a}, and Sl1) were identified fairly rapidly, that for Yk\textsuperscript{a} was elusive. In 2011, Veldhuisen et al.\textsuperscript{2} identified a mutation at c.4223C>T that resulted in the change of threonine to methionine at amino acid position 1408 in Yk(a–) samples. It is interesting to note that the Yk\textsuperscript{a} mutation is found in exon 26, which is at the beginning of a long homologous repeat (LHR-D), while all the other known Knops polymorphisms are located in exon 29. Recently, Kretzschmar et al.\textsuperscript{3} found that Yk(a–) examples (p.1408Met) resulted in a twofold increase in susceptibility to leprosy. They postulated that this change altered the CR1 conformation, which adversely affected a terminal cleavage site leaving more CR1 on the cell for attachment to the invading Mycobacterium.

Knops and Malaria

CR1 as an Invasion Receptor

It has been known for some time that Plasmodium species use multiple red blood cell (RBC) ligands to gain entry into the erythrocyte. P. falciparum merozoites have two major groups of proteins used in invasion: erythrocyte-binding–like antigens and reticulocyte-binding–like homolog proteins (PfRhs). Serologists are well aware that the proteins carrying MNS and Ge are involved in parasite invasion. Therefore, it should not come as a surprise that the Knops protein (i.e., CR1) is also an invasion ligand, but for the sialic acid–independent pathway.\textsuperscript{4,5} The binding site has been localized to SCR1, and the Knops antigens in SCR25 do not appear to have any effect on binding.\textsuperscript{6,7} What does appear to be important is erythrocyte CR1 copy number (E-CR1).

Low E-CR1 (Helgeson Phenotype)

The serological null in the Knops system is known as the Helgeson phenotype and most often results from low expression of E-CR1. Pham et al.\textsuperscript{8} suggested that the Helgeson phenotype may also be due to the lack of a high-prevalence KN antigen. Patients with anti-KCAM often have the Helgeson phenotype (J.M.M., unpublished data). There is a genetically controlled expression of E-CR1 that can be detected by Southern blot or polymerase chain reaction–restriction fragment-length polymorphism methods. In individuals of European descent, there is good correlation with a HindIII polymorphism (Q981H), but this correlation has not been well investigated in people of African descent.

In studies of parasite rosetting, erythrocytes with low E-CR1 formed fewer and less stable rosettes. The clinical role for low E-CR1 in malaria was first observed in Papua New Guinea when Cockburn et al.\textsuperscript{9} found that reduced rosetting protected children from severe malaria. In addition to the association with rosetting, the Helgeson phenotype appears to also result in reduced merozoite invasion thru PfRh4.\textsuperscript{6,7} Numerous anti-CR1 monoclonal antibodies exist as well as recombinant soluble CR1 (sCR1).\textsuperscript{10} Both have been shown to inhibit invasion and may be considered for therapeutic use in the future. Because only the terminal portion of CR1 (SCR1) is needed for inhibition, transfusion of this product would not cause alloimmunization of patients to Knops, since these antigens are in SCR25.

Si, McCoy, and KCAM

Serologists knew for years that Si2, namely Si(a–), andMcC(b+) were more common among individuals of African descent. Because of these known differences, it was...
hypothesized that the Knops blood group polymorphisms might be important in malaria. Numerous studies have now been performed throughout Africa as well as South America and Asia.\textsuperscript{11–13} All have shown that Sl2 and McC(b+) are found with the highest prevalence among people of African descent; however, their exact role in malaria remains controversial. Of note, KCAM-negative examples occur in approximately 80 percent of people of African descent, but there have been few studies of its impact on malaria susceptibility or disease progression.\textsuperscript{14}

Two conflicting reports have been published from Ghana. The first study of 150 children found that McC(a–b+) was associated with a reduced risk of severe malaria.\textsuperscript{14} A slightly larger study of 267 patients found no association with Knops antigens, however.\textsuperscript{15} A very large study of over 4000 case and control subjects in Kenya\textsuperscript{16} found Sl2 associated with decreased odds of cerebral malaria and death when α-thalassemia genes were present, although McC\textsuperscript{b} was associated with increased odds. Because of the variability in performing research using human samples, some investigators have used recombinant soluble CR1 fragments for invasion and rosetting studies and found no association with Sl2 or McC\textsuperscript{b}.\textsuperscript{17}

**Conclusions**

The role of CR1 and the Knops blood group in malaria remains unanswered in this author’s opinion. A major problem is that many of the studies have used only molecular methods to assess the role of Sl1/2 and McC\textsuperscript{a/b}. Without RBC phenotyping, it cannot be assured that these genes are expressed and, furthermore, even when expressed, they may be present in low E-CR1 copy numbers. There is good evidence that low E-CR1 (i.e., the Helgeson phenotype) is protective, since it results in lower rates of parasite invasion as well as a lower number of rosettes. The role of the CR1 blood group polymorphism is just now being investigated in other diseases such as tuberculosis, leprosy, and Alzheimer’s disease.\textsuperscript{3,18} Clearly, the Knops system will remain a point of interest for many more years.

**References**


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Inhibition of blood group antibodies by soluble substances

K.M. Byrne, C.M.C. Mercado, T.N. Nnabue, T.D. Paige, and W.A. Flegel

The presence of multiple alloantibodies or an antibody to a high-prevalence antigen in a patient sample can pose challenges in antibody identification. The pattern of reactivity seen on an antibody panel may show various strengths of reactivity by different methods of testing or same strength of reactivity at one or more phases of testing. To ensure proper identification, multiple investigative tools may be used. We review one of these methods—inhbition by soluble substances—which has become an expansion of our toolbox within the past 10 years. Alloantibodies can be inhibited using specific soluble substances. These soluble substances occur naturally in various fluids or can be manufactured. When a patient sample contains multiple antibodies, clinically significant or not, inhibition of one may help determine specificities of others. Specific inhibition of a particular antibody will also help to confirm its presence. *Immunohematology* 2019;35:19–22.

**Key Words:** soluble blood group substance, dilution control, neutralization, soluble peptide, inhibition, recombinant blood group proteins

**Background**

When performing pretransfusion testing, serologic results may indicate the presence of one or more alloantibodies. There are many methods that can be used to identify and separate specificities. One such method is based on the principle of inhibition. The ability to specifically inhibit one antibody may help identify that antibody and allow other antibody specificities to also be identified. Inhibition can aid in the identification of an antibody to an antigen that shows variable expression among individuals, such as anti-P1. Some antibodies can be inhibited by soluble substances such as sugars, proteins, and peptides; examples include ABH, Lewis, P1, Sd, Chido/Rodgers, and I^D_. Human saliva, hydatid cyst fluid, pigeon egg white, human or guinea pig urine, human serum, and human milk have been used as soluble substances to inhibit red blood cell (RBC) antibodies before the 1990s.

Since then, recombinant blood group proteins (rBGPs) have also been shown to be effective in the identification of antibodies to high-prevalence antigens that are single pass and glycosylphosphatidylinositol-linked proteins, thus leading to the detection of underlying alloantibodies. Unlike traditional soluble substances that are found naturally in human and other animal sources, rBGPs are manufactured. The result of the manufacturing is a very specific rBGP that could aid in antibody identification. Gone are the days when the only tools available to the investigational immunohematologist were RBCs, polyclonal antibodies, lectins, and natural inhibitory substances. Recombinant peptides and proteins represent the latest addition to our growing toolbox.

**Principle**

Inhibition takes place when plasma or serum containing an antibody is incubated with a soluble substance (natural or synthetic) of corresponding specificity. Subsequent testing (hemagglutination) reveals the lack of reactivity with RBCs that tested positive before inhibition. Other antibodies, if
present, in treated plasma or serum should remain unaffected and can be identified. The principle of this reaction is based on the inhibition of the antibody by the corresponding soluble protein.

**Indications**

Inhibition of blood group antibodies by soluble substances can aid in the identification of specific antibodies. Antibody activity of known specificity can be selectively “removed” by using the inhibition method, thus leaving behind other antibodies to be identified. Other indications for inhibition are to determine ABH secretor status and immunoglobulin class of anti-A and/or anti-B. Inhibition using rBGP has also helped classify a new blood group antigen, CD59. One may consider using rBGP based on serological clues of the specimen and the availability and specificity of the rBGP.

**Soluble Substances**

One must determine the appropriate soluble substance to use. Today, the choices are many (Table 1). Blood group substances in water-soluble form in tissue fluids and secretions of the body have been known since the 1930s. Agglutination inhibition tests using A and B substances and boiled saliva were being used as early as 1940. In 1996, soluble CR1 produced by recombinant DNA techniques was used to identify Knops system antibodies.

The latest development is the use of soluble proteins to inhibit drugs in the plasma and serum of patients who are treated with monoclonal antibodies. Besides the surface proteins on the target cells, some of this novel drug binds to RBCs. Examples are anti-CD38 and anti-CD47 monoclonal antibody therapy. Both drugs can be inhibited by recombinant soluble proteins, CD38 and CD47 (unpublished results), although their high titers in patients may preclude the effective inhibition in neat plasma samples. A recent article by Velliquette et al. evaluated anti-CD47 (Hu5F9-G4) interference in pretransfusion testing and offered mitigation strategies. Covering the target blood group antigens on the RBC surface is another approach. This novel and attractive alternative will add to our toolbox and should eventually become the topic of another review.

**Procedure**

Obtain plasma or serum sample for testing. If necessary, process the substance (Table 2). Once ready for testing, the most common procedure starts with labeling two test tubes: one for the sample and one for the dilution control. Combine test sample and soluble substance into the tube labeled “sample.” To the tube labeled “dilution control,” combine test sample and inert substance. Incubate both tubes for a specific time and temperature determined by the known “ideal” for the target specificity. After incubation, test samples against previously reactive RBCs selected by phenotype. Inhibition has occurred when the “sample” is nonreactive and the “dilution control” is still reactive. These results confirm that inhibition of antibody has taken place and the lack of reactivity was not caused by dilution. If the “dilution control”

<table>
<thead>
<tr>
<th>Blood group system</th>
<th>ISBT number</th>
<th>Antibody inhibited</th>
<th>Immunoglobulin class</th>
<th>Soluble substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>001</td>
<td>Anti-A; anti-B</td>
<td>IgM; IgG</td>
<td>Human saliva (secretor)</td>
</tr>
<tr>
<td>H</td>
<td>018</td>
<td>Anti-H</td>
<td>IgM</td>
<td>Human saliva (secretor)</td>
</tr>
<tr>
<td>Lewis</td>
<td>007</td>
<td>Anti-Leα; anti-Leβ</td>
<td>IgM</td>
<td>Human saliva (secretor)</td>
</tr>
<tr>
<td>P1PK</td>
<td>003</td>
<td>Anti-P1</td>
<td>IgM</td>
<td>Hydatid cyst fluid, pigeon egg albumin</td>
</tr>
<tr>
<td>901 series</td>
<td>001</td>
<td>Anti-Sdα</td>
<td>IgM&gt;IgG</td>
<td>Human urine or saliva, guinea pig urine</td>
</tr>
<tr>
<td>Chido/Rodgers</td>
<td>017</td>
<td>Anti-Ch; anti-Rg</td>
<td>IgG</td>
<td>Pooled plasma</td>
</tr>
<tr>
<td>Cromer</td>
<td>021</td>
<td>Most Cromer antibodies</td>
<td>IgG</td>
<td>Human serum, plasma, or concentrated urine</td>
</tr>
<tr>
<td>Scianna</td>
<td>013</td>
<td>Anti-Sc1 (Seltsam et al.)</td>
<td>IgG</td>
<td>Recombinant protein</td>
</tr>
<tr>
<td>CD59, Lutheran, Yt, Dombrock, Chido/Rodgers, Cromer, Knops, JMH</td>
<td>035, 005, 011, 014, 017, 021, 022, 026</td>
<td>See Anliker et al. and Seltsam et al.7</td>
<td>Usually IgG</td>
<td>Soluble recombinant proteins from eukaryotic expression systems</td>
</tr>
</tbody>
</table>

ISBT = International Society of Blood Transfusion.
Inhibition of blood group antibodies

is nonreactive, the test is invalid. The hemagglutination test can be done in tubes as described here or by other methods such as column agglutination or solid phase if recommended by the manufacturer or validated in-house.

Limitations

A positive reaction when testing the sample plus soluble substance may indicate that additional alloantibodies are present in the sample. Testing additional RBCs using the sample plus soluble substance may be indicated to determine possible additional antibody specificities. It may not be possible to test low-titer antibodies because of the required dilution that cannot be achieved. If one wants to use specific rBGPs, they are commercially available if the molecular and genetic basis are known. It may not always be possible to inhibit high-titer antibodies, particularly monoclonal antibody drug formulations, because the required high concentration of the soluble substance cannot be achieved.

Quality Control

The dilution control containing the sample plus inert substance should result in a positive reaction when tested against an RBC positive for the corresponding antigen to the antibody under investigation. The lack of reactivity in the dilution control indicates dilution of weakly reactive low-titer antibody and invalidates the test.

Precautions

Use caution when using any of these soluble substances. Follow the procedure as written and test using the recommended test system, such as tube or gel method. For example, if one chooses to change from testing in tube to gel method, the nonstandard test method should be validated before use. Immunoglobulin class (IgG versus IgM) should be considered when evaluating an unexpected result, such as a false positive.

Summary

Inhibition has proven to be useful in separating, identifying, and detecting alloantibodies that may be present in a patient’s sample. Traditional sources (saliva, plasma, and urine) of soluble substances and anti-drug proteins can be used to inhibit antibodies, allowing for their detection and identification. The use of rBGPs has expanded our ability to inhibit a greater number of antibody specificities and can be used in different assays to detect and identify distinct antibodies.

Acknowledgments

We thank Debrean A. Loy, Marina U. Bueno, and Kshitij Srivastava for providing the inhibition of anti-CD47 by soluble protein (unpublished results).

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Table 2. Preparation of soluble substances

<table>
<thead>
<tr>
<th>Source material</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig urine</td>
<td>After collection of urine, boil for 10 minutes. Dialyze for 48 h at 4°C against multiple changes of pH 7.4 PBS. Centrifuge, aliquot, and freeze.19</td>
</tr>
<tr>
<td>Human milk</td>
<td>Collect milk from lactating women, centrifuge at 1000g (serofuge at 3400 rpm) for 10 minutes, remove and discard the cream layer, incubate milk in boiling water for 10 minutes, mix 1 volume of milk with 1 volume of PBS. Aliquot and freeze.2</td>
</tr>
<tr>
<td>Human saliva</td>
<td>Collect 2 mL saliva, boil for 10 minutes, centrifuge at 1000g (serofuge at 3400 rpm) for 10 minutes, harvest supernate, aliquot, and freeze.2</td>
</tr>
<tr>
<td>Human urine</td>
<td>Collect urine from three individuals, pool, centrifuge, dilute with equal volume of distilled water, check pH (dialysis with PBS may be needed to obtain pH between 6 and 8.5), aliquot, and store frozen until needed.2</td>
</tr>
<tr>
<td>Hydatid cyst fluid</td>
<td>Incubate HCF (with scolices) from animal or human sources at 56°C for 1 hour. Dilute 1 volume of hydatid cyst fluid with 9 volumes of PBS. Aliquot and freeze.2</td>
</tr>
<tr>
<td>Pigeon egg albumin</td>
<td>Separate egg white from the yolk. Prepare dilutions of 1:100 to 1:1000 in PBS. Test the dilutions using a potent anti-P1 to determine the best dilution for inhibition studies. Make aliquots of appropriate dilution and freeze.2</td>
</tr>
<tr>
<td>Pooled plasma</td>
<td>Purchase commercial pooled plasma or prepare pooled plasma from six or more donors. Aliquot and freeze.3</td>
</tr>
<tr>
<td>Recombinant protein</td>
<td>May be commercially available.5–11,15</td>
</tr>
</tbody>
</table>

PBS = phosphate-buffered saline.
References


Important Notice About Manuscripts for Immunohematology

Please e-mail all manuscripts to immuno@redcross.org.

Attention: SBB and BB Students

You are eligible for a free 1-year subscription to Immunohematology.

Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to immuno@redcross.org.
This update of the Lutheran (LU) blood group system (Daniels G. Lutheran. Immunohematology 2009;25:152–9) describes six new antigens of the Lutheran system (International Society of Blood Transfusion system 5). These antigens are numbered LU22 to LU27, resulting in a total of 25 antigens in the system. The molecular background of LU7 is also described. New KLFI mutations responsible for In(Lu) have been identified, and the gene responsible for the X-linked form of Lu\textsubscript{mod} has been identified. Immunohematology 2019;35:23–24.

Key Words: Lutheran, BCAM, CD239, KLFI, GATA1

New Antigens of the Lutheran System and Molecular Basis of LU7

Since publication of the original review in 2009,\textsuperscript{1} the molecular basis for LU7 has been resolved,\textsuperscript{2} and six new high-prevalence antigens have been added to the Lutheran (LU) blood group system (Table 1). The antigen-negative phenotype of each of these new antigens, except LU22, results from homozygosity for one or two nucleotide changes in the Lutheran gene. LU22 is more complex, however. LU22 expression requires the presence of both Arg77 (Lu\textsuperscript{b}) and Arg75 on the same molecule. The LU:–22 propositus, who made anti-LU22, was Lu(a+b+•w) and heterozygous for codons 75 and 77. Cys75 with Arg77 results in weak Lu\textsuperscript{b} and no LU22; Arg75 with His77 results in Lu\textsuperscript{a}, no Lu\textsuperscript{b}, and no LU22; Arg75 with Arg77 results in expression of Lu\textsuperscript{b} and LU22.\textsuperscript{3}

Lu\textsubscript{mod}

Since the discovery that the Lu\textsubscript{mod} In(Lu) phenotype resulted from heterozygosity for mutations in the KLFI gene, many more mutations responsible for that phenotype have been identified and are listed in Daniels,\textsuperscript{4} Singleton et al.,\textsuperscript{5} and Fraser et al.\textsuperscript{6} Flow cytometric analyses revealed reduced expression of OK (CD147) and LW (ICAM4) blood group antigens, in addition to those previously reported, in red blood cells (RBCs) of the In(Lu) phenotype.\textsuperscript{10}

The molecular background of a previously described Lu\textsubscript{mod} phenotype with an X-linked mode of inheritance in a single Australian family was described by Singleton et al.\textsuperscript{11} The Lu\textsubscript{mod} propositus had a mutation in the X-linked gene for the erythroid transcription factor GATA1. The c.1240T>C mutation converts the translation termination codon to an arginine codon, predicting a GATA1 protein with an extraneous 41 amino acids at the carboxy terminus. In addition to the Lu\textsubscript{mod} RBC phenotype, the propositus had a hemoglobin count slightly below normal, a low platelet count, and a history of bruising.

New Nomenclature

The Lutheran gene is now named BCAM, and the Lutheran protein is now named the basal cell adhesion molecule or CD239. It is located on chromosome 19 at 19q13.32 (19:44.81-44.82 Mb).

References

Table 1. New antigens of the Lutheran system and molecular basis of LU7

<table>
<thead>
<tr>
<th>Number</th>
<th>Antigen</th>
<th>Molecular basis of antigen-negative phenotype</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nucleotides</td>
<td>Exon</td>
</tr>
<tr>
<td>LU1</td>
<td>Lu*</td>
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<tr>
<td>LU2</td>
<td>Lu*</td>
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<td>LU3</td>
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<tr>
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<td>Lu4</td>
<td>1. c.524G&gt;A</td>
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<tr>
<td></td>
<td></td>
<td>2. c.524G&gt;T</td>
<td>5</td>
</tr>
<tr>
<td>LU5</td>
<td>Lu5</td>
<td>c.326G&gt;A</td>
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<tr>
<td>LU6</td>
<td>Lu6</td>
<td>c.824C&gt;T</td>
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<tr>
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<td>Lu7</td>
<td>c.1274A&gt;C</td>
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<td>Lu8</td>
<td>c.611T&gt;A</td>
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<td>LU9</td>
<td>Lu9</td>
<td>c.824T&gt;C</td>
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<tr>
<td>LU11</td>
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<td>Not known</td>
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<td>Lu12</td>
<td>1. c.99-104del</td>
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<td>2. c.419G&gt;A</td>
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<td>LU18</td>
<td>Ax*</td>
<td>c.1615A&gt;G</td>
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<td>LURC</td>
<td>c.223C&gt;T</td>
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<td>LUIT</td>
<td>c.469G&gt;A</td>
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<td></td>
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<td>1289C&gt;T</td>
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<tr>
<td>LU24</td>
<td>LUGA</td>
<td>c.212G&gt;A</td>
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<td>LUAC</td>
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<td>LU26</td>
<td>LUBI</td>
<td>c.1495C&gt;T</td>
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<tr>
<td>LU27</td>
<td>LUYA</td>
<td>c.1184G&gt;A</td>
<td>9</td>
</tr>
</tbody>
</table>

*LU22 expression requires the presence of both Arg77 (Lu(a)) and Arg75 on the same molecule.

IgSF = immunoglobulin superfamily.


Geoffrey Daniels, PhD, retired, previously Head of Diagnostics, International Blood Group Reference Laboratory, Bristol, UK, geoff.daniels.bris@outlook.com.
**September 19, 2019**

**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, MD. There is no registration fee, but advance registration is encouraged. Contact Karen Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184, e-mail: kbyrne@cc.nih.gov, or visit the Web site: http://www.cc.nih.gov/dtm/research/symposium.html

**September 20, 2019**

**9th Annual Red Cell Genotyping Symposium 2019: Patients First**
The Department of Transfusion Medicine, National Institutes of Health (NIH) Clinical Center, and Versiti are co-hosting this symposium on the NIH campus in Bethesda, MD. For information, registration fee, and advance registration, contact Natasha Leon, Versiti, P.O. Box 2178, Milwaukee, WI 53021-2178, e-mail: Natasha.leon@versity.org, or visit the Web site: https://Versiti.org/rcg2019
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) 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
Grand Hyatt San Antonio
June 2 - 3, 2019
San Antonio, Texas

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

REGISTRATION FEES:

<table>
<thead>
<tr>
<th>Registration</th>
<th>Fee</th>
</tr>
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<tbody>
<tr>
<td>Advance Registration</td>
<td>$200.00</td>
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<tr>
<td>(before March 31, 2019)</td>
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<tr>
<td>Registration</td>
<td>$250.00</td>
</tr>
<tr>
<td>(after March 31, 2019)</td>
<td></td>
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</tbody>
</table>

Registration for AIMS opens FEBRUARY 1st!

AIMS registration includes the afternoon sessions of ENGAGE, the SCABB Annual Meeting.

Visit https://www.scabbregistration.org/aims for the most up to date information on AIMS.
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 2019 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
## 2019 Educational Courses

<table>
<thead>
<tr>
<th>Dates</th>
<th>Programs</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 13</td>
<td>Webinar</td>
<td>Online</td>
</tr>
<tr>
<td>March 7-8</td>
<td>TSEC</td>
<td>Atlanta, GA</td>
</tr>
<tr>
<td>April 10-12</td>
<td>Hands-On (Molecular)</td>
<td>San Marcos, TX</td>
</tr>
<tr>
<td>May 8</td>
<td>Webinar</td>
<td>Online</td>
</tr>
<tr>
<td>June 6-7</td>
<td>TSEC</td>
<td>Seattle, WA</td>
</tr>
<tr>
<td>July 17-19</td>
<td>Hands-On (Molecular)</td>
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<td>August 7</td>
<td>Webinar</td>
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<td>September 12-13</td>
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<tr>
<td>October 9</td>
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</tr>
<tr>
<td>November 6-8</td>
<td>Hands-On (Serology)</td>
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</tr>
<tr>
<td>December 5-6</td>
<td>TSEC</td>
<td>Dallas, TX</td>
</tr>
</tbody>
</table>

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

For more information, please contact the Grifols Academy of Immunohematology at:

Email: TSEC@grifols.com; Phone: 1-833-835-3439

All dates and locations are subject to change.
Educational Programs 2019

SAVE THE DATE

Dear Colleague,

The Grifols Academy of Transfusion Medicine is pleased to announce its 2019 educational schedule. This extensive educational offering exemplifies Grifols' commitment to support continuing education in the field of transfusion medicine, and addresses comments and suggestions provided during past sessions. The Grifols Academy of Transfusion Medicine is approved as a provider of continuing education programs by the American Society for Clinical Laboratory Science (ASCLS) P.A.C.E.® program and the Florida Board of Clinical Laboratory Personnel. All programs offer C.E. credit.

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 for 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
Level: Advanced
Continuing Education Credits: 10 - 13 hours P.A.C.E.® credits

Immunohematology Workshop (Hands-On)
This course presents the molecular basis and serological characteristics of blood group antigens and applies various molecular techniques to interrogate red cell antigen polymorphisms. During three days, participants will divide their time between classroom lectures and hands-on practice in our training laboratory located in San Marcos, TX. Participants will utilize advanced serological and molecular techniques to resolve complex cases.

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 technology and transfusion medicine.

Level: Beginner, intermediate, and advanced
Continuing Education Credits: 1 hour P.A.C.E.® credit

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

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

*All dates subject to change
Online Specialist in Blood Bank (SBB)
Certificate and Masters in Clinical Laboratory Management Program
Rush University | College of Health Sciences

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

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 2019. 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:  
Laurie Gillard, MS, MLS(ASCP)SBB  
Director of the Specialist in Blood Banking Program  
Assistant Professor, Department of Medical Laboratory Science, Rush University  
312-942-2402 (o) | 312-942-6464 (f) | Laurie_Gillard@rush.edu

Denise Harmening, PhD, MT(ASCP)  
Director of Curriculum, Denise_Harmening@rush.edu.
600 S. Paulina Street | Suite 1021 AAC | Chicago, IL 60612
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Marion Reid
Ian Shine

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S. Gerald Sandler, MD
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Georgetown University Hospital

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

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

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

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

Who are SBBs?

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

<table>
<thead>
<tr>
<th>Professional growth</th>
<th>Job placement</th>
<th>Job satisfaction</th>
<th>Career advancement</th>
</tr>
</thead>
</table>

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.

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<th>California</th>
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<tr>
<td>Florida</td>
<td>Academic Center at OneBlood</td>
<td>St. Petersburg, FL</td>
</tr>
<tr>
<td>Illinois</td>
<td>Rush University</td>
<td>Chicago, IL</td>
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<td>Indiana</td>
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<td>Louisiana</td>
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<td>University Medical Center New Orleans</td>
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<td>National Institutes of Health Clinical Center</td>
<td>Bethesda, MD</td>
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<td>San Antonio, TX</td>
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<td>Milwaukee, WI</td>
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Revised March 2019
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<td>HLA-A, B, C, and DR typing</td>
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<td>HLA-disease association typing</td>
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<td>Paternity testing/DNA</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>IgA Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgA testing is available to do the following:</strong></td>
</tr>
<tr>
<td>• Identify IgA-deficient patients</td>
</tr>
<tr>
<td>• Investigate anaphylactic reactions</td>
</tr>
<tr>
<td>• Confirm IgA-deficient donors</td>
</tr>
<tr>
<td>Our ELISA for IgA detects protein to 0.05 mg/dL.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>National Reference Laboratory for Blood Group Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunohematology Reference Laboratory</strong></td>
</tr>
<tr>
<td>AABB, ARC, New York State, and CLIA licensed</td>
</tr>
<tr>
<td><strong>24-hour phone number:</strong></td>
</tr>
<tr>
<td>(215) 451-4901</td>
</tr>
<tr>
<td>Fax: (215) 451-2538</td>
</tr>
<tr>
<td><strong>American Rare Donor Program</strong></td>
</tr>
<tr>
<td><strong>24-hour phone number:</strong></td>
</tr>
<tr>
<td>(215) 451-4900</td>
</tr>
<tr>
<td>Fax: (215) 451-2538</td>
</tr>
<tr>
<td><a href="mailto:ardp@redcross.org">ardp@redcross.org</a></td>
</tr>
<tr>
<td><strong>Immunohematology</strong></td>
</tr>
<tr>
<td><strong>Phone, business hours:</strong></td>
</tr>
<tr>
<td>(215) 451-4902</td>
</tr>
<tr>
<td>Fax: (215) 451-2538</td>
</tr>
<tr>
<td><a href="mailto:immuno@redcross.org">immuno@redcross.org</a></td>
</tr>
<tr>
<td><strong>Quality Control of Cryoprecipitated–AHF</strong></td>
</tr>
<tr>
<td><strong>Phone, business hours:</strong></td>
</tr>
<tr>
<td>(215) 451-4903</td>
</tr>
<tr>
<td>Fax: (215) 451-2538</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Donor IgA Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Effective tool for screening large volumes of donors</td>
</tr>
<tr>
<td>• Gel diffusion test that has a 15-year proven track record: Approximately 90 percent of all donors identified as IgA deficient by this method are confirmed by the more sensitive testing methods</td>
</tr>
</tbody>
</table>

**For additional information:**
Kathy Kaherl
at (860) 678-2764
e-mail:
Katherine.Kaherl@redcross.org
or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Avenue
Farmington, CT 06032
Diagnostic testing for:
- Neonatal alloimmune thrombocytopenia (NAIT)
- Post-transfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

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

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

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

National Reference Laboratory for Specialized Testing

National Neutrophil Serology Reference Laboratory

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

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

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

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

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

American Red Cross Biomedical Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107
I. GENERAL INSTRUCTIONS
Before submitting a manuscript, consult current issues of Immunohematology for style. Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. Each component of the manuscript must start on a new page in the following order:
   1. Title page
   2. Abstract
   3. Text
   4. Acknowledgments
   5. References
   6. Author information
   7. Tables
   8. Figures
B. Preparation of manuscript
   1. Title page
      a. Full title of manuscript with only first letter of first word capitalized (bold title)
      b. Initials and last name of each author (no degrees; ALL CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
      c. Running title of ≤40 characters, including spaces
      d. Three to ten key words
   2. Abstract
      a. One paragraph, no longer than 300 words
      b. Purpose, methods, findings, and conclusion of study
   3. Key words
      a. List under abstract
   4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
      a. Introduction — Purpose and rationale for study, including pertinent background references
      b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
      c. Materials and Methods — Selection and number of subjects, samples, items, etc., studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patients’ names or hospital numbers.
      d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
      e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
   5. Acknowledgments: Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
   6. References
      a. In text, use superscript, Arabic numbers.
      b. Number references consecutively in the order they occur in the text.
   7. Tables
      a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of…) and use no punctuation at the end of the title.
      b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
      c. Place explanation in footnotes (sequence: *, †, ‡, §, ¶, **, ††).
   8. Figures
      a. Figures can be submitted either by e-mail or as photographs (5 ×7” glossy).
      b. Place caption for a figure on a separate page (e.g., Fig. 1 Results of…), ending with a period. If figure is submitted as a glossy, place first author’s name and figure number on back of each glossy submitted.
      c. When plotting points on a figure, use the following symbols if possible:
         ○ ● △ □ ¥△
   9. Author information
      a. List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable. Provide e-mail addresses of all authors.

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

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

Send all manuscripts by e-mail to immuno@redcross.org
A. For describing an allele that has not been described in a peer-reviewed publication and for which an allele name or provisional allele name has been assigned by the ISBT Working Party on Blood Group Allele Terminology (http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/)

B. Preparation

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

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

C. Text

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

2. Materials and Methods
   Description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patient names or hospital numbers.

3. Results
   Complete the Table Below:

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

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

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