This issue of *Immunohematology* is supported by a contribution from Grifols Diagnostics Solutions, Inc.

**GRIFOLS**

www.grifols.com

*Dedicated to advancement and education in molecular and serologic immunohematology*
<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>Blood Group Review</td>
<td>An update on the JR blood group system</td>
<td>L. Castilho</td>
</tr>
<tr>
<td>45</td>
<td>Serologic Method Review</td>
<td>Heat elution: a modification of the Landsteiner-Miller method</td>
<td>C. Dean-El and N. Quraishy</td>
</tr>
<tr>
<td>48</td>
<td>Blood Group Review</td>
<td>An update on the Scianna blood group system</td>
<td>P.A.R. Brunker and W.A. Flegel</td>
</tr>
<tr>
<td>51</td>
<td>Review</td>
<td>Rh immune globulin: an interfering substance in compatibility testing</td>
<td>T.S. Casina, S.G. Sandler, and S.M. Autenrieth</td>
</tr>
<tr>
<td>61</td>
<td>Blood Group Review</td>
<td>An update on the MNS blood group system</td>
<td>L. Castilho</td>
</tr>
<tr>
<td>63</td>
<td>Serologic Method Review</td>
<td>Albumin-indirect antiglobulin test</td>
<td>J.R. Hamilton</td>
</tr>
<tr>
<td>65</td>
<td>Blood Group Review</td>
<td>An update on the Lewis blood group system</td>
<td>M.R. Combs</td>
</tr>
<tr>
<td>67</td>
<td>Blood Group Review</td>
<td>An update on the H blood group system</td>
<td>E.A. Scharberg, C. Olsen, and P. Bugert</td>
</tr>
<tr>
<td>69</td>
<td>Tribute</td>
<td>Tribute to S. Gerald Sandler, MD, FACP, FCAP, on his retirement</td>
<td>S.J. Nance</td>
</tr>
<tr>
<td>71</td>
<td>Announcements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>Advertisements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Instructions for Authors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Subscription Information</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
“The painting rises from the brushstrokes as a poem rises from the words. The meaning comes later,” said Joan Miro, who used abstract and self-referential motifs which paralleled the avant-garde poetry of his time, including that of Max Jacob and lifelong friend, Tristan Tzara. A contemporary of Magritte, de Chirico, and Dali, Miro went beyond absurd juxtapositions and distortions of ordinary objects to pioneer his own enigmatic brand of surrealism. His process called for both careful sketching as well as a more automatistic and “subconscious” application of paint. In *The Birth Of The World* (1925), semiotic elements invoke the inchoate stages of creation, a primeval ocean of delicate organism-like details and geometric forms. This issue of *Immunohematology* features a review by Casina, Sandler and Autenrieth addressing the interference of RhIG with serologic testing.

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

L. Castilho

This update of the JR blood group system (Castilho L, Reid MR. A review of the JR blood group system. Immunohematology 2013;29:63–8) reports new ABCG2 alleles encoding Jr(a−) and Jr(a+/w−) phenotypes, the predominant alleles encoding the Jr(a−) phenotype, and new functional aspects of the ABCG2 glycoprotein. The JR blood group system (International Society of Blood Transfusion system 32) consists of one antigen: Jra.

**Key Words:** JR blood group system, ABCG2, Jr

### Update on the JR System

The JR blood group system consists of one antigen, Jr, which is of high prevalence in all populations. Anti-Jr has caused transfusion reactions and been involved in hemolytic disease of the fetus and newborn. The Jr antigen is located on ABCG2 transporter, a multipass membrane glycoprotein, which is encoded by the ABCG2 gene on chromosome 4q22.1.

Several null alleles of ABCG2 (nonsense, deletions, and insertions) are responsible for the Jr(a−) phenotype, and the weakened expression of Jr has been linked to missense changes. Since publication of the original review in 2013,13 new alleles of ABCG2 causing the Jr(a−) phenotype have been identified (Table 1), and 9 different alleles of ABCG2 have been reported to encode weak or unclear phenotypes (Table 2).

The complete list of alleles associated with Jr(a−) altered phenotypes or Jr phenotype unconfirmed is available at http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology. The molecular bases of Jr(a−) and Jr(a+/w−) and predicted amino acid changes are also available at http://www.erythrogene.com.

Despite the multiple alleles in ABCG2, the c.376C>T (Gln126Stop) nucleotide change represents more than 90 percent of the Japanese Jr(a−) phenotype, which explains the higher prevalence of the Jr(a−) phenotype in the Asian population.9–11 The c.706C>T nucleotide change in ABCG2 is more frequently detected in white Jr(a−) individuals, more specifically in Jr(a−) individuals of the European Roma communities.10,11 Despite the extended genetic heterogeneity in Jr(a−) individuals, the majority of these individuals are homozygous for a single nucleotide change.

### Table 1. Alleles encoding the Jr(a−) phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Nucleotide change (exon)</th>
<th>Amino acid</th>
<th>Ethnicity/origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jr(a−)</td>
<td>c.420_421insA (5)</td>
<td>p.Gln141Thrfs*16</td>
<td>Pakistani</td>
<td>2</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.986_987delTA (9)</td>
<td>p.Ile329Argfs*19</td>
<td>Pakistani</td>
<td>2</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.263+1G&gt;A (Intron 3)</td>
<td>r.sp?1</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.289A&gt;T (4)</td>
<td>p.Lys9Ter</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.565_566del (6)</td>
<td>p.Gly189fs</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.1515del (13)</td>
<td>p.Ala506fs</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.1723C&gt;T (14)</td>
<td>p.Arg575Ter</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.1768_1790insT (15)</td>
<td>p.Ala59fs</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>27-kb deletion in noncoding Exon 1 and the promoter region of ABCG2</td>
<td></td>
<td>Asian</td>
<td>4</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.21T&gt;C (2)</td>
<td>p.Met1Thr</td>
<td>Asian</td>
<td>5</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.[421C&gt;A; 546C&gt;T] (5, 13)</td>
<td>p.[Gln141Lys; Thr182Arg]</td>
<td>Asian</td>
<td>5</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>c.439C&gt;T (4)</td>
<td>p.Arg147Trp</td>
<td>Turkish</td>
<td>6</td>
</tr>
<tr>
<td>Jr(a−)</td>
<td>17-bp deletion including exons 3–5 of ABCG2</td>
<td></td>
<td>African</td>
<td>7</td>
</tr>
</tbody>
</table>

†RNA was not analyzed, but the change is expected to affect splicing.

### Table 2. Alleles encoding Jr(a+/w−)/ and unclear phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Nucleotide change (exon)</th>
<th>Amino acid</th>
<th>Ethnicity/origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jr(a+/w−)</td>
<td>c.383A&gt;T (5)</td>
<td>p.Asp128Val</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Jr(a+/w−)</td>
<td>c.1589G&gt;A (16)</td>
<td>p.Asp620Gly</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.[421C&gt;A; 440G&gt;A] (5)</td>
<td>p.[Gln141Lys; Arg147Gln]</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.[421C&gt;A; 456C&gt;T] (5)</td>
<td>p.[Gln141Lys; Thr153Gln]</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.1384G&gt;A (12)</td>
<td>p.Gly462Arg</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.1819G&gt;C (16)</td>
<td>p.Cys608Arg</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.1820+1g&gt;a (Intron 15)</td>
<td>r.sp?1</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.1841T&gt;G (16)</td>
<td>p.Leu614Trp</td>
<td>Asian</td>
<td>3</td>
</tr>
<tr>
<td>Unclear</td>
<td>c.1714A&gt;C (14)</td>
<td>Ser572Arg</td>
<td>White</td>
<td>8</td>
</tr>
</tbody>
</table>

†RNA was not analyzed, but the change is expected to affect splicing.
Results of new studies on the associations of variants of *ABCG2* and diseases revealed that *ABCG2* c.421C>A can be affiliated with traumatic brain injury outcomes and that non-synonymous allelic variants of *ABCG2* have a significant effect on earlier onset of gout and the presence of a familial gout history. Fujita et al. demonstrated that *ABCG2* expression levels are higher on cord red blood cells (RBCs) than on adult RBCs, and therefore the clinical course of fetal anemia with anti-Jr might be influenced by the expression levels of these antigens in erythroid lineage cells.

As synonymous and non-synonymous single nucleotide polymorphisms in the gene sequence of *ABCG2* continue to be described, additional diversity within the JR blood group system is expected.

**References**


**Manuscripts**

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

C. Dean-El and N. Quraishy

Elution removes antibodies coating red blood cells and allows for serologic testing of the recovered antibodies. Heat elution may be used in the investigation of ABO hemolytic disease of the fetus and newborn, in the detection of weak A and B antigens in combination with adsorption-elution using polyclonal antibodies, and for the resolution of interfering IgM agglutinating antibodies. *Immunohematology* 2019;35:45–47.

**Key Words:** DAT, direct antiglobulin test, elution, heat elution, ABO, IgM antibodies, HDFN

**Principle**

Antibodies and antigens fit together in a “lock and key” mechanism, stabilized by van der Waals forces, hydrogen bonds, and electrostatic and hydrophobic interactions. The *avidity* of an antibody correlates with its binding strength—for example, the IgM pentamer with its 10 antigen-binding sites has high antigen avidity in contrast to the monomeric IgG. The *affinity* of an antibody for a given antigen depends on the binding capacity of a single antigen-binding site. The interaction between the antibody and its antigen is reversible and is affected by the ionic strength, temperature, and pH of the testing environment. The process of removing an antibody attached to red blood cells (RBCs), either in vivo or in vitro, is termed *elution*, and the recovered, concentrated antibody in solution is called an *eluate*.

Heat elution, the first RBC elution procedure, was described by Landsteiner and Miller in their studies on chimpanzees. The original method involved a 5-minute incubation at 56°C of antibody-coated RBCs in normal saline, but resulted in eluates with low antibody levels. Currently, a modified Landsteiner-Miller heat elution method described by Judd and discussed in the AABB Technical Manual is used.

The exothermic reaction for formation of antigen (Ag)–antibody (Ab) complexes is represented by the following equation:

\[
\text{Ag} + \text{Ab} = [\text{AgAb}] + \text{calories}
\]

An increase in temperature on the Ag–Ab complex results in intensification of the thermal motion of atoms and molecules driving the reaction in reverse and causing dissociation of the Ag–Ab complex.

Additionally, heat causes a conformational change in proteins, resulting in a loss of the “lock and key” interaction between antigen and antibody.

**Indications**

Elution studies may be performed on patient samples having a positive direct antiglobulin test (DAT) due to auto- or alloantibodies, due to drug-induced antibodies, or in the investigation of hemolytic disease of the fetus and newborn (HDFN). They may also be used in combination with in vitro adsorption studies to identify weakly expressed blood group antigens and for the identification of multiple alloantibodies.

### Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Saline (ice-cold for IgM cold agglutinins)</td>
<td>• 56°C water bath or heat block</td>
</tr>
<tr>
<td>• 6% bovine albumin</td>
<td>• 12 × 75 mm test tubes</td>
</tr>
<tr>
<td>• Reagents for testing, including group A, B, and O RBCs, if applicable</td>
<td>• Centrifuge</td>
</tr>
<tr>
<td></td>
<td>• Optical aid</td>
</tr>
<tr>
<td></td>
<td>• Timer</td>
</tr>
</tbody>
</table>

**Procedural Steps**

- Wash patient RBCs four to six times with saline. Use ice-cold saline washes for IgM cold agglutinins.
- Remove and place the final wash in a clean, labeled test tube.
- Place an equal volume of washed RBCs and bovine albumin in another clean, labeled test tube. Cap the tube, and mix the contents thoroughly by gently inverting the tube.
- Incubate the tube at 56°C for 10 minutes. Periodically, invert the tube, and mix the contents during incubation.
- After incubation, centrifuge the tube at 900–1000 g for 2–3 minutes (use a heated centrifuge if available).
- Transfer supernatant eluate into a clean, labeled test tube.
- Test the eluate and final wash in parallel, using the appropriate test phase(s) in a manner that is best for detection of the antibody, following the laboratory’s standard operating procedure.

RBCs = red blood cells.
Indications for heat elution include:

- Investigation of ABO HDFN. Elution is rarely required, however, because the diagnosis is generally made from clinical findings consistent with HDFN in an ABO-incompatible mother and newborn.2
- Identification of weak A and B antigens by a combination of adsorption and elution methods. Polyclonal anti-A or anti-B is adsorbed onto the RBCs, and heat elution is used to harvest the adsorbed-bound antibody to determine the presence of a weak A or B antigen.8
- Identification of IgM cold autoantibodies. Heat elution has been described as an effective method for the identification of IgM cold autoantibodies2,9 when compared with acid stroma (digitonin), organic solvent, and Lui freeze–thaw elution methods.

Procedure

Preparing the Eluate

Before performing the elution, the sensitized RBCs must be washed5,6 thoroughly four to six times with large volumes of saline. Use ice-cold saline to prevent dissociation of cold-reactive IgM antibodies. The wash step is critical for removing cell-free antibody while retaining RBC-bound antibody. After the last wash is performed, harvest the supernatant wash into a clean, labeled test tube.

Place an equal volume of washed packed RBCs and 6 percent bovine albumin in a labeled 12 × 75 mm test tube. A preferable starting volume is 1 mL or 20 drops of each. Cap the tube, and mix the contents well by gently inverting the tube. Immediately place the tube in a 56°C water bath or heat block, ensuring complete submersion of the tube in the heated water. Incubate for 10 minutes, with periodic agitation of the tube. Centrifuge the tube at 900–1000 g for 2–3 minutes. If available, use a centrifuge that is heated to 56°C.

Transfer the supernatant eluate into a clean, labeled 12 × 75 mm test tube. The eluate is now ready for testing using standard antibody identification methods. Perform testing immediately after elution, since eluates are not stable.5

Testing the Eluate

Test the eluate using the appropriate test phase(s) in a manner that is best for detection of the antibody, including a reading at room temperature if applicable. Include group A, B, and O cells if testing for ABO antibodies. Test the recovered last wash in parallel with, and in the exact same manner, as the eluate.

Use clean test tubes during the elution procedure. Contaminated test tubes carry the risk of antibody adhering to proteins on the contaminated test tube surface and being reintroduced to the last wash or eluate.2

Quality Control

The last wash must be tested in parallel with the eluate and must be negative for the eluate results to be valid. A positive last wash indicates the presence of unbound antibody due to inadequate washing of the sensitized RBCs. In that case, the elution procedure must be repeated after more thorough washing of the RBCs.

Another option for quality control is to perform a DAT on the RBCs after the heat treatment, and compare it with the pre–heat treatment DAT. A negative DAT post–heat treatment indicates successful elution.


Limitations

The heat elution method is simple to perform but has limited applications, as described under Indications. Drawbacks of heat elution include the following:

- There is the possibility of marked hemolysis of the eluate. The red color of the eluate can make agglutination difficult to read during testing.
- Heat can denature the RBC membrane and affect clinically significant blood group antigens, weakening or destroying them; thus, the RBCs used in the elution procedure should not be used for phenotyping.10
- Heat elution is not an effective method for removal of warm autoantibodies or alloantibodies, other than ABO.2,11

As with any elution procedure:

- Inadequate washing of the RBCs before the elution method will result in interference from unbound antibodies.
- There is a risk of the Matuhasi-Ogata phenomenon.2,5
References


Celeste Dean-El, MS, SBB(ASCP)CM, Immunohematology Supervisor, Cleveland Clinic, Cleveland, OH, and NurJehan Quraishy, MD, Transfusion Medicine Staff (corresponding author), Q6-2, Clinical Pathology, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, quraishn@ccf.org.
This update of the Scianna blood group system (Brunker PA, Flegel WA. Scianna: the lucky 13th blood group system. Immunohematology 2011;27:41–57) provides the recent work on the genetic variation of ERMAP across more world populations, the elucidation of the molecular basis of an historical serologic case, new cases of antibodies in the system, the development of new serologic reagents, and new discoveries in the biology of the erythroid membrane associated protein (ERMAP). Although genetic variation in ERMAP has been extensively cataloged, nonsynonymous variants associated with alloantigens have remained limited, and no new antigens have been identified. The first case of a severe hemolytic transfusion reaction to anti-Sc2 has recently been reported, highlighting the importance of pursuing the possibility of antibodies to low-prevalence antigens via indirect antiglobulin testing as a routine component of all transfusion reaction investigations. The expanding use of molecular testing in blood centers and transfusion services has uncovered a wider population distribution of Scianna antigens and heightened the awareness of this blood group system. The International Society of Blood Transfusion recognizes seven antigens in the Scianna blood group system 13. Immunohematology 2019;35:48–50.

Key Words: Scianna, ERMAP, Radin

New ERMAP Variants, Haplotypes, and Population Distributions

The International Society of Blood Transfusion recognizes seven antigens in the Scianna system, each of which results from genetic variations that have rather low minor allele frequencies in all studied populations. In recent years, several groups have mined public genetic databases and cataloged the variation in known blood group genes. Using the 1000 Genomes data, the Erythrogene project found 357 nonsynonymous mutations in extracellular regions of known blood group genes that are putative undiscovered antigens, 46 of which were in ERMAP. Review of the 1000 Genomes data 2 years later identified another variant seemingly confined to East Asian populations that is proposed as a possible new antigen target (Trp177Ter). In addition, the broader population sampling in the 2018 analysis revealed Sc2 in South Asians for the first time and expanded detection of the rare SC−:5 and SC−:7 variants that were previously confined to isolated probands to populations.

A true tour de force of ERMAP variation included meticulous haplotype determination via long-range polymerase chain reaction of 50 blood donors of diverse ethnicity. This group carefully cataloged and empirically determined ERMAP haplotypes, including single nucleotide polymorphisms (SNPs) in both coding and non-coding regions and found several novel variations in the non-coding regions. Consistent with prior reports, all coding region variation was present at low frequency, with the exception of the 76C>T (His26Tyr) variant, which had a minor allele frequency of 15 percent. This SNP was found at an even higher frequency in donors of Hispanic ethnicity (44%) and was in significant linkage disequilibrium with the 54C>T (Leu18Leu) synonymous variant.

The Pacific Null Variant Is Definitively Resolved at the Molecular Level

The molecular basis of the Scianna null phenotype had previously been determined in two Scnull individuals from California, but index cases from Pacific Islanders had only been characterized serologically. Hue-Roye and colleagues unified these two patient groups in their report showing that the SC*994C>T nonsense variation was shared in all cases. They further reported that a patient with an anti-Sc3 tolerated uneventful transfusions, providing further evidence that it is usually a clinically insignificant alloantibody.

New Antibody Case Reports: The First Severe Hemolytic Reaction from Anti-Sc2 and Clinical Relevance in Pregnancy

This update provides an opportunity to include a clinical case abstract from 2005 that had been inadvertently overlooked in the initial review. Hurstell and Banks reported a case of clinically relevant hemolytic disease of the fetus and newborn (HDFN) attributed to anti-Sc2 that was detected on routine neonatal direct antiglobulin testing. Like the previously reported anti-Sc2 HDFN case, the maternal antibody detection test was negative, as expected for testing using a screening cell panel lacking low-prevalence antigens.
An important recent report of an acute hemolytic transfusion reaction due to anti-Sc2 provides an excellent example of the potential adverse consequences of categorizing low-prevalence antibodies as "clinically insignificant." In their patient with a previously identified anti-Sc2, electronic or immediate-spin crossmatches were performed rather than compatibility testing using an indirect antiglobulin test because of an assumption that anti-Sc2 was not clinically significant. The patient subsequently experienced fever, rigors, nausea, and abdominal pain requiring an inpatient admission when she was transfused with blood from the same donor implicated in her seroconversion with anti-Sc2. Moreover, this patient’s plasma showed a phagocytic index greater than 50 percent in a monocyte monolayer assay (the normal control with an insignificant phagocytosis is 5% or less) that correlated very well with clinical relevance.

To further add to our tally of reports of clinical consequences of Scianna system alloantibodies, a case of severe fetal anemia was reported recently in which anti-Sc4 (or anti-Rd) was implicated. Clinically significant reports involving anti-Rd, including the five cases in the initial description of this antibody by Rausen et al. in 1967, have all been associated with cases of HDFN. Again, strategies designed to detect antibodies to low-prevalence antigens were used in this case, including neonatal direct antiglobulin testing and paternal typing. Given the apparent association between anti-Rd and HDFN, which is now strengthened by this additional report, Scianna system low-prevalence antigens should be near the top of the list when investigating cases of fetal anemia with a negative maternal antibody detection test.

**Broadened Range of Serologic Reagents for Scianna Antigens by Recombinant Proteins**

Genetic testing continues to play a major role in defining Scianna variants in patients and donors, but new serologic reagents such as recombinant blood group proteins offer a complementary strategy to solving complicated immunohematologic problems. The high-prevalence Scianna system antigens Sc1, Sc3, Sc4 (STAR), Sc5 (SCER), and Sc6 (SCAN) can now be detected using CE-marked recombinant proteins. Particularly in specimens containing mixtures of antibodies that include antibodies to a high-prevalence antigen, these reagents can be useful tools to remove the high-prevalence antibody and permit alloantibody identification by standard methods.

**Discoveries and Future Therapies Based on ERMAP Biology**

The erythroid membrane associated protein (ERMAP) is a butyrophilin-like transmembrane protein, categorized within the immunoglobulin superfamily. Although other butyrophilins have defined immunologic functions, ERMAP still remains somewhat elusive. An exciting new feature of ERMAP genetics that unites it with other blood group genes is the recent discovery of a binding site for the erythropoietic transcription factor KLF-1 in the two alternative ERMAP gene promoters. ERMAP itself was also recently found to directly inhibit T-cell functions by decreasing cell proliferation and decreasing cytokine secretion, leading to exciting proposals to use soluble ERMAP as an immune system regulator in patients with autoimmune or neoplastic diseases. The functions of the “lucky 13th” blood group in essential processes such as erythropoiesis and immunity could help explain the generally low-frequency and population-specific genetic variation seen in ERMAP. Mutations that interfere with such vital functions would likely be deleterious. There continues to be interest and enthusiasm for continuing work on the Scianna system antibodies and their underlying diversity, revealed through continued patient case reports and cutting-edge approaches like “big data” genetics and recombinant protein serology.

**References**


Patricia A.R. Brunker, MD, DPhil (corresponding author), Medical Director, American Red Cross, Biomedical Services, Greater Chesapeake and Potomac Region, 4700 Mount Hope Drive, Baltimore, MD 21215, patricia.brunker@redcross.org; and Willy A. Flegel, MD, Chief, Laboratory Services Section, DTM/CC/NIH, Bethesda, MD.
Rh immune globulin: an interfering substance in compatibility testing

T. S. Casina, S. G. Sandler, and S. M. Autenrieth

Immunoglobulin therapy that interferes with pretransfusion testing may complicate the interpretation of test results and adversely affect patient management. Rh immune globulin (RhIG) should be considered an interfering immunoglobulin therapy when it is detected in an antibody detection test of a sample from a patient who has been treated with RhIG. Frequently, detection occurs in mother's or newborn's plasma. Because an antenatal injection of RhIG is indicated for pregnant Rh-negative women, anti-D is detected frequently by today's highly sensitive antibody screen methods when the mother's plasma is tested subsequently at delivery. Ascertaining the source of anti-D is complicated by the inability of routine clinical laboratory methods to distinguish anti-D due to RhIG from alloimmune anti-D. A combination of qualitative and quantitative test methods, as well as a complete clinical history, is necessary for accurate diagnosis and patient management. *Immunohematology* 2019:35;51–60.

**Key Words:** RhIG, passive, immune, titration, antenatal, ITP

**Introduction**

Rh immune globulin (RhIG) is administered routinely to Rh-negative,* pregnant women to prevent hemolytic disease of the fetus and newborn (HDFN), to Rh-negative patients who have received Rh-positive blood components, and to patients as a treatment for immune thrombocytopenic purpura (ITP). The result of RhIG administration may be the presence of anti-D in the plasma of antepartum and postpartum Rh-negative women, occasionally in their newborns, and in patients with ITP. The number of therapeutic indications for RhIG has expanded over the more than 50 years since RhIG was introduced in clinical practice. Additionally, the sensitivity of laboratory methods for the detection of blood group antibodies has increased. The likelihood of encountering interference from RhIG has increased significantly, often creating additional work both serologically and administratively. This review will cover three topics related to RhIG: how RhIG is manufactured, clinical aspects of RhIG in therapeutic use, and the impact of RhIG on serologic testing.

**Manufacture of Rh Immune Globulin**

The manufacturing processes used to prepare RhIG have evolved since the introduction of Rh immune globulin in 1968 (Kedrion Biopharma, Melville, NY). Many processes used for the manufacture of RhIG have been based on methods developed by Cohn et al.\(^1\) and Oncley et al.\(^2\) to purify plasma proteins, particularly albumin and immunoglobulin G (IgG). Today, manufacturers of therapeutic immunoglobulins continue to use the previously developed cold alcohol precipitation process, but the process has progressed to optimize protein purification for multiple different proteins, including RhIG, and to enhance safety, such as pathogen testing.

Plasma fractionation starts with the collection of human plasma, typically by plasmapheresis. Plasma units, referred to as source plasma, are stored at −20°C or below and tested for the absence of blood-borne infectious agents, including hepatitis B virus, hepatitis C virus, hepatitis A virus, and human immunodeficiency virus.\(^3\) In the case of source plasma for RhIG, the plasma contains high titers of anti-D from healthy Rh-negative donors (males or females without childbearing potential) who have been immunized with allogenic donor Rh-positive red blood cells (RBCs).

In the typical traditional fractionation process, frozen plasma is thawed at 2°C to 8°C, pooled, and centrifuged to remove cryoprecipitate. The plasma is then treated stepwise with varying concentrations of alcohol and buffers with adjustments to pH and ionic strength to selectively precipitate specific proteins. This process is performed at temperatures below 0°C to prevent protein denaturation. At each step, either the precipitate or supernatant containing IgG is retained until the final step, which contains essentially pure

---

*To be consistent with clinical terminology, the authors use “Rh positive” and “Rh negative” when applying the results of laboratory typing tests to clinical applications. Conventional terminology of “D+” and “D−” are used for laboratory typing results, as is the style of Immunohematology.
IgG containing anti-D. Some manufacturers of RhIG have modified the traditional fractionation process, eliminating some or all of the precipitation stages and replacing them with ion-exchange chromatography to purify the IgG. Traditional alcohol fractionation and some chromatography processes purify all IgG in the plasma including anti-D, whereas other chromatography processes are designed to specifically isolate the anti-D IgG.

All manufacturers of RhIG incorporate at least two virus removal or inactivation steps into the manufacturing processes. The most common processes are:

1. Virus filtration: Virus filters work by size exclusion and are effective at removing both enveloped and non-enveloped viruses. Effective filters will generally have the capability to provide a 4-log (10,000-fold) reduction of typical blood-borne viruses that could be present. 

2. Solvent/detergent treatment: The addition of a combination of a solvent (typically tri-n-butyl phosphate) and detergent (typically Triton X-100) to the manufacturing process has been shown to be very effective in inactivating lipid enveloped viruses. Downstream processing will remove these chemicals after treatment.

3. Process partitioning: Certain specific fractionation or chromatography steps used in the manufacturing process have been shown to remove viruses, although effectiveness may vary depending on the type of virus.

4. Heat treatment: Elevated temperatures, typically 60°C for 10 hours, for defined periods have been shown to effectively inactivate viruses. Stabilizers, such as amino acids or sugars, may be added temporarily to the product to prevent protein degradation.

The final step in the manufacturing process is sterile filtration of the product in the final container, which is generally a single-dose prefilled syringe. Most RhIG products are supplied as a dose of 300 µg (1500 IU) of anti-D, which is sufficient to suppress the immune response to an exposure of up to 15 mL Rh-positive RBCs. Some manufacturers provide a 50-µg (250-IU) dose to be used in cases of spontaneous or induced termination of pregnancy through 12 weeks’ gestation.

After the manufacturing process, RhIG products are extensively tested. Anti-D potency is measured compared with a standard traceable to the World Health Organization standard anti-D immunoglobulin. Several methods are typically used to ensure product purity, including electrophoresis and high-performance liquid chromatography. Microbiologic testing ensures that the product is sterile and non-pyrogenic. This testing, together with extensive screening of the source plasma and viral treatments, ensures that the RhIG is safe and effective.

Clinical Aspects of RhIG in Therapeutic Use

We present three clinical scenarios in which a patient’s prior treatment with RhIG may result in an unexpected, “interfering” laboratory finding of anti-D. In one scenario, an Rh-negative woman received RhIG for antepartum or postpartum immunophrophylaxis, the laboratory was not informed, and the laboratory report of anti-D states or infers D alloimmunization. In the second scenario, anti-D was detected in the plasma/serum of an Rh-negative patient, suggesting D alloimmunization. However, the patient had received RhIG for immunophrophylaxis after transfusion of Rh-positive RBCs or transfusion of whole blood–derived platelets containing a relatively large content of donor’s Rh-positive RBCs. In the third scenario, an Rh-positive patient was treated with intravenous (IV) RhIG for ITP, the laboratory was not informed, and the laboratory reports a positive antibody detection test, with or without identification of anti-D and a positive direct antiglobulin test (DAT). The laboratory report states or infers that the patient has autoimmune hemolytic anemia. In each of these scenarios, a positive antibody detection test without a history of prior administration of RhIG may delay emergency release of RBCs, including RBC units released through an electronic crossmatch process.

Resolving an “Interfering” Laboratory Finding

Sometimes, an unexpected “interfering” laboratory finding of anti-D can be resolved clinically, that is, without additional laboratory testing. In the first scenario, namely, a laboratory finding of anti-D in an Rh-negative woman of childbearing age, the unexpected detection of anti-D may be clarified by asking the patient if she is, or was recently, pregnant and received an injection of RhIG. A negative response may not be accurate, because not all patients are reliable historians. A negative response should be followed up by asking about a possible visit to an obstetrician or emergency department. The woman’s blood sample may have been collected at an outpatient facility. Determining whether she received a recent injection of RhIG may require a review of her electronic medical record, communication with her obstetrician, or communication with an outside ambulatory facility where she received prenatal care.

In the second scenario, the transfusion service detected anti-D, unexpectedly, in the plasma/serum of an Rh-negative
patient. Unknown to the transfusion service, RhIG that is inventoried and dispensed by the hospital’s pharmacy was administered because of concern that a unit of whole blood–derived platelets from an Rh-positive donor appeared to contain an excessive content of RBCs (pink-colored plasma). In some hospitals, RhIG may be administered by policy if whole blood–derived platelets are transfused to an Rh-negative patient, especially to a female patient of childbearing potential or to a candidate for a progenitor cell transplant.

In the third scenario, the unexpected detection of anti-D in an Rh-positive child or adult may be clarified by asking if the patient had received an injection of IV RhIG to treat ITP, a low platelet count, bruises, or spontaneous bleeding.

The following discussion expands on the scientific basis and clinical presentations of these three scenarios in which administration of a therapeutic product may interfere with the correct interpretation of a serologic test result in a transfusion service.

A study conducted between 2006 and 2008 at the Canadian Blood Services, Edmonton, identified 91 women with anti-D in perinatal serum samples in the absence of a report of RhIG prophylaxis or previously identified anti-D. The authors distributed a survey to the women’s physicians asking about RhIG administration and for a report of sensitizing events, including prior transfusions. Of 91 responses, 44 (48.3%) of the identified D antibodies were due to passive RhIG; 36 (39.6%) were due to immune anti-D; and 11 (12.1%) were undetermined. The causes of immune anti-D were varied, raising concern for failure to adhere to specific guidelines and protocols, including failure to treat with RhIG at the time of delivery or at the time of amniocentesis or abortion, or failure of a single dose of RhIG to protect against transplacental hemorrhage. The study revealed the difficulty the perinatal laboratory encountered in obtaining accurate and reliable patient information. The authors recommended that each new prenatal patient with anti-D be followed up at the time of antibody discovery to obtain more accurate patient information. They also recommended periodic quality audits or surveys to monitor adherence to policies for RhIG administration.

**Determining RhIG Dosage Postpartum**

In the United States, standard obstetrical practice requires an injection of RhIG for all non-alloimmunized Rh-negative women who deliver an Rh-positive newborn, including a newborn with a serologic weak D phenotype. Clinical trials established that immunoprophylaxis with RhIG within 72 hours of an Rh-negative woman delivering an Rh-positive newborn can prevent alloimmunization to D. A standard 300-µg dose of RhIG will prevent alloimmunization in an Rh-negative woman whose delivery of an Rh-positive newborn is associated with a fetomaternal hemorrhage (FMH) of ≤30 mL fetal whole blood or ≤15 mL fetal RBCs. The dose (i.e., the number of 300-µg vials/syringes of RhIG administered for immunoprophylaxis) is determined by the outcome of the following four steps.

**Screen Mother’s Peripheral Blood for Fetal RBCs**

The first step in determining the postpartum dose of RhIG in an Rh-negative woman is to perform a rosette fetal blood screen test, which is a qualitative serologic assay used to detect fetal Rh-positive RBCs in maternal Rh-negative blood. A suspension of maternal RBCs is incubated with reagent anti-D, which will bind to D+ fetal RBCs, if present. Indicator D+ RBCs are added, which bind to anti-D–coated fetal D+ RBCs in a rosette pattern. Although there are alternative laboratory methods for screening for the presence of fetal RBCs in a sample of the mother’s peripheral blood, most laboratories in the United States use a commercially marketed kit for this function. A negative rosette fetal blood screen test indicates the absence of, or only a minimal, FMH (≤10 mL) requiring no more than the standard injection of one 300-µg dose of RhIG for immunoprophylaxis. A positive rosette fetal blood screen test indicates the likelihood of a greater FMH and requires further testing by a quantitative assay, typically an acid elution (Kleihauer-Betke) or, uncommonly, a flow cytometric assay.

**Determine the Percentage of Fetal RBCs in Maternal Blood**

If the initial rosette fetal screen test indicates a greater FMH than warrants a single dose of RhIG, the second step would be to determine the percent of fetal RBCs in a sample of the mother’s peripheral blood. In the United States, nearly all laboratories quantify FMHs by testing the mother’s postpartum peripheral blood sample using one of several commercially marketed kits for an acid-elution assay (Kleihauer-Betke). The acid-elution assay is based on the principle that hemoglobin A (HbA, adult Hb) can be distinguished from HbF (fetal Hb) on a peripheral blood smear that has been immersed in citric acid. Citric acid elutes HbA from RBCs, whereas HbF is acid-resistant and remains within the RBC. Staining a sample of the mother’s postpartum blood on a peripheral blood smear will color fetal RBCs deep red, because they retain their full content of HbF. In contrast, the mother’s RBCs, from which HbA has been eluted, stain light pink (“ghosts”). Acid elution is a
simple test that can be performed without specialized training in general laboratories, but it is tedious and has a subjective endpoint. The percent of fetal cells in the mother’s circulation is determined by counting the number of darkly staining fetal RBCs in a 2000-RBC scan of a peripheral blood smear. The acid-elution assay cannot be used in women whose RBCs have an increased content of HbF, which occurs if the mother has a coexistent hemoglobinopathy, thalassemia, aplastic anemia, or stress erythropoiesis.\textsuperscript{13}

Flow cytometry for HbF or D+ RBCs offers a more accurate and reproducible method to quantify an FMH in an Rh-negative woman.\textsuperscript{14} However, few hospitals can afford flow cytometric testing for FMHs because of the high cost for a low-volume service that requires a flow cytometer as well as skilled and proficient personnel to be available 24/7.\textsuperscript{14}

\textbf{CALCULATE THE VOLUME OF AN FMH}

The third step in this process is to use the results of the acid-elution test to calculate the volume of the FMH in the mother’s total blood volume using the following formula:

\[
\text{FMH (mL fetal whole blood)} = \frac{\text{(number of fetal cells counted ÷ number of RBCs counted)}}{\times \text{maternal total blood volume (mL)}}
\]

\textsuperscript{1Most laboratories count a total of 2000 RBCs and assign an arbitrary total blood volume of 5000 mL.}

\textbf{ESTIMATE THE DOSE (NUMBER OF VIALS) OF RHIG}

Once the FMH volume is determined, the final step of estimating the dose of RhIG can be performed. One 300-µg dose of RhIG administered within 72 hours after delivery of an Rh-positive newborn will protect an Rh-negative mother from D alloimmunization if there has been an FMH of ≤30 mL whole blood. Based on that formula, most laboratories in the United States will calculate the dose of RhIG using the method of the AABB Technical Manual,\textsuperscript{15} as follows:

\[
\text{Number of 300-µg vials/syringes of RhIG} = \frac{\text{(volume of FMH [mL of whole blood] ÷ 30 mL)}}
\]

Recognizing the inherent imprecision of the acid-elution assay and the pitfalls in the calculation, the editors of the AABB Technical Manual provide the following precautionary adjustment in this formula\textsuperscript{15}:

- When the number to the right of the decimal point is 5 or greater, round up to the next number and add one dose of RhIG (e.g., if the calculation comes to 2.8, give 4 doses).

\textbf{Detecting Anti-D After Postpartum RhIG Administration}

Often, we are asked how long after a postpartum injection of RhIG will anti-D be detectable in the mother’s peripheral blood. This answer depends on the size of the dose, the volume of the FMH, and the sensitivity of the laboratory’s test method. A dose of multiple vials/syringes of RhIG will persist longer than a dose of one vial/syringe. A large-volume FMH consisting of many D+ fetal RBCs will adsorb more anti-D from the circulation than a small-volume FMH. A highly sensitive solid-phase automated analyzer will detect anti-D in a mother’s postpartum plasma/serum sample up to 6 months after a large dose of RhIG. An antibody detection test performed by a relatively insensitive manual tube method may fail to detect anti-D only a few weeks after an injection of only one 300-µg dose of RhIG.

\textbf{Anti-D After Routine 28- to 30-Week Antepartum RhIG Administration}

Clinical trials have demonstrated that adding an antepartum injection of RhIG at 28–30 weeks’ gestation to a postpartum injection of RhIG in an Rh-negative woman who has delivered an Rh-positive newborn will reduce the risk of D alloimmunization from approximately 16 percent to 0.1 percent.\textsuperscript{11} In the United States, it is standard practice to administer a 300-µg antepartum dose of RhIG at 28–30 weeks’ gestation to all Rh-negative pregnant women. However, only approximately 38 percent of fetuses of an Rh-negative mother who were fathered by an Rh-positive man will be Rh-negative, and the mother does not require D immunoprophylaxis.\textsuperscript{16} For this reason, many obstetricians practicing outside of the United States avoid the cost and injections of RhIG, relying on a determination of the fetus’ D type by testing for maternal cell-free DNA (cfDNA).\textsuperscript{17,18} In the United States, a prospective observational study for cfDNA in 520 non-alloimmunized Rh-negative pregnant women had one false-negative result (0.32%).\textsuperscript{16} Analysis of the results revealed that the one error was not in cfDNA testing but instead was caused by a mislabeled blood sample tube. Correcting for the mislabeling, the adjusted false-negative \textit{RHD} result was reported to be 0.0 percent (95% CI 0.00–1.22%). Advocates of cfDNA testing are likely to cite the false-negative result as 0.0 percent. Others,
including these authors, who experience the practicality of day-to-day hospital operations, recognize that adding a step in the procedure that requires collecting, labeling, and reporting the cfDNA result for an additional blood sample is more likely to include the operational error in the results and consider the comprehensive false-negative result to be 0.32 percent. If RHD is not detected in the mother’s cfDNA, the fetus is assumed to be Rh-negative, and RhIG is not administered.

In the Netherlands, a prospective cohort study of a nationwide program compared results of fetal RHD testing with serologic cord D typing results for 25,789 pregnancies. Sensitivity for detection of fetal RHD was 99.94 percent and specificity was 97.74 percent. There were nine false-negative results, of which two were due to technical failures. Those authors concluded that RHD testing at week 27 of pregnancy as a part of an antenatal screening program is highly reliable and can be used to determine antenatal and postnatal immunoglobulin administration. In the United States, most obstetricians assume that the current practice of a routine 28-week antenatal injection of RhIG is 100 percent effective, although there are neither studies nor data to support this assumption. Obstetricians hesitate to change from a practice that is believed to be 100 percent effective in preventing D alloimmunization to a new practice that relies on cfDNA testing, which has been demonstrated to have false-negative results, no matter how few.

In our Transfusion Service laboratory, when a routine highly sensitive solid-phase antibody detection test is performed when an Rh-negative mother is admitted for delivery, we regularly detect residual anti-D in her plasma/serum, which is 8–10 weeks after a conventional 28- to 30-week injection of RhIG. If the newborn is Rh-negative, it is not unusual for a manual tube test to detect residual anti-D in maternal plasma with a 2–3+ graded reaction using R$_r$R$_r$ reagent screen RBCs. There are reports that highly sensitive column gel and solid-phase antibody detection tests have detected residual anti-D from a one-dose injection of RhIG as long as 95 days and possibly 180 days after routine antepartum immunoprophylaxis. In a study using an indirect antiglobulin test (IAT, by tube method) to detect anti-D after antepartum injections of RhIG, 35.6 percent of samples had detectable anti-D at delivery, 54 percent at <76 days after injection, and only 2.4 percent had anti-D detected 76–95 days after injection of RhIG.

### Anti-D After RhIG Immunoprophylaxis During Pregnancy

During an Rh-negative woman’s pregnancy, an injection of RhIG may be administered for D immunoprophylaxis for a suspected or proven FMH as the result of termination of pregnancy (abortion), placenta previa, amniocentesis, chorionic villus sampling, percutaneous umbilical blood sampling (PUBS), other obstetrical manipulative procedure (e.g., version), or abdominal trauma. Vials/syringes of RhIG, containing only 50 µg (250 IU) of anti-D, are commercially available in the United States and will suppress the immune response to an FMH of ≤2.5 mL Rh-positive RBCs. However, many hospital pharmacies stock only 300-µg doses of RhIG, avoiding the cost of maintaining two inventories and, more importantly, avoiding the risk of issuing the wrong RhIG product because 50- and 300-µg doses of RhIG may have similar brand names (e.g., MICRhOGAM versus RhoGAM; Kedrion Biopharma). Using the larger 300-µg dose of RhIG increases the likelihood that anti-D will be detected in the mother’s plasma by an antibody detection test performed any time before delivery.

### Anti-D After Post-Transfusion RhIG Administration

Anti-D may be detected in an Rh-negative patient’s plasma after RhIG was administered to prevent D alloimmunization after exposure to transfused Rh-positive RBCs. That situation may occur if a unit or part of a unit of Rh-positive RBCs was transfused in error. Also, RhIG may have been administered if a unit of whole-blood–derived platelets from an Rh-positive donor containing a significant volume of residual RBCs was transfused to an Rh-negative recipient. Although RhIG is frequently administered in this situation, the number of Rh-positive RBCs is considered too few to require routine administration of prophylactic RhIG. In these situations, IV RhIG is the preferred product because a relatively large therapeutic dose can be administered in a small volume without concern for the discomfort of a large intramuscular or subcutaneous injection, which is the usual mode of administration of RhIG.

### Anti-D After IV RhIG Administration for ITP

IV RhIG may be administered to induce a temporary and reversible “medical splenectomy” to treat thrombocytopenia in Rh-positive adults or children with ITP. The rationale is that an IV infusion of only 50–75 µg anti-D can coat autologous D+ RBCs, inducing phagocytosis and causing a
Concentration of RhIG given

These factors include the following:

1. Concentration of RhIG given

Clinical Aspects of RhIG in Therapeutic Use in this review. RhIG passively acquired through its use, as described under response by the patient, whether alloantibody or autoantibody. The initial explanation is that the antibody is one of an immune when anti-D is encountered in other patient populations, the associated with pregnancy due to RhIG administration. Often

Serologic Challenges

The encounter of true alloimmunization due to D+ RBCs resulting in anti-D is generally considered an unusual event in today’s pretransfusion testing setting, especially with current practices in place in transfusion therapy and obstetrical services to prevent the development of the antibody. Nevertheless, the detection of anti-D often prompts questions and actions by the transfusion service to determine whether the presence of anti-D is related to an immune response to D+ RBCs via transfusion or pregnancy or autoantibody formation or for another non-immunologic reason. When the patient is a female of child-bearing age, the initial explanation that comes to mind is whether this is a passively acquired antibody associated with pregnancy due to RhIG administration. Often when anti-D is encountered in other patient populations, the initial explanation is that the antibody is one of an immune response by the patient, whether alloantibody or autoantibody.

There are many factors that contribute to the detection of RhIG passively acquired through its use, as described under Clinical Aspects of RhIG in Therapeutic Use in this review. These factors include the following:

1. Concentration of RhIG given

2. Mode of administration (intramuscular, IV, or subcutaneous)

3. Pharmacokinetics of the formulation of RhIG

4. Half-life of RhIG

5. Clinical reason for administration

6. Timing of testing after dose(s)

7. Antibody detection test method

In the initial serologic findings of a sample in which RhIG is a factor, the reactivity of the tests can range from negative to barely detectable weak reactivity to strong reactivity. Consideration must be given to the listed factors and their role in the serologic picture that may be presented. The dose plays a significant role in how long the anti-D could be detected. Pharmacokinetics of the formulation, generally demonstrated by flow cytometric methods, confirms that a maximum concentration is achieved within hours to up to 10 days depending on route of administration. IV administration delivers anti-D at its highest concentration in the shortest amount of time. The antibody’s in vivo half-life may vary from 20 to 31 days, with IV administration having a shorter half-life than intramuscular administration. This difference is likely due to the adsorption dynamics from interstitial tissue.

Serologic Detection of Anti-D Due to RhIG

The clinical applications for RhIG, described in the previous section of this review, show that the serologic detection of anti-D due to RhIG may vary depending on the application. D+ RBCs in the circulation adsorb antibody, decreasing the concentration of antibody available, affecting the reactivity observed in serologic tests. In pregnancy, using the standard dose of 300 µg, the presence of detectable anti-D once maximum concentration is achieved is frequently detected in antibody detection tests within several weeks of its administration. In the antenatal application, the amount of antibody given is intended to provide protection against infrequent and generally small-volume bleeds that may occur over a 12-week period. There is little likelihood that all of the antibody will disappear serologically unless FMH bleeds that occur are more than of a very minor volume. If a potential for FMH occurs (any trauma, termination, amniocentesis, or PUBS) before the 28- to 30-week mark for receiving the standard dose, the patient will require continued protective support with an additional dose(s) of RhIG every 12 weeks after the incident until term delivery. Testing of a patient with RhIG who has been treated earlier than normal in the pregnancy could result in an unexpected detection of anti-D. This scenario causes a significant increase in error potential
due to the assignment of an immune status to the identified anti-D when, in fact, the anti-D is passive.

On occasion, newborns will demonstrate a weak positive DAT or a positive antibody detection test as the result of RhIG crossing the placenta from the mother who received it antenatally.

The detection of alloantibodies of clinical relevance is highly desirable in the prevention of potential hemolytic transfusion reactions and prediction of possible HDFN. Unfortunately, the ability to distinguish between the antibody present in RhIG and that of a patient sensitized and producing allo–anti-D is, in practicality, impossible by current basic antibody detection tests. Because RhIG is made from polyclonal anti-D from multiple donors (as discussed in the Manufacture of Rh Immune Globulin section of this review) and is essentially the same antibody formed by a patient who is alloimmunized to D, the ability to determine the source of antibody is challenging.

Clearly, having a reliable and detailed patient history can be invaluable for interpreting the laboratory result when an Rh-negative patient presents with anti-D. When there is a history of administration of RhIG, laboratory practice, particularly in obstetrical patients, is to avoid the presence of the passively acquired anti-D using a D– set of selected reagent RBCs to complete antibody detection/selected cell antibody identification to rule out the presence of any other potentially clinically significant antibodies. The results reported by laboratories typically would state that the “presence of anti-D is likely passively acquired from RhIG.” Even with the knowledge of RhIG administration, the question remains, “Is there a possibility that the anti-D that is present has an alloimmune component?” Without clarity on patient history, however, making this supposition needs to be considered carefully, and further investigation may be required.

**Methods for Distinguishing Passive Versus Immune Anti-D**

Recommendations and suggested methods that attempt to distinguish passive versus immunized anti-D exist. The AABB *Technical Manual* notes that if there are questions about the origin of the anti-D, titration studies may provide information. Generally, the titer result when only passive anti-D is present rarely exceeds 4. Titer endpoints of 16 and above are indicative of the possibility of sensitization to D. However, the titer result should be followed by a cautionary statement that the result may be affected by the test method, RBC phenotype used in the test, technical variability, timing of RhIG dose, and concentration of the dose, therefore it must be carefully evaluated. Because the development of a recently formed anti-D is often predominantly IgM, and RhIG is IgG, the presence of reactivity at the saline phase of testing is indicative of active sensitization. This reactivity could be confirmed by IgM inactivation using sulfhydryl compounds (2-mercaptoethanol or dithiothreitol).

In Australia and New Zealand, the recommended dose of RhIG is 100–125 µg (500–525 IU) for antenatal RhIG prophylaxis administered at 28 and 34 weeks of pregnancy. The Australian and New Zealand Society of Blood Transfusion Guidelines for testing in the antenatal and perinatal setting suggest additional test protocols to distinguish passive versus active sensitization and are based on the detection of anti-D when both a confirmed history of RhIG administration within 6 weeks of the test and the reactivity strength and agglutination scores meet certain criteria. For those results showing a reaction grade of <2 or hemagglutination score <8, the assumption is made that the presence of anti-D is passive. The clinical report typically states that the results suggest that this is likely passive, although the possibility of an early immune response cannot be excluded using serology. Anti-D present within 6 weeks of the RhIG administration with reaction grade ≥2 or score ≥8 indicates that further study including titer, quantitation, and repeat testing should be considered. For those tests that show anti-D presence more than 6 weeks after the administration of antenatal RhIG or when there is no evidence of RhIG administration, further study by titration, quantitation, and repeat testing are indicated. Titers of ≥32 are considered a clinically relevant level in the guidelines and warrant quantitation to determine the IU/mL concentration of anti-D present.

The British Standards in Hematology–Transfusion Task Force established guidelines related to distinguishing between passive and immune anti-D. Like the Australian and New Zealand guidelines, the UK guidelines offer a two-dose RhIG antenatal regimen at 28 and 34 weeks of pregnancy. The focus of recommendations by the British Standards in Hematology to ascertain passive versus immune anti-D presence is to apply a quantitative approach using a continuous flow analyzer with reference to a known standard. For continuous flow, rarely does passively administered RhIG exceed 0.4 IU/mL unless doses exceeding 1500 IU have been administered. Results between 4 and 15 IU/mL are considered a moderate risk for HDFN, and those higher than 15 IU/mL indicate a high risk for HDFN.
Several studies have been undertaken to determine the capability of serologic testing to distinguish passive versus active anti-D. Each of the studies has used time of injection, dose, gestational week, reaction strength, and various test methods (polyethylene glycol tube test [PEGT], gel, and solid phase [SP]) in an attempt to reach an analytical approach to establish the presence of active immunization. Two of the studies suggested that the PEGT was potentially more reliable as a predictive test for active immunization than gel and SP. The potential that alloimmunization should be considered based on strong reactivity in PEGT and gel during the later weeks (weeks 36–40) of pregnancy was more reliable compared with SP testing because both PEGT and gel would be expected to show less reactivity if only passive anti-D is present. The SP method was not possible to use because of the variability of the test method and the lack of striation of test results over time to establish the threshold criteria.

One of the studies suggested in their conclusion, “However, it should be noted that it is not possible to definitively determine whether the anti-D detected is active or passive in this manner. If there is any doubt, RhIG should always be administered to patients who would otherwise qualify. Nevertheless, considering anti-D reaction strength may be of value when devising reporting comments and requesting repeat testing.”

Another study by Irving et al. used a slightly different approach involving testing with enzyme-treated RBCs to identify a serologic way to distinguish passive from active sensitization. Samples (N = 273) with known anti-D detected by column agglutination tests and clear histories of the origin of the anti-D were selected for testing. This group then tested each sample by a low-ionic-strength saline–IAT and enzyme–IAT (both by tube method) and compared the score change between the methods. A change (0–12 score range) of ≥2+ was considered significant (Table 1).

<table>
<thead>
<tr>
<th>Score difference</th>
<th>Immune (n = 68)</th>
<th>Passive (n = 213)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2+ = Positive</td>
<td>TP = 19</td>
<td>FP = 0</td>
</tr>
<tr>
<td>≤1+ = Negative</td>
<td>FN = 41</td>
<td>TN = 213</td>
</tr>
</tbody>
</table>

Compiled from Irving et al.

LISS = low-ionic-strength saline; IAT = indirect antiglobulin test; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

Sensitivity and specificity as well as predictive value were calculated. The sensitivity of the test algorithm was 59 percent, with specificity of 100 percent; the positive predictive value was 100 percent and the negative predictive value was 84 percent.

The serologic picture in clinical cases that involve the use of RhIG for treatment to prevent anti-D formation as the result of transfusion of Rh-positive RBCs in error or in Rh-positive platelets to an Rh-negative individual demonstrate reactivity that is influenced by the concentration of RhIG given and the amount of Rh-positive RBCs transfused. A positive antibody detection test and a typically weakly positive DAT result may be encountered when there are sufficient D+ RBCs and antibody present to elicit a positive DAT.

Although the use of RhIG in ITP treatment has waned in use because of the black box warning and the availability of other solutions, the serologic picture is likely similar to that seen with transfused Rh-positive RBCs treated with RhIG. Additionally, beyond anti-D, other passively acquired antibodies may be seen in patients soon after treatment with IV RhIG.

In both situations, this finding can lead to a perplexing picture, without evidence that the patient has been treated with RhIG. In this situation, identification of anti-D or potentially other passively acquired antibodies can result in erroneous conclusions and the patient being burdened with an antibody (allo or auto) that may have to be honored into the future. Therefore, we see the need to pursue the various information channels—communication with clinicians, health care records, and even outside health care entities are necessary to gain resolution to these uncertain cases.

**Conclusion**

Anti-D collected from stimulated donors used in the manufacture of RhIG is indistinguishable by standard immunohematologic tests from anti-D formed by patients exposed to D by transfusion or pregnancy. Unfortunately, the expectation to serologically solve with total confidence the source of the presenting anti-D without some level of additional investigative work is a precarious position. Some serologic methods are of value in identifying patients who are sensitized to D. Using test methods that provide a quantitative picture versus a qualitative picture are important, in some situations, to make the distinction between passive and immune anti-D. Nevertheless, great care is necessary in labeling a patient as sensitized, especially if there is any chance...
that a misinterpretation may have occurred. Additionally, avoiding misinterpretation of test results and collecting clinical patient information, such that proper treatment to prevent sensitization in the patient is achieved, is just as critical to consider.

Ultimately, having the right information about the patient—transfusion history and clinical history paired with the serology and other testing methods—to call upon whenever the interference of RhIG is encountered in basic serology is critical to determine the proper course of patient treatment. If in doubt, especially in patients in whom prevention of the development of an immune anti-D is the goal, it is better to err on the conservative side and provide prophylaxis to the patient.

References


This update of the MNS blood group system (Reid ME. MNS blood group system: a review. Immunohematology 2009;25:95–101) reports three new antigens of the MNS system numbered MNS47, MNS48, and MNS49; new glycoporphin (GP) variants associated with silent and weak expression of MNS antigens; and the results of new studies on associations of MNS antigens with band 3, Rh proteins, and malaria. The addition of these three antigens brings the total number of antigens in the MNS system (International Society of Blood Transfusion system 2) to 49. Immunohematology 2019;35:61–62.

Key Words: MNS blood group system, GYP A, GYP B, GYPE, glycoporphin

Update on the MNS Blood Group System

The MNS blood group system is highly complex, with 49 antigens currently recognized by the International Society of Blood Transfusion.1 All antigens are carried by glycoporphin A (GPA), glycoporphin B (GPB), or multiple glycoporphin (GP) variants resulting from unequal crossover or gene conversion events between GYP A and GYP B genes.2 GYPE, the other glycoporphin gene family member, does not encode detectable antigens on the red blood cell (RBC) surface but has been shown to be involved in gene rearrangements encoding hybrid proteins.3 The homology of the three genes of the GYP locus facilitates the occurrence of cross-over events between them, generating new alleles and variants. Since publication of the original review,4 three new antigens have been added to the MNS system (Table 1). MNS47, also named SARA, is a low-prevalence antigen on GPA encoded by a single nucleotide change c.240G>T in exon 3 of GYP A, which changes p.Arg80Ser.5,6 MNS48, also named KIPP, is a low-prevalence antigen encoded by the same GYP(B-A-B) hybrid that produces Mur, Hil, MUT, and MINY, but the resulting GP(B-A-B) hybrid has p.Ser51, which distinguishes this protein from other known GP(B-A-B) hybrids that have p.Tyr51.6,7 MNS49, also named JENU, is a high-prevalence antigen on GPB encoded by GYP B, defined as an epitope within the amino acid sequence between positions 38 and 49 (SYISSQTNGETG)9 that is absent from the GP.Mur hybrid as it is disrupted by the insertion of hybrid exon 3.1,8

New alleles and hybrid genes associated with MNS antigen expressions have also been identified, and the complete list of MNS antigens and alleles is available at http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology. The molecular bases and predicted amino acid changes of glycoporphin variants and antigens are also available at http://www.erythrogene.com.

Another addition to the MNS system was the identification of new GP variants associated with silent and weak expression of MNS antigens. It has been shown that in addition to the three major genetic backgrounds known to drive the S–s–phenotype in black individuals (GYP B deletion, GYP B*P2, and GYP B*NY alleles), gene conversion events between GYP B and GYPE can also abolish the expression of S and s.9 Partial or complete skipping of exon 5 in GYP B was associated with weakened expression or silencing of s, respectively.10,11 Finally, three causal GYP B deletions underlying the S–s–U–phenotype were recently characterized.12

A novel variant called En(IND) insertional GYP A variant was the first new genetic basis for the En(a–) phenotype in 30 years, and it has been found to be associated with the single nucleotide insertion G at position c.314-315 in exon 5 of GYP A, causing absence of GPA.13 Studies on the associations of MNS antigens with band 3 and Rh proteins

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Number</th>
<th>Name</th>
<th>Prevalence</th>
<th>Molecular basis</th>
<th>Protein change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNS47</td>
<td>SARA</td>
<td>Low</td>
<td>GPA c.240G&gt;T</td>
<td>p.Arg80Ser</td>
<td>5, 6</td>
<td></td>
</tr>
<tr>
<td>MNS48</td>
<td>KIPP</td>
<td>Low</td>
<td>GYP(B-A-B) hybrid</td>
<td>p.Ser51</td>
<td>6, 7</td>
<td></td>
</tr>
<tr>
<td>MNS49</td>
<td>JENU</td>
<td>High</td>
<td>GYP B, defined as an epitope within SYISSQTNGETG</td>
<td>Interrupted by the amino acids encoded by the GYP A exon 3 insertion in the GP.Mur hybrid protein encoded by GYP*501</td>
<td>1, 8</td>
<td></td>
</tr>
</tbody>
</table>

An update on the MNS blood group system

L. Castilho
demonstrated that the variant GP.Mur may play a pivotal role in supporting \( \text{Wr}^b \) antigen expression on Mi.III RBCs and affects differential erythroid expression of Rh/RhAG before protein translation.\(^{14,15}\) Recently, Leffler et al.\(^{16}\) demonstrated that the loss of \( \text{GYPB} \) and gain of two \( \text{GYPB-A} \) hybrid genes, which encodes the Dantu antigen, reduces the risk of severe malaria caused by \( \text{Plasmodium falciparum} \) by 40 percent.

Considering the complexity of the MNS system and the new technologies that are emerging, new alleles, variants, and antigens are still expected to be identified.

References

9. Willemetz A, Nataf J, Honier V, Peyrard T, Arnaud L. Gene conversion events between \( \text{GYPB} \) and \( \text{GYPE} \) abolish expression of the \( \text{S} \) and \( \text{s} \) blood group antigens. Vox Sang 2015;108:410–6.

Lilian Castilho, PhD, Professor and Researcher, Hemocentro Campinas, University of Campinas, Rua Carlos Chagas, 480, Caixa Postal 6198, CEP 13081-970 Barão Geraldo, Campinas, SP, Brazil, castilho@unicamp.br.

For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, contact Sandra Nance, by phone at (219) 451-4362, by fax at (219) 451-2538, or by e-mail at Sandra.Nance@redcross.org.

Immunohematology is on the Web!

www.redcrossblood.org/hospitals/immunohematology

For more information, send an e-mail to immuno@redcross.org.
Albumin was the first widely used additive solution for hemagglutination tests. Its major effect is to decrease the repulsive forces that keep red blood cells (RBCs) apart. This effect may enable some RBC antibodies, particularly those in the Rh blood group system, to directly agglutinate antigen-positive RBCs after 37°C incubation. The impact of albumin on antibody binding before detection by an indirect antiglobulin test (IAT) is minimal. Use of albumin in antibody identification may help with separation of RBC antibody specificities in a mixture when one or more antibodies demonstrate reactivity after 37°C incubation. Warm autoantibodies can show decreased reactivity in albumin IATs, allowing recognition of underlying alloantibodies.

Key Words: albumin, IAT, antibody identification

Principle

Albumin is added to serologic tests to overcome forces keeping red blood cells (RBCs) apart and, in doing so, makes hemagglutination reactions more likely to occur. Use of albumin in antibody detection tests began as early as the 1940s before the advent of the antihuman globulin (AHG) phase of testing, when direct agglutination tests were the only available method for visualizing the antigen-antibody reaction. Initially, the reagent was used in albumin layering or albumin replacement methods. These methods were very sensitive when supporting hemagglutination but were not adopted widely in the United States. The albumin method used for routine tube testing in the 1970s and still in use today involves the simple addition of albumin to a RBC suspension/plasma mixture. Two theories as to the mechanism of albumin’s effect exist; one being that the albumin raises the dielectric constant of the test environment. The RBCs’ negative charge (zeta potential) is dispersed, causing the RBCs to come into close enough contact that an antibody molecule can bridge between two RBCs. Alternatively, the albumin molecule disrupts the water molecules associated with the RBC membrane, again allowing RBCs to be in closer proximity to allow for antibody crosslinking. In reality, each mechanism likely plays a role.

The practical effect of albumin’s impact on RBC crosslinking is that certain antibodies may show direct agglutination after a 37°C incubation using albumin that they would not show without its use. Rh system antibodies, in particular, can show increased reactivity after 37°C incubation in albumin tests. It has also been postulated, however, that the final concentration of albumin in the test environment (considering dilution by plasma and RBC diluent) is too low to significantly affect direct hemagglutination.

Reagents/Supplies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent RBCs for antibody detection and/or antibody identification</td>
<td>Test tubes (10 × 75 or 12 × 75 mm)</td>
</tr>
<tr>
<td>22% bovine albumin</td>
<td>Pipettes</td>
</tr>
<tr>
<td>0.9% saline or PBS pH 6.5–7.5</td>
<td>Calibrated serofuge and/or cell washer</td>
</tr>
<tr>
<td>AHG (polyspecific or anti-IgG)</td>
<td>Calibrated timer</td>
</tr>
<tr>
<td>IgG-coated reagent RBCs</td>
<td></td>
</tr>
</tbody>
</table>

RBCs = red blood cells; PBS = phosphate-buffered saline; AHG = antihuman globulin.

Procedural Steps

1. Add two drops plasma or serum to a labeled tube.
2. Add one drop reagent RBC suspension.
3. Centrifuge, and perform immediate spin reading (if desired).
4. Add two drops albumin (per MI).
5. Incubate 30–60 minutes at 37°C (per MI).
6. Centrifuge, and perform 37°C reading.
7. Wash three to four times in isotonic saline.
8. Add one to two drops AHG reagent (per MI).

Compiled from Fung et al.

RBC = red blood cell; MI = manufacturer’s instructions; AHG = antihuman globulin.
**Indications**

The albumin-indirect antiglobulin test (IAT) is a method used in antibody identification studies. The increased tendency for 37°C agglutination when using albumin may allow recognition of antibody specificity before the AHG phase. This step is especially helpful when multiple alloantibodies are present; Rh system antibodies are most likely to react at this test phase. Newly developing antibodies may show increased reactivity in 37°C tests when compared with AHG tests using anti-IgG. Conversely, warm autoantibodies generally have decreased reactivity in albumin-IAT tests. This method may be used to avoid their reactivity while retaining the ability to detect clinically significant alloantibodies when proper incubation time is used.

**Procedure**

Place two drops of patient or donor plasma or serum into a properly labeled tube, followed by one drop of the 2–5 percent RBC suspension to be tested. If an immediate spin reading is desired, centrifuge at the calibrated time for a low-protein test, and gently resuspend while examining the tube for RBC agglutination. Add two drops of 22 percent bovine albumin to the tube. Incubate the tube at 37°C for 30–60 minutes or the incubation time specified in the manufacturer’s instructions. Centrifuge at the calibrated time for a high-protein test. If serum is being tested, the tube can be examined for hemolysis before beginning resuspension of the RBCs. Gently resuspend the RBC button while examining the tube for RBC agglutination. This step is the albumin 37°C reading. Wash the RBCs three to four times with large quantities of isotonic saline. Add one to two drops of antiglobulin reagent as specified by the manufacturer’s insert. Two drops of antiglobulin reagent generally produces a more sensitive test. Centrifuge at the calibrated time for an AHG reading. Gently resuspend the RBC button while examining the tube for RBC agglutination. Add IgG-coated reagent RBCs to all negative tests. Centrifuge, and read for agglutination. Tests that do not show agglutination or show agglutination that is weaker than expected per the manufacturer’s instructions indicate that inadequate washing has occurred and the test is invalid and must be repeated.

**Quality Control**

The albumin reagent should be tested for reactivity on each day of use, utilizing an antibody of known specificity and antigen-positive and -negative RBCs. The tests should give expected results at the AHG phase. If the antibody is known to show reactivity after 37°C incubation, this reactivity should be observed with the antigen-positive RBCs. Quality control of other components of the test system should be performed according to laboratory protocols.

**Limitations**

No single test method, including those using albumin enhancement, will detect all clinically significant alloantibodies. Insufficient incubation time is expected to decrease the sensitivity of the albumin-IAT.

The centrifuge used for the direct agglutination reading after 37°C incubation must have been calibrated for centrifugation of a high-protein test. The centrifugation time for this test is generally longer than the spin time for a low-protein test. Improper centrifugation may result in false results.

**References**


Janis R. Hamilton, MS, MT(ASCP)SBB, Manager, Immunohematology Reference Laboratory, American Red Cross, Southeastern Michigan Region, 1415 Trumbull Avenue, Detroit, MI 48216, jan.hamilton@redcross.org.
This update of the Lewis blood group system (Combs MR. Lewis blood group system review. Immunohematology 2009;25:112–8) describes new information on the clinical significance of Lewis antigens regarding susceptibility of individuals to certain diseases and the possible role of bacteria in Lewis expression. This update also describes recently reported examples of Lewis antibodies causing hemolytic transfusion reactions. No new antigens have been identified in the International Society of Blood Transfusion system 7, leaving the antigen count to stand at six: Le\(^a\), Le\(^b\), Le\(^{ab}\), ALe\(^b\), BLe\(^b\), and Le\(^{ab}\). *Immunohematology* 2019;35:65–66.

**Key Words:** Lewis, fucosyltransferases, antigen, antibody

### Lewis Antigens

Lewis antigen fucosyltransferases are encoded by the *FUT3* gene located on chromosome 19p13.3.\(^1\) The presence or absence of Lewis antigens in an individual can be associated with the individual’s susceptibility to certain diseases and infections. As described in a recent review,\(^2\) non-secretors are more likely than secretors [Le(b+)] to be susceptible to symptomatic cholera,\(^3\) bacterial meningitis,\(^4\) type 2 diabetes mellitus,\(^5\) and type 1 diabetes mellitus.\(^6\) In addition, increased activity of the secretor and Lewis fucosyltransferases seems to be involved in the development and control of cancers of the distal colon.\(^2\)

A recent review\(^7\) discusses the possible role of bacteria in Lewis expression. The intestinal tract switches from sialylated glycans to fucosylated antigens with age and bacterial colonization.\(^8\) It is speculated that the low Le\(^b\) expression on neonatal red blood cells (RBCs) reflects the immature nature of gut flora.\(^7\)

### Lewis Antibodies

Lewis antibodies are usually clinically insignificant and are rarely associated with hemolytic transfusion reactions (HTRs). Three recent reports of Lewis antibodies associated with HTRs have been reported.

A case report from 2013 describes a severe HTR due to anti-Le\(^a\) in a multiply transfused Le(a–b–) patient.\(^9\) After receipt of a crossmatch-compatible Le(a+) RBC unit, the patient developed an acute transfusion reaction with fever, chills, severe back pain, hemoglobinuria, and increased levels of bilirubin, alanine transaminase, and serum creatinine. A monocyte monolayer assay, testing the patient’s serum with the Le(a+) RBCs causing the reaction, was positive.

A case report in 2015 of a possible HTR due to anti-Le\(^a\) was reported in a pregnant patient with sickle cell disease with a 37°C gel-reactive anti-Le\(^a\).\(^10\) The crossmatch was compatible using prewarmed plasma neutralized with Lewis substance. During transfusion, the patient experienced significant dyspnea, hypotension, and hemoglobinuria. Indirect bilirubin and lactate dehydrogenase tests were elevated. The direct antiglobulin test on the post-transfusion sample was negative, indicating the possibility that all incompatible RBCs were cleared.

A case of an HTR due to anti-Le\(^b\), also in 2015, was reported.\(^11\) A pretransfusion sample from a 30-year-old African-American woman showed a negative antibody detection test in solid phase. Nine days after receipt of two computer crossmatch-compatible RBC units, her hemoglobin dropped to 7.9 g/dL. The post-transfusion sample typed as group B, D+, although agglutination with the reagent B RBCs in the reverse grouping was detected and thought to be a cold agglutinin. Electronic crossmatch-compatible RBC units were issued. During infusion, the patient developed signs of an acute HTR (chills, nausea, brown urine). An in vitro hemolytic IgM anti-Le\(^b\) was identified. A tube test–only, room temperature–reactive anti-Le\(^a\) was also identified.

### References


Martha Rae Combs, MT(ASCP)SBB, Technical Director of Immunohematology Reference Laboratory, Duke Hospital Transfusion Service, Room 1720, Box 2928, 2301 Erwin Road, Durham, NC 27710, martha.combs@duke.edu.
An update on the H blood group system

E.A. Scharberg, C. Olsen, and P. Bugert

This update of the H blood group system (Scharberg EA, Olsen C, Bugert P. The H blood group system. Immunohematology 2016;32:112–8) reports 11 new FUT1 null alleles: 8 causative for the H– phenotype (Bombay phenotype, Oh), 3 in para-Bombay cases, and 5 new FUT1 alleles causative for a weak H phenotype (para-Bombay, Hw). The H blood group system (International Society of Blood Transfusion system 18) consists of a single antigen (H) defined by a terminal fucose residue found on red blood cells (RBCs) and in secretions. The H antigen is synthesized on the RBC surface by the FUT1 gene product fucosyltransferase 1. On epithelial cells and in body fluids, the H antigen is synthesized by the FUT2 gene product fucosyltransferase 2.

Key Words: H antigen, Bombay phenotype, O\textsubscript{h}, FUT1

New FUT1 Null Alleles Causing the Bombay Phenotype

Since publication of the original review article in 2016,\textsuperscript{1} 11 new alleles silencing the FUT1 gene were published for H– individuals from different populations (Table 1). The new alleles FUT1*01N.22–*01N.25 were formally assigned by the International Society of Blood Transfusion working party. Zanjani et al.\textsuperscript{2} describe the first homozygous loss of almost the entire FUT1 gene region by a large deletion. Five alleles represented point mutations leading to a single amino acid exchange. Interestingly, a Polish blood donor with the Bombay phenotype was found to be homozygous for a point mutation in the FUT1 start codon.\textsuperscript{3} In one individual with the Bombay phenotype, the FUT1 gene was compound heterozygous for the known FUT1*01N.12 allele and the new frameshift insertion c.791_792insG (FUT1*01N.22).\textsuperscript{4} The new FUT1*01N.23 allele is characterized by the frameshift deletion c.710del G and was found in a compound heterozygous proband with para-Bombay phenotype. It is assumed that the frameshift mutation represents a null allele, and the other allele (c.442G>T; p.Asp148Tyr; FUT1*01W.04) mediates a weak expression of H in this proband. Another individual was compound heterozygous for a frameshift deletion (c.454delG; p.E152fs; FUT1*01N.24) and a nonsense mutation (c.288T>A; p.Tyr96Ter; FUT1*01N.25).\textsuperscript{4} Thus, FUT1 was inactive, but FUT2 was active and caused the secretor and para-Bombay phenotype in this individual.

New FUT1 Alleles Causing the Para-Bombay Phenotype

Five new weak FUT1 alleles were identified in individuals with the para-Bombay phenotype (Table 2). The weak expression of H was mainly associated with missense mutations.\textsuperscript{5–7} The FUT1*02 allele is characterized by the missense variant

<table>
<thead>
<tr>
<th>Allele number (ISBT)</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Type of mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUT1*01N.22</td>
<td>c.791_792insG</td>
<td>p.Met265fs</td>
<td>Frameshift insertion</td>
<td>4</td>
</tr>
<tr>
<td>FUT1*01N.23</td>
<td>c.710delG</td>
<td>p.G237fs</td>
<td>Frameshift deletion</td>
<td>4</td>
</tr>
<tr>
<td>FUT1*01N.24</td>
<td>c.454delG</td>
<td>p.E152fs</td>
<td>Frameshift deletion</td>
<td>4</td>
</tr>
<tr>
<td>FUT1*01N.25</td>
<td>c.288T&gt;A</td>
<td>p.Tyr96Ter</td>
<td>Nonsense</td>
<td>4</td>
</tr>
</tbody>
</table>

ISBT = International Society of Blood Transfusion.
c.35C>T (p.Ala12Val) and was found with a new second missense mutation c.658C>T (p.Arg220Cys) in a para-Bombay individual. Further, an H+ individual was homozygous for a base triplet deletion (c.396_398delCCC), leading to the loss of a single amino acid (p.Pro133del) in the fucosyltransferase 1.

References


Erwin Andreas Scharberg, MD (corresponding author), Director of Immunohematology, Institute of Transfusion Medicine and Immunohematology, German Red Cross Blood Service Baden-Württemberg–Hessen gGmbH, Gunzenbachstr. 35, 76530, Baden-Baden, Germany, A.Scharberg@blutspende.de; Coral Olsen, Senior Scientist, Core Laboratory, Pathlab, Bay of Plenty, New Zealand; Peter Bugert, PhD, Associate Professor, Institute of Transfusion Medicine and Immunology, Heidelberg University, Medical Faculty Mannheim, and German Red Cross Blood Service Baden-Württemberg–Hessen, Mannheim, Germany.

Notice to Readers
All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

Attention:
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.
Tribute to S. Gerald Sandler, MD, FACP, FCAP, on his retirement

It is with great pleasure that I write a tribute honoring S. Gerald Sandler, MD, FACP, FCAP, professor in the Departments of Pathology and Medicine, MedStar Georgetown University Hospital, and an icon in the transfusion medicine scientific arena. He has been a mentor and guide through the years to me and to many others.

Dr. Sandler graduated from Princeton University in 1957, and he continued his education at Harvard and Tufts Universities and at the New York University (NYU) School of Medicine. His internship and fellowship in hematology was at NYU-Bellevue Medical Center. He was also a Commissioned Officer in the U.S. Public Health Service and a Clinical Associate at National Institutes of Health (NIH). Dr. Sandler held positions at Georgetown early in his career as Director of Hematology Laboratories and Blood Bank and as a Senior Lecturer and Professor at the Hadassah-Hebrew University Medical Center in Jerusalem, Israel, where he also served as Head of the Blood Bank for 6 years. He served 3 years as a Medical Officer (reserves) in the Israeli Defense Forces.

I first knew Dr. Sandler because of his roles at the National Headquarters, American Red Cross, beginning over 40 years ago, when he was Associate Vice President and Chief Medical Officer. Starting in 1978, he held senior-level positions in Medical and Laboratory Services and instituted the National Reference Laboratory for Blood Group Serology, for which I became responsible in 1995. In 1984, Dr. Sandler supported the start of the “new” scientific journal, Immunohematology, Journal of Blood Group Serology and Education. He personally edited every issue from 1984 to 2005. When he retired as Editor and Medical Editor of Immunohematology, we convinced him to stay on the Editorial Board, and he continued to write and edit articles until this day.

Immunohematology is fortunate to have had his leadership and support in the beginning and his continued commitment for over 35 years. It was his solo effort in 2005 that resulted in Immunohematology being cited in Index Medicus from 1997 to the present. In the words of Delores Mallory, one of the cofounders of Immunohematology (along with Marion Reid and Sandy Ellisor), “no other individual has had more impact on the quality or course of Immunohematology” (Immunohematology 2009;25:6–8).

I remember him recounting his adventure in the dark subterranean hallways of the National Library of Medicine that he traveled for the inside edge to meet with the Chairman, the person who would approve Immunohematology, Journal of Blood Group Serology and Education, being cited in Index Medicus. And it worked! I could elaborate on all of the marvelous things Dr. Sandler has done for Immunohematology, but he has accomplished so much more.

Dr. Sandler has 228 publications, and still counting—19 of which appeared in Immunohematology and two of which I was a coauthor. His large number of publications is likely due to what I have heard to be his mantra: “If you write an abstract for a meeting, you are obligated to write the article to submit for publication.” His publications span an almost unbelievable variety of topics over the 59 years he has published. Some of the broad areas include coagulation, infectious disease, drug therapy, information systems, test methods, disaster programs, blood components, transfusion reactions, rare donor programs, bone marrow donations, therapeutic apheresis, and the serologic and molecular science of blood groups.

Dr. Sandler has also contributed to the world’s literature in writing publications detailing historic events and people (e.g., John Locke, Thomas Jefferson, and Edgar Allan Poe), and his publications are not limited to medical science. Ahead of his time, in 1962, Dr. Sandler published an article titled “The Gluten-Free Diet.”

An accomplished editor, Dr. Sandler sits on the Immunohematology Editorial Board as well as on the editorial boards of seven other journals. His committee memberships, too many to detail here, include committees of AABB, American Red Cross, American Society of Hematology, College of American Pathologists, Center for Biologics Evaluation and Research, International Society of Blood Transfusion (ISBT), Magen David Adom, Medical Society of the District of Columbia, Platelet Disorder Support Association, and Progenika.

Dr. Sandler has received 13 awards from seven different institutions. Many of these awards were from Georgetown University, which included Faculty Teacher of the Year, Excellence in Residency Training, Resident’s Award for Faculty of the Year, and the
Dr. Michael Adams Award for Exemplifying *Cura Personalis* through Dedication and Commitment to Medical Education. It is perhaps even more prestigious to be recognized by your own institution than an outside one. However, he has also been designated an Honorary Member of ISBT and has received the Charles E. Walter Award from the Mid-Atlantic Association of Blood Banks, Richard J. Davey Lectureship in Immunohematology and Blood Transfusion from NIH, Harold Oberman Memorial Lectureship from the University of Michigan Medical School, and the U.S. Food and Drug Administration Commissioner’s Special Citation “In recognition of sustained excellence in cooperation and creativity in problem solving related to the challenge of rapid implementation of anti-HIV donor testing nationwide.”

Dr. Sandler is an avid reader and has accepted invitations to write 16 book reviews for journals, including those for book titles *Immune Hemolytic Anemias* and *Bloody Brilliant!*

On a more personal note, I have rarely met someone as energetic in his pursuit of his interests, as gregarious in committee work, and as personally engaging and hardworking as Dr. Sandler. We, speaking now on behalf of the Editors and the Editorial Board, thank Dr. Sandler for his tireless and effective work for *Immunohematology*, and we wish him well in retirement and his future travels. We feel certain that he will be as energetic in retirement as he has been in his career!

*Sandra J. Nance*

*Editor in Chief, Immunohematology, Journal of Blood Group Serology and Molecular Genetics*

---

**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.

---

**Important Notice About Manuscripts for Immunohematology**

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

---

**Notice to Readers**

*Immunohematology* is printed on acid-free paper.

---

**For information** concerning *Immunohematology* or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@redcross.org.
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, Clinical Center, National Institutes of Health (NIH), 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)^CM^SBB
Program Director
E-mail: lblagg1@jhmi.edu
Phone: (410) 502-9584

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

Phone (410) 955-6580
Fax (410) 955-0618
Web site: http://pathology.jhu.edu/department/divisions/transfusion/index.cfm
Online Specialist in Blood Bank (SBB)
Certificate and Masters in Clinical Laboratory
Management Program
Rush University  |  College of Health Sciences

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

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 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
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>
<td>San Marcos, TX</td>
</tr>
<tr>
<td>August 7</td>
<td>Webinar</td>
<td>Online</td>
</tr>
<tr>
<td>September 12-13</td>
<td>TSEC</td>
<td>Boston, MA</td>
</tr>
<tr>
<td>October 9</td>
<td>Webinar</td>
<td>Online</td>
</tr>
<tr>
<td>November 6-8</td>
<td>Hands-On (Serology)</td>
<td>San Marcos, TX</td>
</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

<table>
<thead>
<tr>
<th>2019 TSEC Schedule*</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2-8</td>
</tr>
<tr>
<td>June 6-7</td>
</tr>
<tr>
<td>September 12-13</td>
</tr>
<tr>
<td>December 5-6</td>
</tr>
<tr>
<td>Atlanta, GA</td>
</tr>
<tr>
<td>Seattle, WA</td>
</tr>
<tr>
<td>Boston, MA</td>
</tr>
<tr>
<td>Dallas, TX</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2019 Hands-On Schedule*</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 10-12</td>
</tr>
<tr>
<td>July 17-19</td>
</tr>
<tr>
<td>November 6-8</td>
</tr>
<tr>
<td>Molecular</td>
</tr>
<tr>
<td>Molecular</td>
</tr>
<tr>
<td>Serology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2019 Webinar Schedule*</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 13</td>
</tr>
<tr>
<td>May 8</td>
</tr>
<tr>
<td>August 7</td>
</tr>
<tr>
<td>October 9</td>
</tr>
</tbody>
</table>
The Discovery and Significance of the Blood Groups

Marion Reid
Ian Shine

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

John Gorman MD,
Lasker Award 1980

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

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

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

Phyllis Walker, MT(ASCP)SBB,
San Francisco

Marion E. Reid
&
Christine Lomas-Francis

BLOOD GROUP ANTIGENS & ANTIBODIES
A GUIDE TO CLINICAL RELEVANCE & TECHNICAL TIPS

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

Sandra J. Nance

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

Immunohematology

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

AVAILABLE NOW!
To order, call 718-784-9112
or visit
www.bloodgroups.info
www.sbbpocketbook.com

The Discovery and Significance of the Blood Groups
by Marion Reid & Ian Shine
Illus. Hardcover/Full color/7.5”x10”/214 pp./$36.00
ISBN 978-1-59572-422-9
What is a certified Specialist in Blood Banking (SBB)?
- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

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

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

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

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

However: In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam.
Conclusion: The BEST route for obtaining an SBB certification is... to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

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.
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
Dexter Facey (215) 451-2545
Dexter.Facey@redcross.org

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

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
CLIA licensed

Molecular Immunohematology Testing

Internationally recognized, CLIA-licensed and AABB accredited molecular immunohematology lab offering:
- Tailored testing solutions with low, medium, and high resolution to best suit your patient-specific needs
- Skilled lab professionals at the forefront of molecular research and testing ready to provide expert consultation
- Convenient, single point ordering for service requests and electronic reporting
- FDA-approved tests coupled with lab-developed assays to increase the accuracy and sensitivity of the services
- Tests include
  - RBC phenotype prediction using Human Erythrocyte Antigen (HEA) panel
  - RHD genotyping for detection of RhD variants (including weak, partial, and D

American Red Cross Biomedical Services
National Molecular Laboratory
700 Spring Garden Street
Philadelphia, PA 19123-3594
(215) 451-4917
nationalmolecular@redcross.org
CLIA licensed
Reference and Consultation Services

Antibody identification and problem resolution
HLA-A, B, C, and DR typing
HLA-disease association typing
Paternity testing/DNA

For information, contact:
Zahra Mehdizadehkashi
at (503) 280-0210

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

IgA Testing

IgA testing is available to do the following:
• Identify IgA-deficient patients
• Investigate anaphylactic reactions
• Confirm IgA-deficient donors

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

For additional information contact:
Dexter Facey (215) 451-2545
or e-mail:
Dexter.Facey@redcross.org

or write to:
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Dexter Facey

IgA Testing

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

For additional information:
Lauren Smith
at (860) 519-4017
e-mail:
Lauren.Smith@redcross.org

or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Avenue
Farmington, CT 06032

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

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

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

National Reference Laboratory for Blood Group Serology

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

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

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

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

B. Preparation
1. Title: Allele Name (Allele Detail)
   ex. RHCE*01.01 (RHCE*ce48C)
2. Author Names (initials and last name of each [no degrees, ALL CAPS])

C. Text
1. Case Report
   i. Clinical and immunohematologic data
   ii. Race/ethnicity and country of origin of proband, if known
2. Materials and Methods
   Description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patient names or hospital numbers.
3. Results
   Complete the Table Below:

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

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

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

D. Acknowledgments

E. References

F. Author Information
   List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable.
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
Subscription Application

United States:  □ Institution . . . $100
               □ Individual . . . $50
               □ Students . . . $40 (free for 1 year with letter of validation)

Outside United States:  □ Institution . . . $100
                        □ Individual . . . $60
                        □ Students . . . $50 (free for 1 year with letter of validation)

FIRST NAME ___________________________  LAST NAME ___________________________

INSTITUTION NAME: (For Institutional Subscriptions) _______________________________________

DEPARTMENT/DIVISION _________________________________________________________________

STREET ADDRESS ________________________________________________________________

CITY, STATE, ZIP CODE, COUNTRY ____________________________________________________

EMAIL _________________________________  PHONE (Required) __________________________

□ Check if home address used

PAYMENT METHOD

□ Check enclosed*  
*Make check payable in U.S. dollars to THE AMERICAN RED CROSS. Mail this form with check to:

Immunohematology
P.O. Box 40325
Philadelphia, PA 19106

□ VISA  □ Mastercard

NAME (As it appears on credit card) _______________________________________________________

Email this form to immune@redcross.org or mail this form to the address above.

**DO NOT include credit card billing information on this form. Once this completed form is received, the requester will be contacted by phone for the credit card billing information.