

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 22, NUMBER 2, 2006



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Immunohematology

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WITH MANY THANKS

Dedication to Marilyn K. Moulds

It is with great admiration and gratitude that we dedicate this issue of *Immunohematology* to Marilyn K. Moulds. Her knowledge and teaching skills have driven her career forward from a laboratory position at Minneapolis War Memorial Blood Bank, when, in 1973, she received her specialist in blood bank certification, to the positions of supervisor, director, and then vice president of consultation and education services at Gamma Biologicals, Inc., in Houston, Texas, where she has spent more than 30 years performing serologic testing, researching new antigens and antibodies, publishing articles, speaking at blood bank meetings, and teaching students.

Marilyn has been involved in discovering new antigens, evaluating the effects of enhancement reagents, and identifying new antibodies; she authored and coauthored many publications over the years. These included articles on the effects of treating Kell and LW antigens with AET; the use of PEG when performing autoadsorptions for the detection of underlying alloantibodies; the analysis of high-incidence antigens such as SCER, SCAN, and MAM; a case report of a hemolytic transfusion reaction caused by anti-Do^b; and the identification of 15 novel A and B subgroup alleles. She continues to be a wonderful supporter of *Immunohematology* as an editorial board member, peer reviewer, and author, claiming that it is “the journal for the bench tech.” She has contributed many articles to *Immunohematology* over the years.

Marilyn has been instrumental in the study of high-titer, low-avidity (HTLA) antibodies and in generating an assortment of well-categorized antisera. She is known for her marvelous memory and can recall patient cases she worked on over the years—a great help when donors went to different blood banks for their donations or patients were admitted to different hospitals.

Marilyn’s love for teaching and sharing her knowledge of blood banking has been exemplified by her dedication to the blood bank school at Gamma, from which hundreds (and possibly thousands) of medical technologists and MDs graduated over the years. Students from all over the world attended the immunohematology school at Gamma Biologicals, Inc.

Her love for sharing her knowledge has also been seen in her acceptance of invitations to speak at blood clubs, as well as state, national, and international blood bank meetings, including those sponsored by the

AABB, American Society of Clinical Pathologists (ASCP), International Society for Blood Transfusion (ISBT), and Invitational Conference of Institutional Immunohematologists (ICII). No group is too large or too small for Marilyn. She has also been an active member of organizations such as the AABB, South Carolina Association of Blood Banks (SCABB), and ISBT, to name a few. She has received many awards over the years, including the L. Jean Stubbins Memorial Lecture Award in 1979 and the Larry L. Trow Memorial Education Award in 1991, both of which were given by SCABB, as well as the Ivor Dunsford Award in 1996 and the Sally Frank Award in 1999, both given by the AABB. In 2005, she received the Jack B. Alperin award from the University of Texas Medical Branch for Outstanding Support of the Specialist in Blood Bank Technology Program.

Marilyn’s enthusiasm for her work is depicted in a story of a serologic study of the HOV antigen in an extended family in Minnesota. Marilyn was in charge of contacting the family members and keeping records and her coworker drew the blood samples. They traveled miles around “small town” Minnesota, collecting a total of 30 samples, including one from a 105-year-old woman. They borrowed some lab space and were up half the night processing the samples for the DNA.

Marilyn’s love for people and for the past resulted in her requesting that all of the photographs from the ICII meetings be shipped to her when she took over the historian position with ICII. She keeps track of everyone, especially the retired folks.

Marilyn’s contributions to the blood banking field have been tremendous and she will be greatly missed. Her influence will continue in the students she taught, the staff members she managed, and the peers she supported.

Many of the articles in this issue of *Immunohematology* were authored or coauthored by Marilyn and we dedicate this issue to her and to her long and distinguished career. We wish her the best as she pursues life beyond the serology laboratory and the public forum. She will always be a blood banker at heart and for that we are grateful.

The Editorial Staff
Immunohematology

The Redelberger antigen: a family study, a family story

N.A. LANG, M.K. MOULDS, AND G.E. COGHLAN

The Redelberger antigen (Rb^a) was first discovered in 1974 on the RBCs of a blood donor who was an employee of the Community Blood Center in Dayton, Ohio. The discovery was made as a result of the investigation of a reagent contamination problem. Two examples of the Rb^a antigen were subsequently identified in the United Kingdom, but no “new” examples have been identified in the United States or Europe. Anti-Rb^a is a commonly occurring antibody, often found in combination with other antibody specificities, especially in combination with other antibodies to low-incidence antigens. *Immunohematology* 2006;22:48–51.

Key Words: Rb^a, Redelberger, low-incidence antigen, reagent contamination

The discoveries of antigens of low incidence have historically occurred as a result of one of several scenarios. These scenarios include the following: an infant suffering from HDN due to a maternal antibody against a low-incidence antigen of paternal origin, a patient who experiences an unexpected transfusion reaction, a patient whose serum has an unexpected antibody detected in a screening or compatibility test, an individual whose RBCs react unexpectedly with routine blood grouping reagents, and the antithetical antigen to a defined high-incidence antigen. Investigations of low-incidence antigens are often time-consuming, and, unfortunately, often dismissed by blood bankers because low-incidence antigens are perceived as only of academic, not practical, interest.

This article will review the discovery of a very rare antigen, Redelberger, or Rb^a. It will also document the subsequent discoveries of the Rb^a antigen in the United States and how all of the individuals in the United States whose RBCs carry Rb^a can be traced back to the original propositus.

Background

In March 1974, Richard Redelberger donated a unit of blood at the Community Blood Center in Dayton, Ohio. Mr. Redelberger was a frequent blood donor who was employed, quite coincidentally, by the Community Blood Center of Dayton as a donor services

coordinator. Mr. Redelberger's RBCs routinely typed as group B, D-, but on this particular donation, his RBCs reacted 3+ at the antihuman globulin phase of testing with a commercial anti-CDE reagent supplied by Gamma Biologicals, Inc., of Houston, Texas. Other lot numbers of Gamma anti-CDE reagent did not react with Mr. Redelberger's RBCs nor did anti-CDE reagents from other commercial manufacturers. Gamma was contacted about this potential contamination problem and the unidentified antibody was traced to the anti-E component of the reagent.¹

The anti-E component of the anti-CDE reagent and samples of Mr. Redelberger's RBCs were sent to many reference laboratories around the world. Initially, it was thought that the antigen present was the previously identified Bishop antigen (Bp^a). However, with further testing, all investigators soon agreed that the antigen present on the Redelberger RBCs was unique. Mr. Redelberger's RBCs reacted with the Tillett serum (which contains many antibodies to low-incidence antigens), as did the RBCs of a random donor from the North London Blood Transfusion Center in London, England. RBCs from this donor, Mrs. NM, gave the same pattern of reactions with a battery of antisera for low-incidence antigens as did those from Redelberger; Mrs. NM was subsequently determined to be the second individual identified as possessing RBCs with the “new” antigen. A third donor, Mrs. SR, was found at the Wales Blood Transfusion Center in Cardiff. The RBCs from this donor reacted with only one of several anti-E sera. Further investigation revealed that her RBCs gave the same pattern of reactions with antibodies to low-incidence antigens as did those of Redelberger and Mrs. NM.¹

The Redelberger antigen was studied in all three families and was found to be autosomal dominant in its inheritance.¹ Richard Redelberger was thrilled to learn that a “new” antigen had been discovered on his RBCs. Always the master of the one-liner, Richard declared that he knew all along that he could “never be a

bishop!” The antigen was subsequently named in his honor and abbreviated as Rb^a according to the conventions of the time.

No new examples of the Redelberger antigen were reported for many years in either the United States or Europe. However, anti-Rb^a was found with some frequency, suggesting that the antibody is usually naturally occurring. It has been demonstrated that most examples of anti-Rb^a are direct agglutinins and predominantly IgM. In one study, 6200 donor sera were screened for the presence of anti-Rb^a and six examples were found.^{1,2} The incidence of anti-Rb^a is much higher in sera containing multiple antibodies, especially when multiple antibodies to low-incidence antigens are present. Anti-Rb^a is especially common in sera that contain anti-Bp^a and anti-Wr^a.¹

At the American Red Cross/AABB Immunohematology Reference Laboratory (IRL) conference held in Memphis, Tennessee, in April 2004, Marilyn Moulds of Gamma reported that an apparent new Rb^a propositus had been identified. The individual, RT, was a healthy blood donor who had donated a unit of RBCs at the Blood Connection in Greenville, South Carolina. The most probable Rh genotype of his RBCs was R₂R₂ and they were subsequently used by Gamma as part of a reagent RBC screening duet. Within days of the release of the screening duet, Gamma received numerous customer complaints about this particular RBC reacting with many patient sera when subsequent antibody identification studies did not detect any alloantibodies. One hospital reported seven reactive sera with four of the seven patients reporting no history of transfusion. A second hospital reported that five of six patient sera reacted with the RBC over a weekend! Marilyn’s investigation focused on the identification of an antigen of low incidence on the RBCs in question and, with the assistance of Gail Coghlan of the Rh Laboratory, University of Manitoba, Winnipeg, in Manitoba, Canada, they were found to be Rb(a+)

In the audience at the IRL conference that day was Nancy Lang, the lead technologist in the IRL at the Community Blood Center/Community Tissue Services in Dayton, Ohio. Nancy listened to the facts of Marilyn’s discovery with particular interest since she personally knew of the discovery of the Redelberger antigen in Dayton. Upon returning to work on the Monday morning after the conference, Nancy opened an e-mail message from a local blood donor, GK. The message, in part, read:

“I received an e-mail from my sister in Greensboro, NC, who indicated that her son in Greenville, SC, was recently told that he had a particular antigen in his blood associated with the Diego Blood Group . . . I know that many years ago the Dayton Blood Center told my mother that she was a carrier of some rare type of blood component but none of us knew what this meant . . . I was wondering if you would be willing to test my blood for this antigen since I am a regular donor. Apparently, the test involves Rb(a)—whatever that may be.”

What timing! Nancy called GK and was able to confirm that GK’s mother was Richard Redelberger’s sister. Nancy’s second phone call was to Marilyn Moulds to tell her the Rb^a discovery was not a “new” family in the United States!

A Manufacturer’s Perspective

Often, blood bankers are simply annoyed at the finding of an antibody directed at a low-incidence antigen when performing routine serologic procedures. Very little, if any, effort is put forth in attempting to identify the specificity of this antibody. Seen as an isolated event, identification seems unnecessary and not useful, so the reactivity is ignored unless a pattern or trend is noted. As a result, potential “new” low-incidence antigens are not identified as frequently as they might be. There is one instance where identification might be pursued, however, and that is in the case of potential for clinical HDN in a current pregnancy or for future pregnancies.

Manufacturers of commercial RBC products and antisera have an entirely different perspective, however. As illustrated in the case of the R₂R₂ donor above, an antibody to a low-incidence antigen can be quite common, although the incidence of the antigen itself is very low. Anti-Wr^a, for example, is analogous to anti-Rb^a in that the antibody is quite common while the antigen itself is very uncommon. The use of a screening or panel (antibody identification) RBC that possesses a low-incidence antigen can prove to be quite frustrating for the unsuspecting customer of the product when the corresponding antibody occurs with some frequency. Complaints are generated and sent to the manufacturer, which launches an investigation of the antigen on the RBC.

The presence of an antibody to a low-incidence antigen is not of great concern to the manufacturer of commercial antisera if the incidence of the antigen is extremely low. In fact, commercial reagent antisera

only need to be screened for the presence of antibodies directed at rare antigens that occur in 1 percent or more of the random population. However, commercial antisera "contaminated" with anti-Rb^a led to the initial discovery of the Rb^a antigen and the accidental discovery of the Rb^a antigen in another member of the Redelberger family was also the result of an anti-D contamination problem. That story follows.

AR was a female blood donor from Florida who had donated to the 6-gallon benchmark. Donor records showed that she had been typed as D- on all donations until the last. At that time, in August 2003, the RBCs of AR reacted weakly in the weak D test with one source of anti-D reagent, which was a human polyclonal reagent. Her RBCs did not react with anti-D reagent used in an automated test and did not react in tube tests that used monoclonal blend anti-D reagent.

An investigation was launched by the manufacturer of the anti-D reagent that caused the positive weak D test result. The RBCs of AR did not react with six monoclonal anti-D reagents from different manufacturers but did react with three of 12 human sera containing multiple antibodies directed at low-incidence antigens. Several antibodies were in all three reactive sera. After testing with her standard panel of sera containing multiple antibodies, Gail Coghlan of the Rh Laboratory suggested that this donor's RBCs might also be Rb(a+). Donor RBCs, known to be D-, Rb(a+), from a liquid nitrogen collection were then tested and found to react with the same human polyclonal anti-D reagent that reacted with AR's RBCs. Another lot number of anti-D reagent that contained serum from the same donor was also found to react with AR's RBCs and with the D-, Rb(a+) frozen RBCs.

The referring donor center in Florida was contacted to obtain more information on AR. AR indicated that she knew she was positive for the Redelberger antigen and produced the paperwork from the original study. Her mother was a sister of Richard Redelberger!

The Rb^a Antigen and Antibody Today

The Rb^a antigen was assigned to the Diego blood group system in 1996 and its ISBT symbol (number) is DI6 (010006).³ The molecular basis for the Rb^a antigen was published by Jarolim et al. in 1997.⁴ They showed that individuals whose RBCs carry the antigen have a point mutation in anion exchanger 1 that leads to the replacement of proline with leucine at amino acid 548.

The antigen is expressed on cord RBCs and the effect of enzymes on the antigen is variable. The antigen deteriorates upon storage at 4°C but survives storage in liquid nitrogen.¹ Anti-Rb^a is predominantly IgM but can also be IgG.^{1,3} The antibody does not bind complement and its clinical significance is doubtful.³ Five Rb(a-) women, who gave birth to Rb(a+) children, did not make anti-Rb^a.^{1,2} No other data are available.

Summary

The Rb^a antigen is of extremely low incidence and has only been discovered in three families in the world.³ All examples of the Rb^a antigen found in the United States since 1974 can be traced back to the original propositus, Richard Redelberger, for whom the antigen is named (Fig. 1). Anti-Rb^a has been found to be a frequent, naturally occurring antibody, however, and is often found in combination with other antibodies, especially those directed to antigens of low incidence.

Addendum

The authors have been in contact with the Redelberger family in regard to the publishing of this article. While completing the article, an e-mail message was received from family member JS, a grandnephew of Richard Redelberger. The message, in part, read as follows:

"...It made me start thinking about the bone marrow donation that I did a couple years ago. The recipient was a non-relative that matched antigens with mine. Do you think that it could mean that the recipient also has the Redelberger antigen?"

No, JS, the recipient did not have the Redelberger antigen at the time you were selected as the marrow donor. But the recipient does have the Redelberger antigen now!

Who says blood banking is boring????

Acknowledgments

We thank the family of Richard Redelberger for its enthusiastic cooperation over the years in the study of the Rb^a antigen. The Redelberger family's altruism has been demonstrated by several generations of faithful volunteer blood donors throughout the United States. Their spirit and dedication to providing the gift of life to others is a great legacy to Richard and to his mission of donor recruitment. We also want to acknowledge the

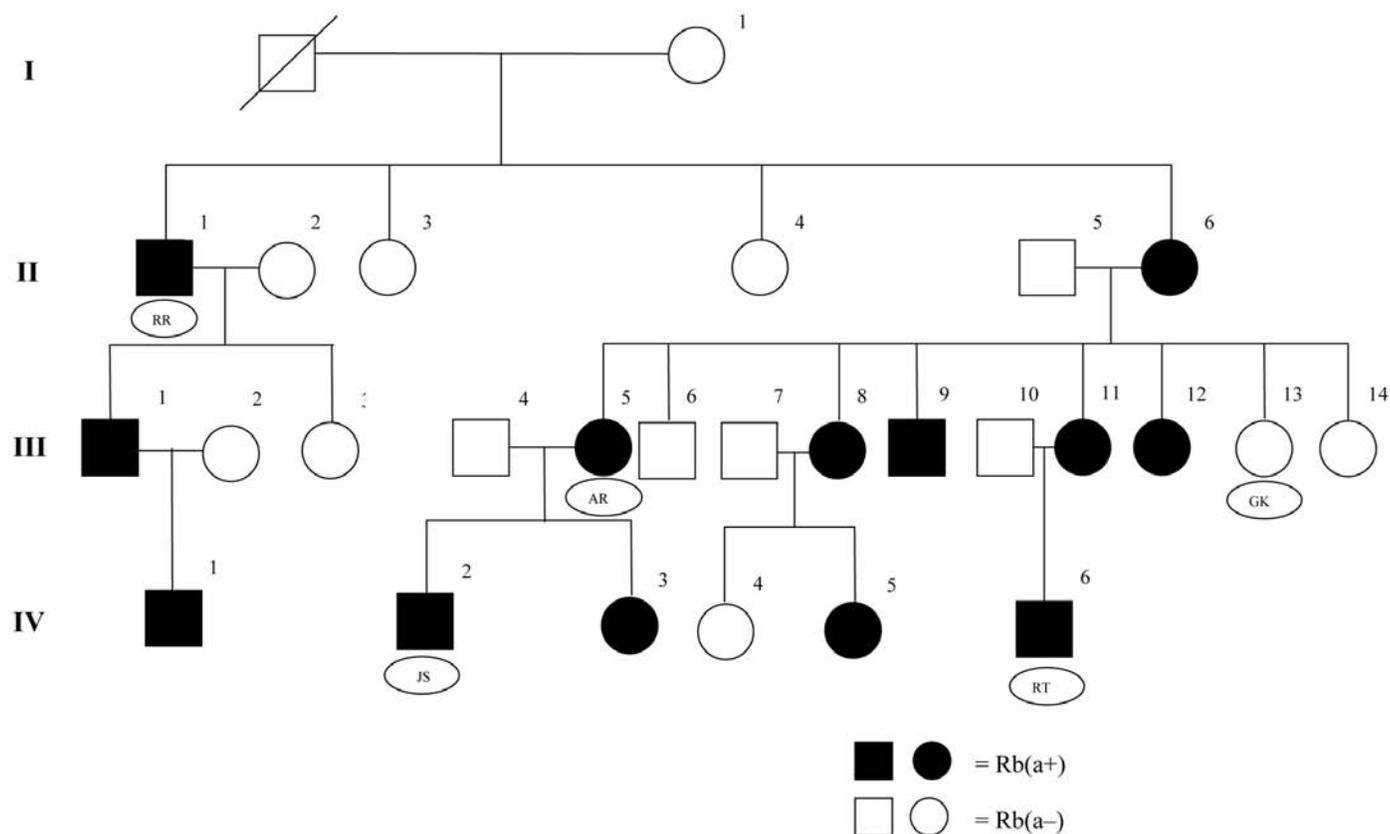


Fig. 1. Pedigree chart of the Redelberger family.

efforts of Delores Mallory and Joan Bare of the Community Blood Center in Dayton for all their hard work in the initial investigation of this “new” low-incidence antigen and for their sharing of samples with other investigators all over the world. Delores continues to keep in touch with the family even today.

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Review: monoclonal reagents and detection of unusual or rare phenotypes or antibodies

M.K. MOULDS

Monoclonal antibodies have been used in the formulation of commercially available blood grouping reagents since the early 1990s. It became apparent early on that introducing them into routine use along with, or instead of, human- or animal-derived reagents could and did lead to discrepant reactions. These discrepancies most often came to light when confirming a blood type obtained previously with human- or animal-source reagents or when using two or more sources of a reagent from the same or another manufacturer to perform blood typing or antibody detection or identification testing. A number of factors contribute to differences in reactivity of reagents that are of the same specificity but are from more than one source. One factor is the use of different clones of the same specificity to manufacture blood bank reagents. Another is the effect of the various diluents used by different manufacturers to formulate reagents that contain the same clone(s). In addition, RBCs having unusual or rare phenotypes can cause discrepant reactions when performing phenotyping. Discrepant reactions can also occur because of patient or donor antibodies that react in an unusual manner when antiglobulin tests are performed with monoclonal antihuman globulin (AHG) versus rabbit AHG reagent. It is important to know the identity of the unusual or rare phenotypes and antibodies and to be able to recognize the different types of reactions that will be observed when using more than one reagent of the same specificity. Most importantly, one must be able to interpret reactions correctly and establish the true blood type of the RBCs or specificity of the antibodies. This review will describe situations in which the use of monoclonal reagents from more than one source or manufacturer, or comparison with results of human- and animal-source reagents, resulted in discrepancies with unusual or rare phenotypes or antibodies. Many of the samples described in this review were sent to the reference laboratory at Gamma Biologicals, Inc., in Houston, Texas, which later became ImmucorGamma with sites in Norcross, Georgia, and Houston, Texas. *Immunohematology* 2006;22: 52-63.

Key Words: monoclonal antibodies, monoclonal reagents, unusual or rare phenotypes or antibodies

For a background on the introduction of monoclonal antibodies as reagents to the field of blood banking, the reader is referred to a very comprehensive review by Marjory Stroup in a 1990 publication of *Immunohematology* titled "A review: the use of monoclonal antibodies in blood banking."¹ In addition, two presentations by John Case, in 1992 and 1993,

provide a very good overview of monoclonal antibodies of varying specificities used as blood bank reagents.^{2,3} He also presented an excellent paper on dealing with reagents made from human and monoclonal Rh antibodies as the Sally Frank Lecture at the AABB meeting in 1998, titled "Monoclonal/polyclonal reagents. Some practical differences in performance."⁴ This present review will bring us to the monoclonal reagents in use today in the United States and to unusual situations involving rare blood types and antibodies. I should point out here that even though some of the clones used in the tube reagents are the same as those used in the MTS gel cards distributed by Ortho-Clinical Diagnostics, Raritan, New Jersey, for use in the ID-Micro Typing System (Ortho-Clinical Diagnostics), I have not included any test results or publications on the reactions obtained with unusual phenotypes using the gel technique, as I do not have firsthand knowledge of how they do react.

The two most important blood group systems are ABO and Rh. Blood samples from all blood donors, prenatal patients, and transfusion recipients are routinely tested for A, B, and D antigens. Some unusual or rare phenotypes that have been found to give discrepant or variant typing results in the ABO system are group O with missing anti-A or anti-B in plasma, A subgroups and rarely B subgroups, A subgroup B and AB subgroup, B(A), and group A with acquired B antigen. In the Rh blood group system, weak D, partial D, and RhCE variants (special reference to R₀^{Har} and Crawford), weak C (r^s), Rh32 (R^N) and others, E^v and E variants, c variant Rh:-26, and e variants (in particular, hr^B-) have given discrepant or variant typing results. In the Kell blood group system, K1 variants can give different typing results depending on whether human or certain monoclonal reagents are used. Other monoclonal reagents that can give discrepant test

results, when compared with tests performed using human- or animal-source reagents, with unusual phenotypes are anti-M, -N, -P1, -Le^a, and -Le^b. And lastly, the monoclonal anti-IgG and anti-C3 reagents can give varying results compared with those obtained with rabbit antihuman globulin (AHG) reagents when testing unusual or rare antibodies. Throughout the paper, the reader is referred to Table 1 for the list of clones present in each of the monoclonal-based reagents discussed.

ABO Reagents

To be or not to be. Is it a group O with missing isoagglutinin, A subgroup, B subgroup, A subgroup B, AB subgroup, B(A), or group A with acquired B antigen? These are the questions to be answered.

During a period of four years, more than 30 samples were tested to resolve ABO discrepancies using serologic and molecular techniques at two independent laboratories, the reference laboratory at ImmucorGamma in Norcross, Georgia/Houston, Texas, and the laboratory of Dr. Martin L. Olsson in the University Hospital, Lund, Sweden. Many of the samples that gave unusual ABO grouping results were included in a paper published in 2001 by Dr. Olsson and collaborators, of which I was one.⁵ Some of these samples were tested only by serologic techniques but most were tested by both.

One set of samples that proved to be most interesting and diverse gave the same serologic pattern of reactivity but different ABO genotypes by molecular testing. Forward grouping showed that these samples did not react with ABO monoclonal reagents (anti-A, -B, and -A,B) from all three manufacturers (Immucor, Norcross, GA; Gamma Biologicals, Inc., Houston, TX; and Ortho-Clinical Diagnostics), suggesting the RBCs to be group O. Yet, the serum or plasma, when tested with A and B reagent RBCs, demonstrated expected strong reactivity with B RBCs but lacked the expected reactivity with A RBCs, suggesting the RBCs to be group A. Molecular genetic analyses for *ABO* yielded two patterns. In one pattern, no *A* allele could be demonstrated, yet there was no explanation for the lack

Table 1. Clones used in the manufacture of reagents.

	GAMMA		IMMUCOR		ORTHO		Gel card
	Clone	Ig class	Clone	Ig class	Tube		
					Clone	Ig class	
Anti-A	BIRMA 1	IgM	BIRMA 1 A26A2	IgM IgM	MH04 3D3	IgM IgM	BIRMA 1
Anti-B	GAMA110	IgM	ES4 B95.3 LB-2	IgM IgM IgM	NB10.5A5 NG10.3B1 NB1.19	IgM IgM IgM	LB-2
Anti-A,B	BIRMA 1 ES4 ES15	IgM IgM IgM	BIRMA 1 ES4 ES15	IgM IgM IgM	MH04 3D3 NB10.5A5 NB1.19	IgM IgM IgM IgM	ES4 ES15
Anti-D	GAMA401 GAMA401 F8D8	IgM IgM IgG	Series 5 TH28 MS26 Series 4 MS201 MS26	IgM IgG IgM IgG	MAD2 HUMAN	IgM IgG	MS201
Anti-C	MS24	IgM	MS24	IgM	MS24	IgM	MS24
Anti-E	GAMA402	IgM	MS12	IgM	C2	IgM	MS258 MS260
Anti-CDE			MS201 MS258 MS24	IgM IgM IgM			
Anti-c	951	IgM	MS33	IgM	MS42	IgM	MS33
Anti-e	MS16 MS21 MS63	IgM IgM IgM	MS16	IgM	MS16	IgM	MS16 MS21 MS63
Anti-Le ^a	GAMA701	IgM	LM112/161	IgM	LM112/161	IgM	
Anti-Le ^b	GAMA704	IgM	LM129/181	IgM	LM129/181	IgM	
Anti-Jk ^a					MS15	IgM	
Anti-Jk ^b					MS8	IgM	
Anti-K1	MS56	IgM	MS56	IgM			
Anti-M	M2A1	IgM	F23	IgM			
Anti-N	12E.A1	IgG1					
Anti-P1	OSK17	IgM					
Anti-IgG	16HB	IgM					
Anti-C3b	055A.305GA MA003	IgM			F7G3	IgG1	
Anti-C3d	053A.714GA MA004	IgG1			C4C7	IgG1	

of expected anti-A in the plasma. Thus, the patients and donors were genetically group O. In the other, an *A* allele was detected, indicating the patients and donors were genetically group A, even though their RBCs did not react with all anti-A, -B, and -A,B reagents. The samples gave the same serologic picture but different ABO genotyping results. This study reinforces the fact that one should not assume either that the RBC grouping is the correct ABO blood type or that the

serum or plasma reverse grouping is the correct type. It could be either, according to ABO genetic studies.

Several samples that were determined to be A subgroups gave discrepant results with different anti-A,B reagents when attempting to resolve the discrepancy between the ABO RBC grouping and the serum or plasma grouping. The anti-A and anti-B reagents from all three manufacturers did not react on forward grouping with the RBCs, suggesting that they were group O, but the plasma of the patients and donors on reverse grouping contained a strong expected anti-B and lacked the expected anti-A, suggesting that the plasma was group A. The anti-A,B reagent from two manufacturers (Immucor and Gamma Biologicals, Inc.), which contained a blend of the same three clones of anti-A, anti-B, and anti-A,B, did react with the RBCs. The anti-A,B reagent from a third manufacturer (Ortho-Clinical), containing a blend of two different clones of anti-A and two different anti-B clones, did not react (Table 1). The reactivity with the anti-A,B reagents from Immucor and Gamma was determined to be because of the anti-A,B clone ES-15 in the anti-A,B blended reagent. This clone has been described as being very efficient at picking up A_x and A subgroup RBCs.^{6,7}

Genomic analysis was performed on samples from three blood donors in the study that gave discrepant serologic ABO grouping results.⁸ RBCs from two of the donors reacted weakly with only one anti-A reagent (Ortho-Clinical) and reacted strongly with all three manufacturers' anti-B reagents, suggesting an A subgroup B. The plasma of one donor contained weakly reactive anti-A and no anti-B and genomic analysis revealed an A^x hybrid (*A-O^v*) allele found in A_x samples. The other donor sample had a strong anti-A and no anti-B in the plasma and genomic analysis revealed the donor to be heterozygous for the *B(A)-I* allele. It was concluded that this latter case had the originally described variant with an A703G mutation as compared with normal *B* alleles.⁹ The RBCs from these two donors gave the same serologic picture but gave different serum or plasma grouping and genomic analysis. The serum grouping makes a clear distinction between A subgroup B and B(A) phenotype. The B(A) phenotype was first described in 1986 in an abstract¹⁰ and again in a paper¹¹ by Beck et al. The anti-A clone (MH04) in the Ortho anti-A reagent that was very efficient in detecting A_x RBCs was also shown to react weakly with some group B RBCs.

Group A With Acquired B Antigen

In 1992, Beck and Kowalski in the communications section of *Immunohematology* reported on monoclonal anti-B reagents composed of the clone ES4 reacting with RBCs from group A persons because of the acquired B phenomenon.¹² In that same year, there were two abstracts on this subject.^{13,14} Five cases in 8 months were observed in the reference laboratory of Beck at the Community Blood Center in Kansas City. Previously, this laboratory had only seen two or three such cases per year. The hospital-based laboratory of Pedreira et al. identified 13 patients in 9 months. None had been detected in the 8 months before beginning the use of a monoclonal anti-B reagent that contained ES4. It was determined that acidification of the anti-B reagent to pH 6 would reduce the number of samples with acquired B antigens that would be detected. Reagents containing ES4 were manufactured by Gamma, Immucor, and the former BCA (Biological Corporation of America, West Chester, PA). Ortho's anti-B reagent did not contain this clone and did not react with RBCs that were group A with acquired B.

The Gamma monoclonal anti-B reagent containing ES4 was first introduced into blood banking in November 1990. For two years (1991–1992), six cases (three per year) of acquired B were investigated by the Gamma reference laboratory. At that time, Immucor and BCA also manufactured an anti-B reagent formulated with ES4. The reagents manufactured by the three companies varied in pH from pH 7 (Gamma) to pH 6.5 (Immucor) to pH 6.0 (BCA). In May 1993, acidified anti-B reagents (pH 6.0) were approved by FDA for manufacture by both Gamma Biologicals, Inc., and Immucor. The Gamma reference laboratory had eight cases referred in 1993, 1994, and 1995 (2–3 per year). From 1977 to 1990 (14 years), 28 cases of acquired B were investigated; again two cases per year. The Gamma reference laboratory did not see an increase in the number of acquired B samples because of the use of monoclonal anti-B reagent containing ES4 from what had been referred to the laboratory before the introduction of the reagent containing ES4 (personal observations). In April 1996, a monoclonal anti-B reagent manufactured by Gamma (containing clone GAMA-110) was introduced to the market; this clone did not react with acquired B RBCs. Gamma discontinued manufacturing the anti-B reagent made from ES4. Immucor still markets an acidified anti-B reagent made with ES4. It is important to note that with group A acquired B there is an ABO discrepancy

between the forward (RBC) grouping and reverse (serum) grouping in cases where the anti-B reagent contained ES4 (except in one rare exception). The RBCs react strongly with anti-A reagent and react more weakly with anti-B reagent but the serum or plasma reacts strongly with B RBCs and weakly or not at all with A₁ RBCs, suggesting group A with unexpected reactivity of the RBCs with anti-B reagent. Immucor also now has a tube reagent anti-B made from a clone that does not react with acquired B RBCs.

These cases are not all inclusive of the types of ABO discrepancies seen with the use of ABO monoclonal reagents. However, they give the reader a sense of the types of unusual samples that have been encountered in a routine hospital, prenatal screening laboratory, or donor center.

Rh Reagents

Anti-D

After A and B antigens of the ABO blood group system, D is the most important blood group antigen in routine blood banking. To enable measures to be taken to avoid immunization to the D antigen and to assure the identification of all recipients who should be given only D- blood, testing for the D antigen is an important laboratory routine. The D- phenotype occurs with an incidence of approximately 15 percent in Whites and 9 to 10 percent in Blacks. The term D^u was originally coined in 1946 to describe variable reactivity of certain bloods with a battery of sera containing saline-reactive anti-D.¹⁵ It has since been replaced by the term "weak D" to describe forms of the D antigen that may not be agglutinated directly by anti-D reagents but require an IAT to detect them.^{16,17} With the introduction of powerful monoclonal anti-D reagents, many bloods formerly classified as weak D (D^u) have been reclassified because they have been found to show strong direct agglutination with the newer reagents. Individual monoclonal anti-D reagents may differ in regard to their reactivity with RBCs of this kind. A suitable IgG anti-D reagent (for use by IAT) is still required to detect some examples of the weak D phenotype, and the partial D phenotype known as Category VI. RBCs of apparently D- donors are generally tested for weak D by converting negative tests with anti-D reagents to an AHG phase and then reading the test again. Testing for weak D on samples from transfusion recipients and prenatal patients is not required¹⁸ and it has been suggested that the test only be performed on cord bloods of D-mothers and on samples from blood donors.

Reagents for D typing in the United States currently consist of four reagents for tube testing and one for MTS (Ortho) gel cards. Gamma-clone Anti-D and Immucor Series 4 and 5 Anti-D monoclonal reagents each contain a blend of an IgM clone and an IgG clone. Ortho's tube monoclonal anti-D reagent contains a blend of an IgM clone and human polyclonal IgG. Series 4 and 5 of Immucor contain the same IgG clone and the MTS (Ortho) gel card contains the same IgM clone used in the Immucor Series 4 (Table 1).

Weak D and Partial D

Several studies in the United Kingdom, Sweden, Germany, Canada, and the United States have shown that there is great variability in reactivity of monoclonal anti-D reagents with weak D and partial D RBCs because of multiple factors. In one study by laboratories in the United Kingdom and Sweden, RBCs of weak D and partial D phenotypes were tested with 26 IgG and 15 IgM monoclonal anti-Ds. The IgG clone F8D8, used in the Gamma-clone Anti-D blend reagent and MAD-2 IgM clone in Ortho's tube anti-D reagent were part of the study. Reactivity of monoclonal anti-D is dependent on antibody concentration and antibody avidity. D antigen site quantitation was not performed in the study. However, testing the monoclonal antibodies with RBCs of the weak D phenotype suggests that site density has a profound effect on the performance of monoclonal antibodies in the identification of a D variant, especially with monoclonal antibodies of low avidity. Some D variants may be associated with decreased site numbers and lack of reactivity of monoclonal antibodies with these RBCs may be because of a quantitative rather than a qualitative effect.¹⁹ Flegel and coworkers in Germany reported on the molecular basis of weak D.²⁰ Denomme et al. published studies in Canada with two monoclonal anti-D reagents, suggesting the importance of testing samples from prenatal and transfusion recipients with more than one anti-D reagent.²¹ They tested 33,864 samples in 18 months and 57 of those demonstrated an immediate spin discrepancy between two commercial sources of anti-D reagents. This gave a frequency of 1 in 594 or 0.17 percent. RBCs of the Category DVI type 1 were common and were only superseded by the frequency of those of weak D, type 1 (n = 16).

Jenkins and colleagues in the United States tested 1005 D+ donors on the Olympus PK7200 (Olympus America, Inc., Melville, NY), using two different anti-D reagents (one diluted monoclonal blend and one diluted human polyclonal) and detected four samples

with RBCs of the weak D phenotype, for an incidence of 0.4 percent in a primarily White donor population.²²

A very comprehensive review of partial D antigens by Tippett et al. in 1996 showed that monoclonal anti-D, which provides unlimited supplies of reagents, is ideal for defining partial D antigens.²³ The strength of partial D antigen varied within all categories and was most obvious in D^{Va}. Expression of partial D can be affected by the accompanying gene complex. Partial D antigen of D^{Vc} appears stronger than that of D^{Vc}E samples. Variables of antigen (site number, presentation, and accessibility of an epitope) and variables of monoclonal antibody (immunoglobulin concentration, immunoglobulin class, and binding constant) affect reactions of partial D antigens with monoclonal anti-D. Lomas-Francis (personal communication) found that the strength of D antigen expression on weak D and some partial D phenotype RBCs can diminish on storage. Recently, Judd et al. reported on tests performed on partial D RBCs with U.S. FDA-licensed anti-D monoclonal reagents.²⁴ They recommended that tube D tests should not be converted to weak D tests at IAT when performing D typing on samples from pregnant women or potential transfusion recipients.

R₀^{Har}

This is a rare complex that has a weak partial D (DHAR) antigen, very weak e, no G, and low-incidence antigens Rh33 and FPTT. In the original study, only 7 percent of polyclonal anti-D reacted with R₀^{Har} RBCs and R₀^{Har} RBCs were shown to be directly agglutinated by some saline reactive anti-D.²⁵ Several reports of R₀^{Har} producing anti-D are in the literature, including a case of HDN.²⁶⁻²⁸ The R₀^{Har} haplotype comprises only one gene; there is no *RHD* or *RHCE* but only an *RH(CE-D-CE)* hybrid with only exon 5 representing *RHD*.²⁹ The majority of IgM but only a few IgG monoclonal anti-D react with RBCs of the R₀^{Har} phenotype. Although R₀^{Har} is not a common phenotype, it seems that such RBCs have been transfused to D- recipients, yet there are no reports of any adverse effects or stimulation of anti-D resulting from such transfusions.²⁵ The IgM clones GAMA-401 (Gamma reagent), TH28 (Immucor Series 5), and MS201 (Immucor Series 4 and MTS gel cards) will all react with RBCs of the R₀^{Har} phenotype. The MAD2 clone in the Ortho tube reagent does not.²⁴

Crawford

The low-incidence Rh antigen Crawford was reported by Cobb in 1980.³⁰ One lot of a U.S.

FDA-licensed polyclonal anti-D reagent, manufactured by Gamma Biologicals, Inc., was found to contain anti-Crawford. Although Crawford was not proven to be part of the Rh blood group system at that time, there appeared to be a strong association and it was assigned the number Rh43. That would never happen today!!! John Moulds always said it would someday be proven that Crawford was Rh-related and indeed he was correct. Twenty years later the story continues. While investigating 27 samples from October 2000 to May 2002³¹ that gave unexplained reactivity with two commercial monoclonal anti-D reagents from Gamma Biologicals, Inc., and Dominion Biologicals, Ltd. (Dartmouth, NS, Canada), testing showed that most, if not all, of the RBCs were VS+. All 27 samples were from African American persons, except for one from Colombia. After seeing these findings, this author had a revelation!!! Having been at Gamma when the Crawford study was initiated and much testing was performed with the anti-D reagent, a light went on. I literally ran down the hall to Tom Frame's office saying "We need to test Crawford cells"!! Indeed, of the 27 samples that we were able to test, all reacted with the original anti-D plus Crawford reagent. We tested 285 samples from D- African American blood donors from various testing centers (mostly from Tennessee). The RBCs of two donors were found to react with the Gamma monoclonal anti-D reagent and the original anti-D plus Crawford reagent. Interestingly, a total of 500 samples from D- African American blood donors in Houston did not react.

Samples from several of the donors and patients that reacted were sent to Dr. Willy Flegel's laboratory in Ulm, Germany, for molecular studies. A report of the findings will appear in *Transfusion* in 2006.³² It was shown that Crawford is associated with a novel *RHCE* allele, *ceCF*. The Crawford phenotype is present when two amino acid substitutions occur within exon 5, Q (glutamine) at position 233 to E (glutamic acid) and L (leucine) at position 245 to V (valine); the former substitution giving rise to several D-specific epitopes and the latter giving rise to the expression of the VS antigen on these RBCs. The authors proposed that the allele be designated *ceCF*, with *CF* for Crawford. This variant allele was reported by Esteban et al. in 2001 in a pregnant woman from Colombia.³³

A third abstract at the same AABB meeting³⁴ reported on a pregnant woman whose RBCs were originally thought to be R₀^{Har} but were subsequently shown to be of the Crawford phenotype. Two other

abstracts pertaining to Crawford presented data on the molecular background of Crawford occurring with C35 and management of an Rh43 positive donor.³⁶ Westhoff and coworkers³⁵ confirmed the molecular basis for the Crawford antigen and its association with r^{fs} observed in early studies. They raised the possibility that the *RHD-CE-D* hybrid, associated with the r^{fs} haplotype, may contribute to expression of D epitopes in the Crawford background. Slayten et al.³⁶ described a donor with a history of typing as D- at one donor center but on a subsequent donation at another center, the donor's RBCs gave questionable D typing results. The anti-D reagent used at the second center (monoclonal blend from Gamma containing the IgM clone GAMMA401) reacted weakly at immediate spin and did not react at IAT when tested for weak D. Immucor's anti-D reagent did not react with this sample. The donor unit was labeled as D+. The donor was notified of the discrepancy and was told that if the donor was to require a blood transfusion, only D-blood should be transfused. To quote the authors, "The coordination and communication of the donor typing results from the centralized donor testing laboratory and the regional blood center were critical for the correct labeling of the donated products and the management of the donor."

Anti-C

The monoclonal anti-C reagents produced by Gamma, Immucor, and Ortho are all made using the same clone (MS24). The interpretation of test results section of the Gamma package insert for Anti-C (Monoclonal) Gamma-clone (March 2004) states that "The C antigen produced by certain Rh genes may give weaker reactions with Anti-C than is seen with the cells selected as controls. In particular, weaker reactions may be observed (even after incubation of the test) with the C antigen that is a product of r^{fs} (*Cce^s*), R^Z (*DCE*), and r^v (*CE*) genes than the C that is a product of R^1 (*DCE*) and r^1 (*Ce*)." There have been occasions when the Gamma-clone Anti-C reagent reacted weakly and the Immucor and Ortho anti-C reagents did not react. The difference in reactivity between these reagents is because of the second cause of discrepant reactions with monoclonal reagents made from the same clone: differences in the diluents used by the three manufacturers and in potency of the final product. We encountered two situations with r^{fs} and R^N (Rh32).

Cce^s (r^{fs})

RBCs of this phenotype possess a weak C antigen and the low-incidence antigen VS. In Blacks, the VS antigen occurs in 26 to 40 percent; in other populations, it occurs in less than 0.01 percent.³⁷ Recently, the molecular background of VS and weak expression of C in Blacks was published by Faas et al. in the Netherlands.³⁸ They postulated that a hybrid *D-CE-D* transcript, containing exon 4, 5, 6, 7, and (probably) 8 from the *RHCE* gene, is responsible for the weak expression of C in the three donors studied. "The hybrid gene carried a Leu62Phe substitution, as well as the Leu245Val substitution responsible for VS. The gene most probably cosegregates with a *C* allele encoding Cys 16 (normally encoded only by the *C* allele) and Val 245 (responsible for VS antigenicity when encoded by the *RHCE* gene). This explains the combination of weak expression of C and VS positivity that is frequently found in Blacks."

In another report,³⁹ Daniels et al. studied RBCs from 100 Black South Africans and 43 Black persons from the Netherlands. The respective frequencies of all VS+ and of VS+V- (r^{fs}) phenotypes were 43 percent and 9 percent in the South Africans and 49 percent and 12 percent in the Dutch donors. All VS+ donors had G733 (Val245) but six with G733 were VS- (four V+^w, two V-). They concluded that it is likely that anti-VS and anti-V recognize the conformational changes created by Val245 but anti-V is sensitive to additional conformational changes created by Cys336.

R^N (Rh32)

The RBCs of a Gamma employee from Puerto Rico (Puerto Rican and Black ethnicity) phenotyped as D+C+E-c+e+ (probable Rh genotype R,r). However, one of the technologists in the reagent manufacturing department found that his RBCs gave a weaker reaction with the Gamma monoclonal anti-C reagent in titration studies than did another RBC of the same phenotype. The RBCs also did not react with Immucor and Ortho anti-C reagents. The RBCs did not react with anti-VS but reacted with two examples of anti-Rh32.

Rosenfield et al.⁴⁰ first described the R^N gene in a Black family as producing a normal expression of D and traces of C and e, but in some studies, R^N has been shown to produce elevated D antigen expression. It has been estimated that Rh32 is present on RBCs of 1 percent of African Americans. Numerous homozygotes have been found because of anti-Rh46 in their serum and others because of weak C and e antigens.⁴¹

Anti-E

All three commercial anti-E tube reagents and the MTS gel card anti-E available in the United States are comprised of different clones. In the limitations section of the package inserts (Gamma Biologicals, Inc., February 2004; Ortho-Clinical Diagnostics, March 1999), the manufacturers state that variant E antigens, such as E^w, may not react with these reagents. One manufacturer (Gamma, February 2004) goes on to say "this limitation is not unique to monoclonal reagents. It applies equally to some polyclonal Anti-E products." The Gamma reference laboratory investigated a donor sample in 2003 that did not react with all three monoclonal anti-E reagents but did react with three human polyclonal anti-E reagents. An E^w+ RBC gave the same pattern of reactivity but the RBCs of the donor did not react with anti-E^w and must express a different E variant. Molecular studies have not been performed on samples from the donor.

E^w

The low-frequency Rh antigen E^w was first described by Greenwalt and Sanger in 1955,⁴² when an antibody in the serum of a White woman caused HDN. Several other examples followed in the 1960s and 1970s.

E variants

Lubenko and colleagues⁴³ reported on a new qualitative variant of E different from E^u (quantitative variant), depressed (C)D(E) gene complex and qualitative variant E^w and 'partial' antigen E^T. The RBCs of their proposita were agglutinated by eight of 12 polyclonal sera, were weakly agglutinated by two, and not agglutinated by the remaining two. One of three monoclonal anti-E reagents tested by flow cytometry did not react, a second bound strongly, and the third bound weakly. Noizat-Pirenne et al.⁴⁴ reported in 1998 on the molecular basis of qualitative E variants and the same author and other colleagues reported in 1999 on the molecular basis of category EIV variant phenotype.⁴⁵

The first group reported that the E antigen is composed of at least 4 epitopes, proline 226 being necessary but not sufficient for full expression of E antigen. In the second report, they confirmed the heterogeneous molecular background responsible for the E epitope alterations and defined category EIV molecular background. As compared to category EI, EII, and EIII, category EIV appears to possess all 4 epitopes although the expression of some appears to

be suppressed. The decreased expression of some E epitopes in category EIV can be explained by conformational modifications induced by the intracellular amino acid substitution at position 201. As a result, categories EI, EII, and EIII could be considered as partial E phenotypes whereas category EIV could be considered as a weak E phenotype. More recently (2001), a study was undertaken in Japan⁴⁶ that tested monoclonal anti-E with 15 E variant samples. These 15 E variant samples were found in screening 140,723 Japanese blood donors for an incidence of 0.011 percent. A new variant (RHEKH) was found. The variants were subdivided into three types; EFM, EKH, and EKK.

Anti-c

The three anti-c tube reagents in the United States contain different clones but the clone used by Immucor (MS33) is also used in the MTS gel card. In the limitations section of the Gamma package insert for Anti-c (Monoclonal) Gamma-clone (June 2005), it is stated "The monoclonal antibody that is the active ingredient of this product has been observed to show negative reactions with the Rh26 antigen, which is a component that is absent from a rare phenotype reported as representing a variant or partial form of the c antigen. This may introduce discrepant results between those observed with this product and other sources of anti-c. Cells lacking Rh26 may react with some monoclonal anti-c reagents. They can also be expected to show reactivity with most polyclonal anti-c reagents, albeit more weakly than those possessing a normally expressed c antigen. The uncommon phenotype that includes a weak c antigen and no Rh26 may show variable incidence in different populations." Rh26 was first reported by Huestis et al. in 1964.⁴⁷ The patient's RBCs typed as probable Rh genotype R₁R₁ (c-) and the serum contained anti-c but the antibody did not react with the RBCs of an Italian donor who typed c+ with anti-c reagents.

The Gamma reference laboratory investigated a sample from a woman whose RBCs were determined to be Rh:-26 when they typed c+ but her serum contained alloanti-c. Her RBCs did not react with the original anti-Rh26 serum.⁴⁸ More recently, it was shown that these RBCs do not react with the Gamma monoclonal anti-c reagent (clone 951) but that they do react with Immucor (clone MS33) and Ortho (clone MS42) monoclonal anti-c reagents. These anti-c clones were part of a study in 1997 in the Netherlands.⁴⁹

Anti-e

The anti-e reagent of Gamma contains three clones (MS16, MS21, and MS63). One of the clones (MS16) is used as the single clone for the Immucor and Ortho anti-e tube reagents. The MTS gel card uses the same three clones that are in the Gamma tube reagent. Most discrepant reactions will be caused by those samples that react with the Gamma anti-e reagent but do not react with Immucor and Ortho tube anti-e reagents. The limitations section of the Immucor package insert for Anti-C, Anti-E, Anti-c, Anti-e Series 1 (11/01) states "The e antigen may be only weakly expressed on the RBCs of some Blacks and such RBCs may react weakly with anti-e. Some hr^s- or hr^B- RBCs may not react with anti-e. Anti-e may give slightly weaker reactions in the absence of the C antigen."⁵⁰ Three other publications can be consulted for more information on e variants.⁵¹⁻⁵³ The Gamma reference laboratory encountered one sample that did not react with Gamma anti-e blended reagent but did react with the Immucor and Ortho anti-e reagents that contain only one clone (MS16). When the three clones in the Gamma reagent were tested separately, the MS16 clone did react with the RBCs but the other two clones did not. When MS16 was blended with the other two clones to make the Gamma anti-e reagent, the potency of the clone was weakened, causing the reagent to not react with what appeared to be a different e variant. Molecular studies have not been performed on this sample.

Anti-K

Immucor and Gamma manufactured monoclonal anti-K reagents that contain the same clone (MS56). The RBCs of a donor that originally typed as K- with a monoclonal anti-K reagent were found to react with a human polyclonal anti-K reagent. Serologic studies indicated that the RBCs from the donor had weakened or altered expression of K antigen. Molecular analysis has not been completed. Skradski et al.⁵⁴ reported in 1994 on a K variant and summarized previous reports of weakened or variant expression of K. Poole and coworkers⁵⁵ reported on serologic and molecular studies on the RBCs of two Swiss blood donors. Their RBCs reacted with various anti-K but did not react with monoclonal anti-K MS56 clone.

Anti-M and Anti-N

Gamma and Immucor both have monoclonal anti-M reagents and use different clones. The Gamma clone

(M2A1) is IgG1 and the Immucor clone (F23) is IgM. In the limitations section of the Gamma package insert for Anti-M (Murine Monoclonal) Anti-N (Murine Monoclonal) Gamma-clone, it states "Variant sialoglycoproteins exist that may, on relatively uncommon occasions, cause aberrant reactions when testing human red blood cells with Anti-M and Anti-N reagents (and also with Anti-S and Anti-s). The presence of an N-like antigen ('N') on glycophorin B (the S/s sialoglycoprotein) is well known to be the cause of weak reactivity between N- cells and Anti-N reagents, which may be mistakenly attributed to the presence of the N antigen itself...when the level of glycophorin B is increased, as occurs in the case of some hybrid sialoglycoproteins, the resulting enhanced expression of 'N' may for all practical purposes be indistinguishable from normal N. Such enhanced expression of 'N' has been noted as a feature that accompanies the low-incidence antigen Dantu, and also occurs with cells classified as Mi III in the Miltenberger subsystem."⁵⁶

A different type of situation arose when the Gamma reference laboratory examined the RBCs of a 1-year-old child whose serum contained anti-M but whose RBCs reacted with the Gamma human polyclonal anti-M reagent and did not react with the Gamma monoclonal anti-M reagent. In this case, the monoclonal reagent gave the result consistent with the antibody in the serum. It turned out that the Gamma human polyclonal anti-M reagent contained anti-Tm, which is directed at a low-incidence antigen associated with the MNS blood group system. The human anti-M reagent is tested at 4°C and the anti-Tm in the reagent was very weak. It is well known that manufacturers cannot eliminate the possibility that human polyclonal reagents might contain antibodies directed at low-incidence antigens and testing on the reagents is only done with common antigens with a frequency of 1 percent or greater; i.e., C^w, Kp^a, Js^a, etc.

Anti-Le^a and Anti-Le^b

Immucor and Ortho use the same clones for their anti-Le^a and anti-Le^b reagents. These reagents are very temperature sensitive and the manufacturers' package inserts (directions for use) should be followed closely as to optimum temperature and incubation time for each reagent. Gamma uses different clones.

A weak positive reaction with a monoclonal Anti-Le^b reagent and Le(a+) RBCs of any ABO group should not be considered negative as this may represent the

Le(a+b+) phenotype. The evaluation of these unusual reactions may require secretor studies. The Le^b reaction of Le(a+b+) RBCs may range from very weak to strong. The Le(a+b+) phenotype is common in Orientals and Polynesians but uncommon in Europeans. It occurs in persons of African origin but there is no data on the incidence. The Le(a+b+) phenotype has been established by serologic, immunochemical, and genetic means.⁵⁷ The strength of the Lewis antigens may be diminished during pregnancy and with newborns⁵⁸ or in patients with cancer and other diseases.⁵⁹

Anti-P1

Gamma manufactures the only FDA-licensed monoclonal anti-P1 reagent, using clone OSK17. In the interpretation of test results in the package insert for Anti-P1 (Murine Monoclonal) Gamma-clone (March 2003), there is a special note that states "The monoclonal Anti-P1 reagent is somewhat more potent than most polyclonal reagents of the same specificity. This may result in an unexpected positive reaction when the cells being tested have a comparatively weak P1 antigen that is not being reliably detected by polyclonal reagents."^{60,61}

Monoclonal Antihuman Globulin Reagents

Anti-IgG

Gamma manufactures a monoclonal anti-IgG reagent using clone 16H8. This clone is also used in the polyspecific AHG reagent along with a monoclonal anti-C3b,-C3d. Immucor no longer makes a rabbit anti-IgG reagent and Ortho manufactures a rabbit tube anti-IgG reagent. The Ortho polyspecific AHG reagent is a blend of rabbit anti-IgG and monoclonal anti-C3. The anti-IgG clone 16H8 in the Gamma reagents reacts with an epitope on the CH3 domain of the Fc region of human IgG. Examples of pure IgG4 subclass antibodies may not be detected by this reagent. Pure IgG4 antibodies are very uncommon. They have not been reported to cause hemolytic transfusion reactions or HDN. Autoanti-JMH is the one most often seen. All except five of seven JMH antibodies, one of one anti-Rg, and two of nine Yt^a antibodies were detected by the monoclonal AHG reagent.⁶²

Bell and Johnson reported on the use of the monoclonal anti-IgG and PEG to reduce the detection of "high-titer, low-avidity" antibodies.⁶³

Anti-C3b,-C3d

Gamma and Ortho manufacture monoclonal anti-complement reagents using different clones; Immucor does not make this reagent. In the Gamma package insert (February 2005) under the test method section for this reagent there is a statement "Note: Weak anti-complement reactions are often enhanced if the washed cells and Anti-C3b,-C3d are incubated for a short period before centrifugation. A delay of five minutes before proceeding to Step 5 may improve the detectability of a weak C3 coating, although the greater potency of this Anti-C3 reagent over conventional ones generally results in better reactivity at the immediate-spin phase."⁶⁴

Additional Reading

Three other references on monoclonal antibodies and unusual samples can be consulted for more extensive information. The wonderful, **heavy**, blue book *Applied Blood Group Serology*, Fourth Edition, by Peter D. Issitt and David J. Anstee, has a wealth of information on serologic and molecular findings with monoclonal antibodies.⁶⁵ Unfortunately, I have heard that it will not be printed again but fortunately that means we can copy it for our own use! Good luck copying all those pages (1208 in all)! Connie Westhoff wrote an excellent review of the Rh blood group system and summarized serologic and molecular analysis of weak D, partial D, weak C, e variants, etc.⁶⁶ Her list of references includes numerous recent publications on molecular analysis of Rh variants. And, in *Immunohematology*, she wrote a very comprehensive and practical review of the Rh blood group D antigen.⁶⁷ And, finally, I wrote an article for *Advance* magazine on monoclonal antibodies as blood bank reagents with special reference to ABO, D, C, and e, and monoclonal AHG reagents.⁶⁸

Summary

In this review I have attempted to give a summary of recent serologic and molecular studies on unusual samples detected when using monoclonal antibodies as blood bank reagents. It was not possible to list every publication but I tried to list recent papers, which often referred to earlier papers that laid the foundation for work being performed today. In general, reagents made with different monoclonal antibodies give reactions that are the same as or similar to reagents manufactured from human, animal, or lectin (seed) source material. It is when unusual or rare samples are

encountered that users of monoclonal reagents need to know how each reagent from different sources or manufacturers performs. I also selected the samples that most often gave discrepant results with monoclonal reagents. There are many others that I have not covered and the interested reader is directed to the various references for further information.

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Rare blood donors: a personal approach

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The National Blood Group Reference Laboratory (NBGRL) in Israel was established in Jerusalem in 1971 and transferred to Magen David Adom (MDA), National Blood Services in 1995. This laboratory was the inspiration of the first author of this article for over 30 years. The realization of this vision was made possible by the cooperation of colleagues and laboratory workers in blood transfusion services throughout the country. The aim of the service was to provide diagnostic help in resolving immunohematologic problems found in the blood banks and clinics in Israel. In the beginning, only a part-time technician performed the work and testing was done using very limited reagents. The service was expanded by personal visits to all of the 22 blood banks in Israel to explain the aim of this new service and to educate them about the importance of resolving each and every case. One major issue was the cost involved in referring problems but it was decided at the outset that these would be covered by the government to ensure that a workup would be performed for all referred cases. The expansion of the service could not have been achieved without the help of the SCARF program. This voluntary service enabled us to identify the first rare donors in Israel, resolve complex cases, and find compatible blood for our patients. To illustrate the importance of the NBGRL in Israel and the rapid resolution of cases referred, several individual stories are described. The purpose of this review is to show the importance of the NBGRL in identifying rare blood groups and in providing and coordinating services and the importance of keeping in close contact with the rare donors to encourage and promote their donations, which may save lives. *Immunohematology* 2006; 22:64–68.

Key Words: rare blood donors, NBGRL, SCARF, personal approach

The Gerbich (Ge) Story

Two of the earliest samples that we received from SCARF were from a donor whose RBCs were Ge:-2,3 (Yus phenotype) with anti-Ge2 in the serum and a donor whose RBCs were Ge:-2,-3 (Gerbich phenotype) with anti-Ge2,3 in the serum.

In 1976, a sample from a Jewish blood donor (ZY) born in Iraq, whose ABO blood group could not be resolved, was referred from Magen David Adom (MDA) National Blood Services. This donor had never been transfused and no antibodies had been detected in his serum in previous donations. His RBCs typed as group A, D+ and the serologic workup demonstrated the presence of an antibody in his serum directed at a high-

incidence antigen. The RBCs of the donor were subsequently shown to be Ge:-2,3 (Yus phenotype) and his serum contained anti-Ge2. These findings were proved using reagents provided by SCARF. They were confirmed at that time by overseas laboratories including that of the Medical Research Council Blood Group Unit (MRC BGU) in London, then directed by Dr. Ruth Sanger. The family of this donor was examined and it was found that the donor was married to a first cousin whose RBCs were shown to be Ge:2,3,4. However, three of his children were found to be Ge:-2,3 like him. The propositus was employed as a mounted policeman in Jerusalem and was frequently seen riding on his white horse by the staff of the laboratory where the diagnosis of his rare blood was established. Because of the potential injury involved in his work, it was decided to relocate him within the service to a more sedentary position. However, the donor later decided that he wished to return to his post working with the horses in spite of the risks involved. He and one of his sons became regular donors and their units were frozen in MDA for future use. When this son moved to live in the United States, he continued to donate blood there.

In subsequent years, 20 other cases of donors with anti-Ge2 were seen in our laboratory (Table 1); most of them were found in Jews who emigrated from Ethiopia and in Israeli Arabs. In the Ethiopian Jews in Israel, the Yus phenotype (Ge:-2,3,4) was found and the frequency of RBCs with the Ge:-2,3 phenotype may reach 0.015 to 0.1 percent.^{1,2} Only two cases of donors with Gerbich phenotype Ge:-2,-3,4 were found, in one family who emigrated from Iraq.

We have not succeeded in convincing the other Gerbich negative individuals to become regular donors despite numerous letters, telephone calls, and even an invitation to the rare blood meeting, which we will describe later.

Table 1. The Gerbich negative phenotype in Israel

Number	Reason for referral	Sex	Country birth/origin	Gerbich groups	Gerbich antibodies
1a	Blood donor	M	Iraq	Ge:-2,3	anti-Ge2
1b	Family screening	M	Israel	Ge:-2,3	No antibody
1c	Family screening	F	Israel	Ge:-2,3	No antibody
1d	Family screening	F	Israel	Ge:-2,3	No antibody
2	Pregnancy	F	Ethiopia	Ge:-2,3	anti-Ge2
3	Pregnancy	F	Ethiopia	Ge:-2,3	anti-Ge2
4	Blood donor	M	Ethiopia	Ge:-2,3	anti-Ge2
5a	Pregnancy	F	Ethiopia	Ge:-2,3	anti-Ge2
5b	Family screening	F	Ethiopia	Ge:-2,3	anti-Ge2
6a	Pretransfusion	F	Iraq	Ge:-2,-3,4	anti-Ge2
6b	Family screening	F	Iraq	Ge:-2,-3,4	No antibody
7	Pretransfusion	F	Ashkenazi	Ge:-2,3	anti-Ge2
8	Donor screening	F	Ethiopia	Ge:-2,3	No antibody
9	Pregnancy	F	Ethiopia	Ge:-2,3	anti-Ge2
10	Pretransfusion	F	Iran	Ge:-2,3	anti-Ge2
11	Pregnancy	F	Ethiopia	Ge:-2,3	anti-Ge2
12a	Pretransfusion	F	Israeli Arab	Ge:-2,3	anti-Ge2
12b	Family screening	F	Israeli Arab	Ge:-2,3	anti-Ge2
13	Blood donor	M	Israeli Arab	Ge:-2,3	anti-Ge2
14	Blood donor	M	Israeli Arab	Ge:-2,3	anti-Ge2
15a	Pregnancy	F	Ethiopia	Ge:-2,3,4	anti-Ge2
15b	Family and pregnancy	F	Ethiopia	Ge:-2,3,4	anti-Ge2
16a	Pretransfusion	M	Israeli Arab	Ge:-2,3	anti-Ge2
16b	Family screening	M	Israeli Arab	Ge:-2	No antibody
17	Dialysis	F	Ethiopia	Ge:-2,3	anti-Ge2
18	Pretransfusion	M	Israeli Arab	Ge:-2,3	anti-Ge2
19a	Pregnancy	F	Israeli Arab	Ge:-2,3	anti-Ge2
19b	Family	F	Israeli Arab	Ge:-2,3	No antibody
19c	Family	F	Israeli Arab	Ge:-2,3	anti-Ge2
19d	Family	M	Israeli Arab	Ge:-2,3	anti-Ge2
20	Blood donor	M	Israeli Arab	Ge:-2,3	anti-Ge2

The JMH Story

Over the years, the National Blood Group Reference Laboratory (NBGRL) in Israel has identified five samples of blood that appeared to be JMH-. Two of these samples were subsequently found to be JMH variants (Table 2).

In 1980, a woman (MR) was admitted for surgery. An antibody directed at a high-incidence antigen was identified in her serum and no compatible blood units were found for transfusion. Additional testing at the NBGRL showed that this antibody did not react with enzyme (ficin)-treated RBCs. The identity of this antibody could not be established in our service. The

RBCs of the patient reacted with anti-JMH. Samples of blood from this woman were sent to several overseas laboratories and an immediate reply was received from the former Gamma Biologicals, Inc., Consultation and Education Services (headed by Marilyn and John Moulds), notifying us that the antibody maker had a JMH variant phenotype. The RBCs of MR reacted with known anti-JMH but her serum did not react with known JMH- RBCs. Family screening identified a brother of the same ABO group and of the same JMH variant, so he could have been a potential donor if blood had been required for the surgery. This Jewish family immigrated to Israel from Poland. JMH variant antibodies are not considered to be clinically significant.³

Some time after this JMH variant (which was called MR)⁴ was found, an additional JMH variant was examined in a patient who had a terminal illness. This variant was identified as being the same variant as that found on the RBCs of MR. This patient was also a Polish Jewish immigrant.

Many years later Dr. Axel Seltsam described the molecular background that underlies the molecular basis of the JMH variants.⁵

Table 2. The JMH variants in Israel

Number	Reason for referral	Sex	Country birth/origin	JMH groups	JMH antibodies
1a	Pretransfusion	F	Poland	JMH variant	Anti-JMH variant (MR)
1b	Family screening	M	Poland	JMH variant	Anti-JMH variant (MR)
2	Pretransfusion	M	Poland	JMH variant	Anti-JMH variant (MR)

The pp (Tj[a-]) Story

The first finding of a rare blood group in the NBGRL in Jerusalem, which proved to be the pp phenotype, was in 1972.⁶ The blood was from a woman who had been hospitalized for bleeding after a miscarriage. The referring laboratory told us that the RBC typings of the patient tested as group B but the back tests "caused problems!" All RBCs (groups A, B, and O) were agglutinated and, within a short period, these RBCs hemolyzed in tube tests. We had never seen a blood specimen from a pp phenotype patient but this story sounded as though this was the problem. Fortunately, no immediate transfusion was required and the blood specimen was referred to the International Blood Group Reference Laboratory (IBGRL) in the United Kingdom for confirmation. Just before we received the answer to confirm these suspicions, the

patient was interviewed and she told the following story: She had previously been admitted to another hospital when she bled on a number of occasions in the first trimester of her pregnancies. She had only one live child. The previous hospital had told the patient that they were unable to find compatible blood for her and if she was readmitted and hemorrhaged she might die! On questioning the woman further, two important findings emerged. She had a sister living in Israel, who had the same obstetric history and who nearly died after a transfusion. She also told us that she had a cousin living in Israel who was said to have a rare blood type. We were able to follow up with this latter relative and we found that her blood had previously been referred to the MRC BGU, at that time directed by Rob Race, where her blood was found to be group AB, pp.⁷ A small sample of serum from this patient had been stored in a freezer of the referring laboratory and the RBCs of our patient did not react with this serum! The following day we received the confirmation from the IBGRL in the United Kingdom and they arranged for two units of blood to be sent from Umea, Sweden, for our patient. These donors, who were twins, were found in a dance hall and they immediately donated their blood to be dispatched. A few years later, a Swedish newspaper heard this story and decided to bring the recipient and these donors together. The donors were flown to Jerusalem and the recipient arranged a party for them. The group met and communicated with the help of translators.

As soon as our patient was well enough, she began to donate her blood for freezing and she has donated regularly nearly every 3 months. Here was a great ending to a difficult problem. This lady told us, more than once, that after her blood group had been determined and she donated her blood for freezing, she could now go to bed and sleep easily without any worry of what might happen if she ever needed a transfusion.

Our second example of pp phenotype blood was found in a blood donor. He was group AB but unfortunately we were never able to follow up on this young man. It turned out that he had been adopted and did not want to know more relating to his rare blood group and we lost track of him.

The next family turned out to be very special.⁸ This patient was being prepared for a cesarean section and no blood could be found for her. Her blood typed as group B, D+, pp. This was the same blood group as our first patient and it was agreed that some of the frozen units of our first patient would be available if needed.

This new patient also had a history of numerous miscarriages and no live child.

Immediately, the family was investigated and two siblings were found to have RBCs of the pp phenotype. Her brother was group B and her sister was group O. The patient required transfusion, as she had a placenta accreta, and, after delivery of a healthy baby, had to have a hysterectomy. At that time she received one unit donated by her brother. The baby soon developed HDN and needed an exchange transfusion. The sister of the proposita was group O and her blood was used for the exchange transfusion for the baby.

The story relating to the sister was equally interesting.⁹ This woman wanted to have a child but she was unable to continue her pregnancies past the first trimester: a classic story for a woman whose RBCs were of the pp phenotype. For a number of years, this woman called to inquire if any advances to help her have a child were known. The situation was critical for her and for her marriage, as both parents wanted to have children. One day, the patient called saying that she had heard on a midnight radio newscast that a woman in a hospital in Baltimore (who had a story similar to hers) had just delivered her first live child and she wanted to know if this advance could be applied to her situation. Dr. Paul Ness in Baltimore was immediately approached and, indeed, his patient had a similar blood group. With regular plasmapheresis as an inpatient, she was able to continue the pregnancy past the first trimester and she delivered a live child. The protocol used in Baltimore was performed on our patient at the Rambam Medical Center in Haifa, Israel. There were many problems but the patient finally delivered a live, healthy, full-term child who is now a young adult. The meaning of the name of the doctor who had engineered the treatment of the case in Baltimore, Dr. "Ness," in Hebrew translates as "miracle," and for our patient the birth of her child was indeed a miracle.

This story is special in that any transfusion problems in the family were overcome by the cooperation of the siblings. These three siblings have continued to donate their blood for storage in our frozen blood bank.

Over the following years we continued to see more cases of individuals who were of the special group pp. We noted that a great percentage of these patients were immigrants from North Africa, especially from Morocco, where the frequency of the pp phenotype reaches 1 in 55,663.¹⁰

The Anti-Vel Story

The first example of anti-Vel, sent to us in 1976, was in the serum of a pregnant woman who had been admitted to a local hospital for delivery. The hospital was unable to find compatible blood for her before the delivery. The specimen arrived in the NBGRL on a Friday and the urgency for an answer meant that the staff had to stay on to find a solution. We were doubtful that we could solve this case but in the middle of the night we found one last rare RBC sample from SCARF in our collection that lacked the high-incidence antigen Vel. To our surprise, this RBC sample was compatible with the serum of this patient. At that time, there was no blood in Israel and no known blood donors whose RBCs were Vel-. As this woman was near term, the decision was made with the blood bank director and the gynecologists to take an autologous unit from the patient and, after a few days, to take a second unit before her delivery. Near the end of the first donation the patient did not feel well and we positioned her in the Trendelenburg position, only to find that this did not really help her. We realized almost immediately that, in this position, the uterus was pressing on her chest, restricting her air entry, and so we reversed the position. At once the patient felt well and the phlebotomy of the blood unit was completed successfully.¹¹

Through the WHO International Rare Donor Panel in the United Kingdom, we contacted the Red Cross Blood Bank in Toronto, Canada, and arranged to have two units of this rare blood shipped to Israel. Fortunately, the woman delivered a healthy baby and she only received her own predonated blood units. One first cousin was found to be Vel- in the family investigation.

Three additional examples of anti-Vel have been seen over the years and hemolysis was seen with enzyme (ficin)-treated RBCs in all of them.

The Cartwright (Yt) Story

The following summary of the Yt blood group shows how the NBGRL must look out for findings that could have bearing on the distribution of any particular blood group and consider the value of investigating them in the population.

Anti-Yt^a was first detected in the NBGRL in Jerusalem in 1974. Over the subsequent 12-year period, 14 people who had anti-Yt^a in their serum were found among 4470 referrals. This frequency of 1 in 320 was considered much higher than would have been

expected. To investigate the Yt^a and Yt^b frequencies in Israel, anti-Yt^a and anti-Yt^b reagents were required. Anti-Yt^a was available but we needed a supply of anti-Yt^b. A sample of anti-Yt^b was offered by Marilyn and John Moulds, which enabled the testing of the Yt groups of Israeli Jews, Arabs, and Druze. These tests showed a high frequency of the Yt^b allele, which explained the relatively high frequency of samples with anti-Yt^a in our population. The Yt^b allelic frequencies ranged between 0.1005 and 0.1522 in the Jewish communities and were 0.1294 and 0.1429 in the Arab and Druze communities, respectively.^{12,13} These are the highest Yt^b allelic frequencies observed so far in any population tested,⁴ which explains why anti-Yt^a is the most frequently detected antibody directed at a high-incidence antigen in the NBGRL.

In retrospect, it is interesting that the late Dr. Lyndall Molthan (in a written communication) had written that she considered anti-Yt^a as "The Jewish Connection"!

First Rare Blood Donor Meeting

For many years, we considered setting up a meeting of our rare blood donors with the staff of the NBGRL; this finally took place in April 2002. A special program was prepared and invitations sent out. The venue was the MDA National Blood Services and the attendance far exceeded our expectations, showing us that our rare donors understood the importance of this gathering. Each person was registered and received a badge and a folder of detailed information about their blood group. Also included was a request to give permission to be listed on the International Rare Donor Panel managed by the IBGRL in the United Kingdom, to which 43 donors agreed. The meeting began with refreshments to enable the mingling of donors and staff. The more formal program included lectures and active participation of a number of individual rare donors to talk about their personal stories and problems. Throughout the whole meeting our donor room remained open for the rare blood donors and their families to donate. Thirty-two rare donors donated blood and 15 samples were drawn from family members for testing.¹⁴

Today, we have 566 registered rare blood donors, some unique in the world, and more than 1200 frozen units of rare blood are stored in high glycerol solution in freezers at -80°C.

Our beginnings were modest. The service was started in one small room with one physician and one

laboratory worker employed for half of a day. We began with minimal reagents, test tubes, and racks; a simple centrifuge; a refrigerator; and one freezer. It did not take us long to realize how many rare blood groups came to light. This is certainly due to the ingathering of the exiles to Israel from a multitude of different ethnic backgrounds. Clearly, there has been a necessity for additional help for the NBGRL in Israel. This help has always been willingly extended by SCARF and by many colleagues and laboratories all over the world; they examine referred samples of blood and give their advice. If we tried to list and thank all we would surely leave some out, but we do want to mention and thank Marilyn and John Moulds from the former Gamma Biologicals, Inc.; the then MRC BGU in London directed by the late Rob Race and Ruth Sanger and by Patricia Tippet; and the WHO IBGRL in the United Kingdom, managed by Carolyn Giles and later by Joyce Poole and Marion Reid from the New York Blood Center. Their help has proved invaluable to us, to the patients, and to the field of immunohematology in general.

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Case report: DNA testing resolves unusual serologic results in the Dombrock system

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Typing for antigens in the Dombrock blood group system and identifying the corresponding antibodies are notoriously difficult tasks. The reagents are scarce and the antibodies are weakly reactive. When RBCs from family members of a patient with an antibody to a high-prevalence Dombrock antigen were tested for compatibility, an unusual pattern of inheritance was observed: RBCs from the patient's children and one niece, in addition to those from some of the patient's siblings, were compatible. This prompted the performance of DNA-based assays for *DO* alleles and the results obtained were consistent with and explained the compatibility test results. It was possible to study this large kindred because of the cooperation of family members, hospital personnel, and reference laboratory staff. *Immunohematology* 2006;22:69–71.

Key Words: blood groups, Dombrock, transfusion medicine, DNA-based assay, molecular typing

Antibodies to antigens in the Dombrock blood group system are likely to be more clinically significant than is currently documented. This is because of the lack of typed RBCs on antibody identification panels, a dearth of monospecific antibodies with a reasonable strength of reactivity, and the absence of *in vitro* characteristics that are usually associated with delayed hemolytic transfusion reactions.¹ The cloning of the gene for Dombrock and the identification of the molecular basis associated with Dombrock antigens provide other means by which to study them.^{2–5}

The *DOA* and *DOB* alleles can be distinguished by a mutation at nucleotide (nt) 793(A>G) of *DO*, which is predicted to encode a change of Asn265Asp. The Hy- phenotype is associated with a change of G>T at nt 323 (Gly108Val). The *HY* allele also carries 793G, which explains why the Hy- phenotype is invariably Do(a-b+). The Jo(a-) phenotype, encoded by *JO*, is associated with a single-nucleotide change of 350 C>T (Thr117Ile). 350T is on an allele carrying 793A and most Jo(a-) phenotype RBCs are Do(a+).¹

We describe here the use of PCR amplification followed by restriction fragment length polymorphism (RFLP) analyses to resolve unusual serologic results in

a patient with an antibody to a high-prevalence Dombrock antigen.

Case Report

A 55-year-old, group B, D+ African American woman was hospitalized with congestive heart failure (CHF) and anemia. Her antibody screen was negative and she received two RBC transfusions and was discharged with a Hct of 29.6%. Tests performed to identify the cause of the anemia were within the normal range for each test and there were no signs of bleeding or hemolysis. Eight days later, the patient was readmitted with CHF and a Hct of 27%. Two units were ordered but the antibody screen was positive (2+) by the IAT. The antibody reacted with all RBCs tested except a Hy- sample. After 6 days in the hospital, she was discharged with a Hct of 24.7%. Twelve days later, she was readmitted with CHF again, chronic renal failure, and unstable angina. While the antibody was being investigated, the primary care physician was informed that compatible blood was not available and was asked to determine if the patient had siblings. The patient's son arranged for five siblings and 13 other relatives to have blood samples collected for testing with the patient's serum. Two ABO-compatible siblings were crossmatch compatible as were three of her five children and one niece. Compatible blood was transfused without incident. Difficulties with antibody identification, lack of sufficient volumes of anti-Hy and anti-Jo^a, and the unusual inheritance pattern prompted us to perform DNA analysis.

Materials and Methods

Blood samples were collected from consenting family members. The IATs were performed by hemagglutination in tubes.

Table 1. Primers used for PCR-RFLP analyses

Primer	Sequence 5' to 3'	Uncut size	Restriction enzyme (nt)	Restriction fragment size (allele)	
DoF DoR	TACCTCACCTCAGCAATCCAGCTGCTGAGGAGAGAC TTTAGCAGCTGACAGTATATGTGCTCAGGTTCC (annealing temperature 62°C)	368 bp	BseI I (nt 793)	326, 42 (DOA)	268, 58, 42 (DOB)
DoX2F Do378R	TCAGTACCAAGGCTGTAGCA AGTAAAGTCAGAATGAACATTGCTGCACAAT (annealing temperature 58°C)	220 bp	BsaI I (nt 323) Xcm I (nt 350)	120, 92, 8 (wild type) 167, 53 (wild type)	212, 8 (HY) 220 (JO)

Genomic DNA was extracted using the QIAamp DNA Mini Blood Kit (QIAGEN, Valencia, CA). PCR was performed using the following conditions: 100 ng of each primer, 200 μ M of each dNTP, 2.5mM (for nt 323 and nt 350 of *DO*) or 3.0mM MgCl₂ (for nt 793 of *DO*), 1.0 U HotStar Taq DNA polymerase (QIAGEN), and buffer in a total volume of 50 μ l. Amplification was performed in the 9700 thermal cycler (Perkin Elmer, Norwalk, CT) with the following profile: 95°C for 15 minutes; followed by 35 cycles of 94°C for 20 seconds; 55°C (for nt 323 and nt 350 of *DO*) or 62°C (for nt 793 of *DO*) for 20 seconds and 72°C for 20 seconds; then 72°C for 7 minutes.⁵ PCR products were analyzed by electrophoresis in 1% agarose gel.

PCR-RFLP assays for these three polymorphisms were performed using *BsaI*I for the polymorphism at nt 323, *XcmI* for the polymorphism at nt 350, and *BseI*I for the *DOA/DOB* polymorphism at nt 793.^{3,6} The sequence of primers, PCR annealing temperature, restriction enzyme used to digest each PCR-amplified product, and expected restriction-fragment sizes are given in Table 1. Digested products were analyzed by electrophoresis on an 8% polyacrylamide gel.

Results

The results of PCR-RFLP analyses of the three *DO* single nucleotide polymorphisms (SNPs) are shown in Table 2. The patient (II-10), who had one *HY* and one *JO* allele, is predicted to have the phenotype Do(a+^wb+^w) Hy+^w Jo(a-) and to have produced anti-Jo^a. Her two compatible siblings (II-4 and II-7) and one compatible child (III-9)

were *JO/JO*. The other two compatible children (III-8 and III-11) were *HY/JO* and the compatible niece (III-2) was *HY/HY*. Thus, RBCs from all six compatible family members are predicted to be Jo(a-). Of the ABO-compatible but crossmatch-incompatible family members, each had one *DOA* or *DOB* allele together with either an *HY* or a *JO* allele. A summary of ABO types, compatibility testing with the patient's serum by the IAT, and *DO* alleles is given in the pedigree (Fig. 1).

Discussion

The cooperation among family members, hospital personnel, and reference laboratory staff made it possible not only to provide blood for the patient but also to study this large kindred by PCR-based methods. The presence of combinations of *DOA*, *DOB*, *HY*, and *JO* alleles was consistent with the compatibility testing

Table 2. Results of PCR-RFLP analyses

Identification	323 (G>T) Hy+>Hy-	350 (C>T) Jo(a+)>Jo(a-)	793 (A>G) Do ^a /Do ^b	Alleles	Predicted phenotype
II-2	G	T	A	<i>JO/JO</i>	Do(a+b-) Hy+ Jo(a-)
II-4	G	T	A	<i>JO/JO</i>	Do(a+b-) Hy+ Jo(a-)
II-6	G/T	C	A/G	<i>DOA/HY</i>	Do(a+b+W) Hy+W Jo(a+W)
II-7	G	T	A	<i>JO/JO</i>	Do(a+b-) Hy+ Jo(a-)
II-9	G/T	C	A/G	<i>DOA/HY</i>	Do(a+b+W) Hy+W Jo(a+W)
II-10	G/T	C/T	G/A	<i>HY/JO</i>	Do(a+Wb+W) Hy+W Jo(a-)
II-11	G	C/T	G/A	<i>DOB/JO</i>	Do(a+ W b+) Hy+ Jo(a+W)
III-1	G	C/T	G/A	<i>DOB/JO</i>	Do(a+ W b+) Hy+ Jo(a+W)
III-2	T	C	G	<i>HY/HY</i>	Do(a-b+W) Hy- Jo(a-)
III-4	G/T	C/T	G/A	<i>HY/JO</i>	Do(a+Wb+W) Hy+W Jo(a-)
III-5	G	C/T	G/A	<i>DOB/JO</i>	Do(a+Wb+) Hy+ Jo(a+W)
III-6	G/T	C	G	<i>DOB/HY</i>	Do(a-b+) Hy+ Jo(a+W)
III-8	G/T	C/T	G/A	<i>HY/JO</i>	Do(a+Wb+W) Hy+W Jo(a-)
III-9	G	T	A	<i>JO/JO</i>	Do(a+b-) Hy+ Jo(a-)
III-11	G/T	C/T	G/A	<i>HY/JO</i>	Do(a+Wb+W) Hy+W Jo(a-)
III-12	G	C/T	G/A	<i>DOB/JO</i>	Do(a+Wb+) Hy+ Jo(a+W)
IV-1	G/T	C	G	<i>DOB/HY</i>	Do(a-b+) Hy+W Jo(a+W)
IV-2	G/T	C	G	<i>DOB/HY</i>	Do(a-b+) Hy+W Jo(a+W)
IV-3	G	C/T	G/A	<i>DOB/JO</i>	Do(a+Wb+) Hy+ Jo(a+W)

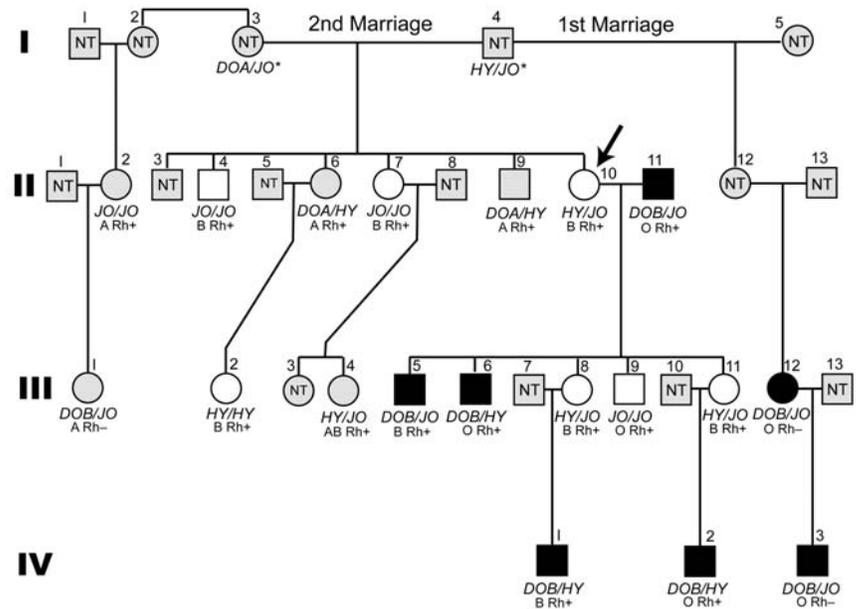


Fig. 1. Pedigree of the family studied. The ABO and Rh types and DO alleles are indicated under the family members who were tested. * = presumed; NT = not tested. Circles represent women and squares represent men. Open symbols indicate serologically compatible family members, black symbols indicate ABO-compatible but serologically incompatible family members, and grey symbols indicate either ABO-incompatible family members or those from whom blood samples were not obtained.

results and provided an explanation for the initial, apparently unusual inheritance pattern of compatible donors. This study also revealed a surprisingly high number of negative alleles (*HY* or *JO* or both) in one kindred.

The Do status of the RBC samples could not be determined because of the lack of appropriate antibodies. This study highlights the value of using PCR-based analyses in conjunction with classic hemagglutination. This is particularly relevant when studying blood group antigens that are expressed weakly and when reagents are scarce. As we have previously advocated,^{1,7} PCR-based analyses can be invaluable for typing reagent RBCs and for screening for antigen-negative donors.

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Problems highlighted when using anticoagulated samples in the standard tube low ionic strength antiglobulin test

A.J. SWEENEY

Within the UK blood transfusion services, there is currently no recommendation for the use of either clotted or anticoagulated samples for antibody identification testing. This report describes three cases in which the detection of IgM antibodies was impeded by the use of anticoagulated samples. Two patient samples, referred for compatibility testing, were both identified as having IgM complement-activating anti-S and the remaining case involved an antenatal patient with IgM complement-activating anti-Vel. In all three cases, the coincidental referral and investigation of both clotted and anticoagulated samples led to the discrepancy in serum and plasma test results becoming apparent. Potential errors in selection of suitable blood for transfusion and appropriate antenatal management were avoided by correct identification of the antibodies present using the clotted samples. *Immunohematology* 2006;22:72-77.

Key Words: antibody detection, plasma or serum, anti-S, anti-Vel, antiglobulin test

The suitability of anticoagulated or clotted samples for pretransfusion testing has been the object of investigation.¹ Kidd antibodies, in particular, have received close scrutiny due to their association with delayed hemolytic transfusion reactions, their ability to activate complement, and the earlier reported difficulty of their detection in systems with reduced sensitivity to complement-activating antibodies.² It is widely accepted that, given the improvements in antibody detection by the antiglobulin test (AGT) resulting from the use of different enhancement media (e.g., LISS, polybrene, and PEG) and the advances in AGT technology (gel and bead techniques), the necessity to detect complement activation by capable IgG antibodies has been reduced and possibly eliminated.³ The general conclusion is there is no significant difference in detection rates when using anticoagulated or clotted samples.⁴

The Welsh Blood Service (WBS) laboratories provide an antenatal antibody screening service for seven hospitals within the region as well as a reference service for serologic investigations for 15 hospitals. The majority of hospital blood banks served use automated sampling systems for RBC typing and antibody investigations and, consequently, use anticoagulated samples.

Subsequently, anticoagulated samples were increasingly referred to the WBS for routine antenatal and patient antibody investigations. Reported here are three cases that serve to emphasize the limitations of anticoagulated samples used by the current testing protocol of the WBS when performing RBC antibody investigations. In each case, the detection of an IgM complement-activating antibody was impeded by the use of anticoagulated samples.

Materials and Methods

Routine tests for antibody identification were performed; they included the standard tube AGT using 1.5% LISS-suspended RBCs incubated at 37°C for 30 minutes, an agglutination test using PBS-suspended RBCs incubated at 18°C for 60 minutes, and a two-stage prepapainized agglutination test incubated at 37°C for 30 minutes. DiaMed column gel ID AGT cards (DiaMed AG, Cressier sur Morat, Switzerland) were used according to manufacturer's instructions. Polyspecific anti-human globulin (AHG) reagent (i.e., combination of anti-IgG and anti-C3d) was used for both the LISS tube test (Lorne Laboratories, Reading, UK) and DiaMed ID cards (DiaMed AG). All tests were performed using plasma and serum samples with appropriate nine-cell panels.

Variation in the pattern of reactivity obtained when testing plasma and serum samples with the panel RBCs by the AGT implied that the detected antibody was a complement-dependent IgM antibody. A series of further tests was performed to confirm the presence of a complement-dependent IgM antibody. Treatment of the serum samples with dithiothreitol (DTT) (Sigma-Aldrich, Steinheim, Germany) was performed to verify the presence of an IgM antibody. Equal volumes of 0.01 M DTT were incubated with the patient's serum for 30 minutes at room temperature. PBS was added to the patient's serum in a second tube and similarly incubated as a control. Doubling dilutions were performed on the patient's untreated serum, DTT-treated serum, and PBS control serum. All dilutions were tested using appropriate RBCs by a LISS-AGT. Reactions were graded macroscopically; due to variation in interpretation of reaction grades, a twofold difference in titer result was considered to be significant.

A two-stage EDTA test was also performed on those anticoagulated patient samples in which a complement-dependent antibody was suspected and when the anticoagulated sample did not react by the tube LISS AGT. A neutralized EDTA solution at pH 7.2 was prepared using 4.0 g of K₂EDTA and 0.3 g of NaOH in 100 mL of distilled water. EDTA solution was added to the patient's plasma sample at a 1 in 10 dilution with incubation at room temperature for 10 minutes. Two volumes of the treated plasma were subsequently incubated with reagent panel RBCs suspended in PBS for 60 minutes at 37°C. After incubation, the RBCs were washed and incubated for 15 minutes with a fresh source of complement. (The complement was derived from a pool of group-compatible non-transfused donor sera that tested negative for the presence of irregular RBC antibodies and was stored frozen within 24 hours of donation.) After additional washing, polyspecific AHG reagent was added and the test was centrifuged and read.

The clinical significance of RBC IgG antibodies can be investigated using a chemiluminescence (CL) test based on the method described by Downing et al.⁵ In the following cases, where appropriate, the CL assay was performed to demonstrate the absence of a clinically significant IgG antibody. Briefly, the patient's serum was incubated separately with antigen-positive and antigen-negative RBCs. After washing, the sensitized RBCs were incubated with the mononuclear cell preparation in the presence of luminol. Monocyte

activation, as indicated by increased chemiluminescence, was measured every 4 minutes for 2 hours. The CL test results were reported as an opsonic index; an opsonic index < 1.2 is indicative of a clinically insignificant IgG antibody.

In each case, compatibility testing was required and performed using serum samples tested with RBCs antigen-negative for the previously identified antibody by a LISS AGT.

Case Reports

Case 1

In 1991, a 50-year-old woman was admitted to a local hospital with a Hb of 8.3 g/dL after a viral infection. Serologic investigations revealed the presence of anti-D and a strongly positive DAT. The patient was diagnosed with autoimmune hemolytic anemia. Transfusion was required on three occasions from 1991 to 1999; the patient received a total of nine units of RBCs. In 1999, a second antibody was detected in the patient's serum and samples were referred to the WBS for antibody identification and compatibility testing. The presence of anti-S and anti-D was confirmed. In January 2002, the patient presented with a Hb of 7.8 g/dL and was referred for compatibility testing presplenectomy. Pretransfusion testing was performed using anticoagulated samples. The anti-S was no longer detectable by the LISS AGT; however anti-S could be detected by a saline 18°C tube agglutination test. Additional anticoagulated and clotted samples were requested to further investigate the initial findings.

The patient's RBCs were grouped as A, D- (rr) and were found to react in the DAT with polyspecific and monospecific anti-C3 AHG reagents; negative reactions were obtained when the RBCs were tested using anti-IgG AHG. Routine antibody identification panels using plasma revealed the presence of anti-D reactive by LISS AGT; the anti-D was not confirmed active by papain tests due to the presence of a nonspecific papain autoantibody. Anti-S was detectable by saline 18°C agglutination tube tests and in the LISS AGT before washing. The anti-S was not detectable after washing, addition of AHG reagent, and centrifugation. The anti-S was also detectable when performing a DiaMed gel card AGT. We suspected the presence of an IgM complement-activating anti-S and we repeated our investigations using serum samples (Table 1).

Antiglobulin test titrations were performed using D+ ss, D- Ss, and D- rr ss LISS-suspended RBCs.

Table 1. Case 1: results of routine antibody identification panels

	Saline 18°C test	Prepapainized test	LISS AGT	DiaMed test
Plasma sample	Anti-S	PNSA*	Anti-D [†]	Anti-D and anti-S
Serum sample	Anti-S	PNSA*	Anti-D and anti-S	Anti-D and anti-S

*An apparently nonspecific papain autoantibody detected.

[†]Before AGT washing, positive reactions giving anti-S pattern were observed.

Table 2. Case 1: results of AGT titrations using untreated and DTT-treated serum*

	RBC phenotype	Dilution					
		Neat	1:2	1:4	1:8	1:16	1:32
Untreated serum sample	R ₁ R ₁ ss	5	4	3	2	2 [‡]	0
	rr Ss	4	3	2	0	0	0
	rr ss	0	0	0	0	0	0
DTT-treated serum sample	R ₁ R ₁ ss	NA	4	3	2	2	0
	rr Ss	NA	0	0	0	0	0
	rr ss	NA	0	0	0	0	0
PBS control serum sample	R ₁ R ₁ ss	NA	4	3	2	0	0
	rr Ss	NA	4	2	0	0	0
	rr ss	NA	0	0	0	0	0

*Reactions graded according to system described in Table 12.2 Guidelines for Blood Transfusion Services in the United Kingdom.¹⁶

[‡]Endpoints of titration in bold figures.

Table 3. Case 1: results of additional testing by AGT

	LISS AGT using polyspecific AHG	LISS AGT using anti-IgG AHG	NISS AGT using polyspecific AHG
Serum sample	Anti-D and anti-S	Anti-D*	Anti-D and anti-S
Plasma sample	Anti-D*	Anti-D*	Anti-D*

*Before AGT washing, positive reactions demonstrating anti-S pattern were observed.

Titration of the patient’s serum demonstrated the anti-D to be detectable at a 1 in 16 dilution and anti-S at a 1 in 4 dilution. DTT treatment of the patient’s serum destroyed anti-S activity such that no reactions were observed in the titrations by the AGT; the anti-D remained detectable at a dilution of 1 in 8. As expected, no reactions were obtained with D- rr ss RBCs (Table 2).

Further testing was performed to confirm the characteristics of the antibody (Table 3). The anti-S was detectable by a normal ionic strength saline (NISS) AGT, therefore excluding the possibility that the detection of the anti-S was a LISS phenomenon as had been previously described with other antibody specificities.⁶ The use of monospecific anti-IgG rendered the anti-S undetectable in the LISS AGT even with the use of a serum sample. Our results confirmed the anti-S to be an IgM complement-activating

antibody. Failure of the patient’s RBCs to react with the anti-S detected by saline 18°C agglutination tests suggested the anti-S to be an alloantibody; this was confirmed by determining the patient’s RBCs to be S- using an in-house IgG anti-S typing reagent.

The patient was transfused with six units of group A, D- (rr) ss RBCs units without incident; postoperative Hb was 13.2 g/dL.

Case 2

In December 2001, a sample was received from an antenatal patient at 11 weeks’ gestation for routine antenatal screening. The patient had two previous pregnancies with no record of any previous transfusions and no irregular antibodies detected during previous antenatal screening. Clotted and anticoagulated samples were received for antenatal antibody screening. Automated testing was performed using the anticoagulated sample with bromelinated RBCs on the Olympus PK7200 (Olympus, Tokyo, Japan) by hemagglutination and using the clotted sample by a solid phase antiglobulin test (SPAT) on the Tecan Genesis RSP 150 (Tecan Schweiz AG, Männedorf, Switzerland). The sample was identified as antibody positive by the SPAT method on the Tecan Genesis RSP 150. The sample was then referred to the Patient Diagnostic Services department for antibody identification and titration as necessary.

Routine antibody identification panels using the patient’s serum sample revealed the presence of an antibody that reacted with all panel RBCs tested by papain and LISS AGT with a negative autocontrol. The patient’s serum was tested using an additional selected-RBC panel to exclude the presence of a combination of the more common antibody specificities and with a panel of RBCs with the following rare phenotypes: Kp(b-), k-, Lu(b-), Yt(a-), Co(a-), and Vel-. The patient’s serum failed to react with the Vel- RBC. Anti-Vel was confirmed by testing with additional examples of Vel- RBCs. No additional RBC antibodies were detected and the patient’s own RBCs typed as Vel-. Due to insufficient serum, further testing by the AGT was performed using the referred anticoagulated sample. Before the AGT washing phase, agglutination was observed throughout the LISS panel when using the anticoagulated sample; however, after washing, the anti-Vel was undetectable by LISS AGT (Table 4).

Vel antibodies are characteristically IgM complement-activating antibodies; therefore, predictably discrepant results were obtained when

Table 4. Case 2: results of routine antibody identification panels

	Saline 18°C test	Prepapainized test	LISS AGT
Serum sample	Anti-Vel	Anti-Vel	Anti-Vel
Plasma sample	Not tested	Not tested	No antibodies detected*
Plasma sample in 2-stage EDTA test	Not tested	Not tested	Anti-Vel

*Before AGT washing, positive reactions were observed throughout panel.

Table 5. Case 2: results of AGT titrations using untreated and DTT-treated serum*

	RBC phenotype	Dilution					
		Neat	1:2	1:4	1:8	1:16	1:32
Untreated serum sample	Vel+	4	3	2 [†]	0	0	0
	Vel-	0	0	0	0	0	0
DTT-treated serum sample	Vel+	NA [‡]	0	0	0	0	0
	Vel-	NA	0	0	0	0	0
PBS control serum sample	Vel+	NA	3	2	0	0	0
	Vel-	NA	0	0	0	0	0

*Reactions graded according to system described in Table 12.2 Guidelines for Blood Transfusion Services in the United Kingdom.¹⁶

[†]Endpoints of titration in bold figures.

[‡]Not available (addition of DTT solution produces an initial 1 in 2 dilution).

testing plasma and serum samples. The plasma sample was further tested by a two-stage EDTA AGT. This technique permits the detection of complement-activating antibodies in plasma samples by the subsequent incubation of IgM-sensitized RBCs with a fresh source of complement. Complement enhancement of the routine tests by a two-stage EDTA AGT revealed the anti-Vel.

Titration of the patient’s serum demonstrated anti-Vel detectable at a 1 in 4 dilution. DTT treatment of the patient’s serum (as previously described) destroyed the anti-Vel activity such that no reactions were observed in the titrations by the AGT (Table 5).

IgM Vel antibodies are not associated with HDN. The clinical significance of anti-Vel with respect to its ability to cause HDN was confirmed using a CL test. CL assay results were negative (opsonic index < 1.2). Results of the serologic investigations confirmed the anti-Vel as an IgM antibody; the CL test results were, therefore, as expected.

Continuous monitoring of the antibody throughout pregnancy was undertaken and samples from the patient were referred on five additional occasions. On each occasion, the antibody specificity and class were confirmed and additional specificities were excluded. Four units of group-compatible Vel- RBCs were kept on standby for the mother before the expected date of

delivery. The patient delivered at 40 weeks’ gestation, without RBC transfusion support. The cord Hb was 21.9 g/dL, bilirubin was 61 µmol/L rising to 90 µmol/L, and the DAT was negative; all parameters indicated no HDN.

Case 3

In November 2003, a 70-year-old man was referred for preoperative investigations and compatibility testing. Anticoagulated and clotted samples were referred and initial investigations were performed on the anticoagulated samples because the clotted samples were inappropriately labeled. No reactions were detected by LISS AGT using the anticoagulated samples; however, a pattern of reactivity was detected by a saline 18°C agglutination test. Further testing was performed comparing the reactivity of the serum and plasma samples.

Initial antibody investigation results using anticoagulated samples suggested the presence of an IgM antibody detected by the saline 18°C agglutination test. The presence of a weak anti-S was confirmed by tube LISS AGT using clotted samples and a DiaMed (DiaMed AG) gel test using both anticoagulated and clotted samples. The anti-S demonstrated typical dosage effects and did not react with all heterozygous Ss RBCs. Titrations using the patient’s untreated, DTT-treated, and PBS control sera were inconclusive due to the lack of reactivity with diluted serum. The patient’s serum did not react with SS panel RBCs when tested by a NISS AGT, indicating that the antibody is LISS dependent.

The patient was successfully transfused with five units of ABO and Rh phenotype compatible, ss RBCs with a posttransfusion Hb of 12.2 g/dL.

Discussion

All three cases demonstrate the failings in the tube LISS AGT to detect complement-activating IgM antibodies when using anticoagulated samples. Coincidental testing of plasma and serum samples demonstrated the differences in antibody activity but it poses the question of how many complement-activating IgM antibodies remain undetected in plasma samples. The clinical significance of the IgM anti-S identified in cases 1 and 3 is unknown. S antibodies of IgM class are rarely reported⁷ and both patients reported here received S- RBCs. Anti-S can cause immediate and delayed hemolytic transfusion reactions and IgG anti-S has been implicated in HDN.⁸ It is

therefore necessary that anti-S be detected during pretransfusion testing.

Anti-Vel is rarely associated with cases of HDN; the Vel antigen is not fully developed at birth and examples of IgG anti-Vel are rare.⁹ Confirmation of the lack of clinical significance of the anti-Vel in relation to HDN was provided by a negative CL test result. Antenatal screening, however, not only allows for the identification of antibodies that may cause problems to the fetus and neonate but also alerts the blood bank of the presence of antibodies that may cause pretransfusion testing problems. Anti-Vel has been implicated in hemolytic transfusion reactions and failure to identify this antibody could result in a hemolytic transfusion reaction if Vel+ RBCs were transfused.^{10,11} In addition, the frequency of Vel- RBCs is 1 in 3711; failure to identify this antibody before delivery could have resulted in significant delay in the provision of compatible blood, if required.

The British Standards and Controls in Haematology Guidelines for pretransfusion compatibility procedures in blood transfusion laboratories do not advocate the use of serum over anticoagulated samples, but they recommend validation of any changes to the sample used for testing.¹² Serum samples, however, were recommended for the investigation of suspected hemolytic transfusion reactions in the annual Serious Hazards of Transfusion report (2001-2002) in recognition of the ability to detect weak complement-binding antibodies in serum samples.¹³ Studies investigating the suitability of samples for pretransfusion testing conclude that there are no significant differences in detection rates when using either plasma or serum.^{14,15} There is no single technique that will consistently outperform all other techniques in the detection of clinically significant RBC antibodies, but each laboratory must be aware of any limitations of the test they perform. Because the WBS is a reference center for RBC serology referrals, room temperature agglutination tests are performed to assist in the identification of cold-reactive antibodies in complex antibody mixtures that may interfere with LISS AGT. Failure to have performed saline 18°C agglutination tests or to have noted the pattern of reactions before washing could have resulted in the antibodies in these case studies remaining undetected in the anticoagulated samples. In each case, the use of a clotted sample tested by both automated and manual techniques performed at the WBS, as well as the application of good serologic practices, facilitated the

detection of these antibodies. The standard DiaMed gel AGT was performed in both cases where anti-S was identified. Contrary to the results of the tube LISS AGT, anti-S was detectable using both serum and plasma samples by the DiaMed AGT. Although there is much debate as to the sensitivity of the DiaMed test when compared with the traditional LISS tube AGT, DiaMed gel tests proved more sensitive for the detection of IgM antibodies when using plasma samples in the cases discussed. The washing phase in the LISS tube AGT disrupts IgM agglutinates and, consequently, the detection of both the anti-S and anti-Vel antibodies using polyspecific AHG relies entirely on the activation of complement. Lack of complement activation when using plasma samples therefore inhibits the detection of these antibodies by the LISS tube AGT. The omission of a washing phase when using DiaMed AGT cards allows IgM agglutinates to remain intact. Therefore, the anti-S and anti-Vel antibodies were detectable in plasma samples when using the DiaMed AGT cards despite the lack of complement activation.

These cases are of interest because of the rare occurrence of anti-S of IgM class and of anti-Vel. They also serve to highlight the benefit of using clotted samples, particularly when performing tube tests. The WBS commenced routine antenatal screening by the DiaMed technique using anticoagulated samples in November 2004 and continues to use the standard tube LISS AGT for the reference work performed. We request the referral of clotted samples for all reference work.

Acknowledgments

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2006 Immunohematology Reference Laboratory Conference

Summary of presentations

For more than 20 years, the AABB and the American Red Cross (ARC) have hosted the Immunohematology Reference Laboratory (IRL) Conference in mid-spring. Initially the conference was jointly hosted by the two organizations; it has since been hosted alternately, with each organization choosing the host city and planning the conference. The conference has been held in various cities throughout the United States, including Atlanta, Chicago, Las Vegas, New Orleans, Memphis, and this year, Orlando. The 2007 IRL Conference will be hosted by the AABB and held in Albuquerque, New Mexico, the weekend of March 27 to 29, 2007.

The conference begins on Friday afternoon with proctor-led case studies discussing advanced immunohematologic investigations, followed by a welcoming reception that promotes connecting and networking with fellow technologists and physicians from around the country and, typically, Canada. Saturday begins with breakfast followed by speaker presentations on various serologic, technical, clinical, administrative, quality, and regulatory issues that affect today's reference laboratories. These presentations extend until late afternoon with scheduled breaks and a provided lunch. The presentations continue on Sunday with the conference ending at noon. Attendees are encouraged to bring posters for viewing and those that do have the opportunity to present the information to all.

Following are summaries of the presentations given at the 2006 IRL conference that was hosted by the ARC from April 28 to 30 in Orlando, Florida.

Reference Laboratory's Joys and Woes— 30 Years (1975–2005)

I had been working for 8 years in blood banking at War Memorial Blood Bank (now known as Memorial Blood Center) in Minneapolis, Minnesota, when I came to Gamma Biologicals, Inc., in Houston, Texas, to work in the consultations service. I began as the supervisor of consultation on August 1, 1975, became the director of consultation and education in 1990, and was named the vice president of consultation and education services in 1996, a position I held until 1998 when I joined Immucor as the vice president of reference and education services.

There are several elements essential to having a successful immunohematology reference laboratory for RBC serology. The first element is to have staff that

is competent, dedicated, enthusiastic, and eager and willing to investigate all types of samples, whether they contain cold- or warm-reactive autoantibodies or a combination of the two, mixtures of alloantibodies, antibodies directed at low- or high-incidence antigens, or even the mundane Lewis, P1, or Bg antibodies. The second element is to have a facility where management is willing to support the many, and sometimes lengthy, investigations associated with resolving complicated serologic problems. Another element is to have the resources of unusual and rare RBCs and antibodies to perform special investigative studies. These are obtained by the collecting and sharing of samples by immunohematologists around the world. The SCARF program (organized by John Moulds) and other exchange programs have certainly helped to accomplish this. In addition, very importantly, a reference laboratory needs blood bankers in transfusion services, prenatal testing laboratories, donor centers, and even reagent manufacturing facilities who are willing to take the time to send samples for further studies. They are the ones who have the patients whose lives can be saved by providing the best possible blood for transfusion. They also have access to families and donors that could aid in furthering our knowledge of blood groups. And, last but not least, a key element to the success of an immunohematology reference laboratory is education. I was very fortunate to be able to attend meetings and seminars worldwide, as well as to be able to give talks on the special cases we investigated and the new methodologies and technologies introduced to blood banking that helped us to resolve the unusual cases.

There are many joys in working in an immunohematology reference laboratory. However, sometimes there are woes, such as regulations, assessments, validations, SOPs, corrective actions, etc. I have learned over the years to deal with these woes as best I can and look back on the good times and not the

bad. I am not always good at taking my own advice, but I do like to give it!!!

It has been a wonderful 30 years and I have many to thank for the support I have had in my career: from my mentors, the facilities that employed me, and the more than 20 staff members who worked with me at Gamma and Immucor and, before that, at War Memorial Blood Bank, Chadron Community Hospital in Chadron, Nebraska, and St. John's McNamara Hospital in Rapid City, South Dakota. Also, I extend a special thanks to the blood bank friends and customers who shared samples with our immunohematology reference laboratory. I wish all of you the best in your careers in the future and hope that there will always be those who have the desire and dedication to work on special serologic problems for the best patient care we can give.

Marilyn K. (Grandstaff) Moulds, MT(ASCP)SBB, Vice President Education, ImmucorGamma, Norcross, Georgia/Houston, Texas.

Serologic Results to Diagnostic Interpretation

Beyond the tests associated with antibody identification studies routinely performed in an immunohematology reference laboratory (IRL) are serologic tests that can lead to diagnosis. Serologic results alone, however, are not diagnostic. Their significance must be reviewed in conjunction with the patient's clinical condition. The following reviews serologic testing the author feels has a direct impact in diagnosis as well as comments on several requested tests that this author believes have limited value.

Detection of Mixed-Field Agglutination

Detecting mixed-field agglutination (2-cell populations) when performing ABO and D typings can be used to assess engraftment of marrow in a transplant recipient. Likewise, detecting mixed field in antigen typing can be used as an aid in assessing the survival of transfused RBCs.

Direct Antiglobulin Test

One of the most useful tests in the investigation of hemolysis is the DAT. Careful analysis of DAT results, in conjunction with the evaluation of serum reactivity, can lead to the diagnosis of warm autoimmune hemolytic anemia, cold agglutinin disease, and paroxysmal cold hemoglobinuria and can allow the physician to plan a course of treatment. Additional

testing can be performed to evaluate patients with so-called DAT-negative hemolytic anemia in an attempt to detect an antigen-antibody reaction. This type of information is valuable to the patient's physician because it can help confirm hemolysis is immune-mediated.

An area in which serologic testing can be misleading is the detection of newly forming antibodies in patients that have been recently transfused. A positive DAT posttransfusion with identification of a new antibody 7 to 14 days after transfusion alerts the laboratory to a possible delayed transfusion reaction. Clinical evidence of hemolysis is the key to differentiate a delayed hemolytic transfusion reaction from a delayed serologic transfusion reaction.

Elution

Elution procedures are performed to determine the specificity of the antibody coating the patient's RBCs. Eluates can confirm alloantibody specificity identified in the patient's serum. In rare cases, newly forming antibody can only be found coating the transfused RBCs in a recently transfused patient. A negative eluate in a patient with a strongly positive DAT (4+) may indicate that the patient has a drug-dependent antibody. Further review of the patient's clinical course and medication history may indicate the patient is experiencing drug-induced immune hemolytic anemia. Drug studies to look for the presence of drug-dependent antibodies will confirm this diagnosis.

Lectin Testing

Testing samples from infants and children with a panel of lectins may be valuable in detecting T-activated polyagglutinable RBCs, particularly in infants with a bacterial infection. However, routinely screening for polyagglutination in newborns with a diagnosis of necrotizing enterocolitis (NEC), for example, is generally not performed. RBCs from normal, healthy infants can show T activation and infants with NEC and possessing T-activated RBCs may show no hemolysis. Most experts believe that testing for polyagglutination should be selectively performed when the neonate has received RBCs or plasma products and has demonstrated hemolysis or an unexplained lack of rise in posttransfusion Hb.

There are rare examples of immune-mediated hemolysis in children because of T-activated RBCs and the child's own anti-T. The exact mechanism of hemolysis is not fully understood.

Ii Antigen Typing

Occasionally the IRL is asked to perform Ii antigen typing on a child. This is generally requested when a physician suspects stressed hematopoiesis. The i antigen is characteristic of fetal erythropoiesis and may be increased in cases of hereditary erythroblastic multinuclearity with a positive acidified serum (HEMPAS), aplastic anemia, and myeloblastic erythropoiesis, for example. There are several issues to consider before performing this typing. The first issue is that antisera are in limited supply. The second issue is that, since the level of I antigen expression increases and i antigen expression decreases with age, samples from children about the same age as the patient to be tested should be used as a control. Also, this testing can only be performed if the child has not been transfused within the past 3 to 4 months. Lastly, there are other tests that will provide more useful diagnostic results than determining the Ii antigen status.

ABO Titration in Transplant Patients

IRLs are being asked to assist transplant programs by performing anti-A and anti-B titration studies for ABO-incompatible kidney transplants and for ABO-incompatible heart transplants in infants.

In ABO-incompatible kidney transplants, the recipient is usually group O and the kidney donor is group A² or B. The procedure accepted by the United Network for Organ Sharing for ABO titration is peculiar to immunohematologists. Patient serum is treated with DTT to destroy IgM anti-A or anti-B and dilutions of patient serum are tested against pooled group A or B RBCs by incubating at room temperature for 10 minutes. If the titer is less than 8, the patient is considered able to receive a kidney from a group A² or B donor. ABO titers are performed periodically before transplant, immediately before transplant, and immediately posttransplant. If the titer increases following transplant, a plasma exchange may be performed to reduce the ABO-antibody titer.

The ABO titration performed to monitor infants less than 1 year old consists of diluting the patient's serum in saline, preparing doubling dilutions, adding known group A or B RBCs, and incubating the mixture at room temperature for 30 to 60 minutes. Interestingly, ABO antibodies often detected in infants are passively acquired from transfusion once maternal antibody clears.

Although more and more is being discovered about blood groups at the molecular and biochemical level, relatively simple serologic tests to detect antigen-

antibody reactions continue to play a major role in diagnosis and monitoring of a patient's disease. However, a serologic test result alone must always be correlated with the patient's clinical condition to provide the utmost value to the patient's physician.

Susan T. Johnson, MSTM, MT(ASCP)SBB, Manager, Immunohematology Services, BloodCenter of Wisconsin, Milwaukee, Wisconsin.

Special Notes When Using Anti-D Monoclonal Reagents

Commercial anti-D reagents using monoclonal antibodies began to be widely used in the early 1990s. Since then, differences among the reactivity of the various reagents with unusual or rare D+ RBCs have led to widespread discussion of the testing appropriate for blood donors, transfusion recipients, and obstetric patients. D+ RBCs giving varying reactions fall into the categories of weak D, partial D including the R₀^{Har} phenotype, and the Crawford (ceCF), ceRT, and the DEL (D_{cl}) phenotypes. Currently, there are several types of anti-D reagents available, including human blend, monoclonal/polyclonal blend, monoclonal/monoclonal blend, and monoclonal IgM used in the gel card. Another influence on the types of reactions observed is the technique used: tube, microplate, slide, gel cards, or automation, to name a few. Also, some reagents react best with these unusual RBCs at immediate spin (IS) and room temperature, some at IS and after incubation at 37°C, and some only in the IAT. In addition, the ethnic background of the person whose RBCs give these unusual varying reactions is significant.

Weak D

The incidence of weak D has previously been reported as 0.23 and 0.5 percent in Europe and 3 percent in the United States. These studies were performed before the introduction of monoclonal anti-D reagents. A study published in 2005¹ reported on the incidence of weak D blood donors typed as D+ by Olympus PK7200 as 0.4 percent (4 of 1005 donors).

Partial D

Judd et al. also reported in 2005² on the reactivity of FDA-approved anti-D reagents with partial D+ RBCs. They compared reactions in tube tests using anti-D reagents from Gamma, Immucor, and Ortho and with Ortho gel cards and found differences among Categories DVa, DBT, and R₀^{Har} RBCs in the various phases of testing.

Crawford (ceCF)

In 2003, Schlanser et al.³ reported on the Crawford phenotype that is present in African Americans and in two individuals from Colombia. RBCs with this unusual phenotype react with two monoclonal/monoclonal blend anti-D reagents but not with other anti-D reagents.

ceRT and Del

Even more recently, there have been several reports (a review, an editorial, and a forum)⁴⁻⁶ that focus on the issue of whether RBCs of blood donors with these phenotypes can elicit the production of anti-D in transfusion recipients and obstetric patients and whether we should be concerned with weak D. These publications can be consulted for the various opinions and other pertinent references.

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Cold Agglutinins

As serologists know, cold agglutinins found in normal individuals may interfere with pretransfusion testing. Cold agglutinins are also found in individuals with disorders including cold hemagglutinin disease (CHD) and paroxysmal cold hemoglobinuria (PCH). Reference laboratory serologists play a key role in helping to distinguish the former benign type of cold

agglutinins from the latter, more clinically significant type.

The focus of this discussion will not be on the serologic management of cold agglutinins. Rather, it will be on three clinically related topics: the association of cold agglutinins with patients undergoing cardiac surgery, with blood donors, and as critical values.

Should one be concerned with cold agglutinins in cardiac surgery patients? Over the last 10 to 20 years, the number of cardiac catheterization and percutaneous coronary intervention procedures has continued to increase, while the number of coronary artery bypass surgery procedures (CABG) peaked in 1997 and has since started to decline. 0.8 to 4 percent of cardiac surgery patients have been found to have some type of cold agglutinins. This compares with an incidence of 1 in 41,000 to 1 in 80,000 individuals with autoimmune hemolytic anemia (15.6% of these individuals have CHD).

The traditional CABG permits a cardiopulmonary bypass (CPB) machine to take over the functions of the cardiac and pulmonary systems (i.e., to pump blood through the body, while supplying oxygen and removing carbon dioxide). It also permits the surgeon to operate on a quiet, bloodless surgical field. Techniques initiated systemic hypothermia to 28 to 32°C, while cold (~5°C) potassium cardioplegic solutions were infused into the coronary arteries via the aortic root. By modifying the temperature or the manner in which cardioplegic solutions are infused, surgeons are now able to avoid complications that potentially may be induced by cold agglutinins.

Perhaps a more preferable way of referring to cold agglutinins is as cold-reactive proteins. There are three categories of cold-reactive proteins that may cause concern in cardiac surgery patients: cryoglobulins, cold agglutinins, and Donath-Landsteiner (DL) antibodies.

Cryoglobulins (Types I-III) are serum proteins that reversibly precipitate in the cold. Four cases of patients with cryoglobulinemia undergoing CPB surgery have been reported in the literature. The outcomes of these cases were all successful even though the techniques used varied. Some used plasma exchange to reduce the patients' cryocrit while others used temperature modifications in systemic or cardioplegic solutions.

Cold agglutinins are antibodies that typically bind to RBC antigens and cause agglutination and complement fixation over a particular temperature range. They are disturbing to the cardiac surgery

patient (and surgeon) for their potential to cause agglutination within the circuit or within coronary arteries that may result in hemolysis or organ damage secondary to agglutination or complement-mediated RBC destruction. Several case reports of hemolysis associated with CPB, as well as ways to avoid complications, have been published both in transfusion-medicine and cardiovascular-surgery literature. All serologists are encouraged to review these papers critically if requested to prescreen cardiac surgery patients for cold agglutinins.

The DL antibody is a biphasic IgG hemolysin that is classically associated with PCH. There is only one reported case in the literature of a patient with the DL antibody undergoing cardiac surgery.

Certain strategies may be considered when managing CPB patients with cold-reactive proteins. If cold-reactive proteins are detected before surgery, the surgical procedure can be modified to maintain normothermic systemic circulation and a crystalloid cardioplegic solution (rather than a blood-based cardioplegic solution) can be used. Plasma exchange may be helpful, for example, in cryoglobulinemia to reduce the cryocrit or in other cases of IgM-only antibodies. Patients with documented CHD may present special concerns. If cold-reactive proteins are not detected before surgery, the surgeon should observe for agglutination during cooling of the cardioplegic unit. If detected at that time, both systemic hypothermia and cold blood or cardioplegia should be avoided.

Individuals can be healthy enough to donate a unit of blood (allogeneic or autologous) and evidence of cold agglutinins may be found in these units, either grossly or microscopically. Units with gross evidence of cold agglutinins must be detected prior to issue. The use of digital imaging visual technology may prove helpful in the near future for communicating information between individuals in different facilities (e.g., does this unit have a clot or cold agglutinin?) and for education.

The College of American Pathologists transfusion medicine checklist now contains the following Phase II requirement: "Are critical values established for certain tests that are important for prompt patient management decisions?" There may be certain critical situations where discussion between the laboratory and the ordering physician should take place with regard to cold agglutinins; these may include evaluating a patient for autoimmune hemolytic anemia, resolving

an ABO discrepancy, and detecting cold alloantibodies that one considers clinically significant or that delay the procurement of blood.

Geralyn M. Meny, MD, Medical Director, American Red Cross Blood Services, Penn-Jersey Region, Philadelphia, Pennsylvania.

Polyagglutination

Polyagglutination is still present but rarely seen because of the use of monoclonal ABO typing reagents. Human-based ABO reagents contained polyagglutinins that interfered with the forward typing of RBC samples from patients with polyagglutinable RBCs. A basic review of polyagglutination will be presented.

Polyagglutinins are considered naturally occurring as they are most likely stimulated by normal flora found in the intestines. There are three types of polyagglutination: microbially induced, nonmicrobially induced, and inherited. The microbially-induced polyagglutination types are T, Th, Tk, Tx, VA, and acquired B. Microbial polyagglutination is transient and once the infection is eradicated, the polyagglutinable state goes away. One interesting note about T polyagglutination is that it may be a useful marker for hemolytic-uremic syndrome. Tk polyagglutination alters ABH, Ii, Lewis, and P1 antigens. Few cases of Th polyagglutination have been identified. Tx polyagglutination is also rare and is found in children with pneumococcal infections. Acquired B is usually found in patients with bowel disorders. The VA stands for Vienna and these polyagglutinable RBCs have reduced expression of the H antigen.

The nonmicrobial form of polyagglutination is Tn. Tn is permanent and irreversible. Tn RBCs acquire a weak A-like antigen. Tn has also been shown to be associated with leukemia.

The inherited forms of polyagglutination are CAD, HEMPAS, NOR, and Hemoglobin M-Hyde Park. HEMPAS stands for hereditary erythroblastic multinuclearity with a positive acidified serum test. HEMPAS RBCs have increased expression of i antigen and normal to increased I antigen expression. CAD 1, CAD 2, and CAD 3 phenotypes have been identified in which only CAD 1 is considered polyagglutinable. RBCs with the autosomally inherited NOR form of polyagglutination were found not to react with cord sera. Polyagglutinable RBCs are usually identified by their reactivity with different lectins and with all adult ABO-

compatible serum or plasma, and by their nonreactivity with cord sera.

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Project Management

Immunoematology reference laboratories (IRL) provide a critical, value-added service to patients and to the healthcare community. The IRL commonly focuses on testing and operational issues; however, IRL staff and supervisors often serve as agents of change as well. Changes within the IRL, such as implementing new techniques or preparing for computer software upgrades, may benefit from application of project management techniques and tools.

Project management is anchored by the project management body of knowledge (PMBOK). The Project Management Institute maintains the guide to the PMBOK, which consists of 44 processes. These processes or activities fall into one of five process groups; initiation, planning, execution, closure, and monitoring or control. The processes or activities can also be grouped by knowledge area; integration, scope, time, cost, risk, human resources, quality, communication, and procurement. Full exploration of the PMBOK is beyond the ability of this review, and therefore, three selected process groups and 12 processes will be explored as an overview of project management.

Project Planning

Project planning includes defining the scope of the project. The scope statement should describe the work that will be done within the boundaries of the project, and where relevant, the work that is out of scope. Goals, objectives, and measurements are also defined during planning. Project goals are commonly established on the basis of one of four elements: schedule, cost, quality, or performance. Objectives will address decisions, inputs, or activities that are essential to meeting one of the project's goals. Measurements should be very specific and should detail how the objective will be measured and the success target value. Critical to measurement design is that the measurements take place during execution of the project; this ensures that corrective action can be taken during the course of the project in time to bring the project back on track. Tasks and activities for the project work are detailed with input from subject matter experts. Resources are assigned to each task or

activity on the basis of the required skills or abilities. Each task or activity is evaluated for an estimated duration and dependencies between tasks and activities are identified. A schedule is constructed by assigning estimated start and finish dates, starting with constrained dates, or with a chosen project start date. Risk planning is initiated by evaluating the scope and schedule as well as other sources, for "things that could go wrong." The identified risks are evaluated for probability of occurrence and impact on the project. Selected risks are assigned mitigation actions. A plan for monitoring the project is designed on the basis of the objectives and measurements, risk plan, and other component planning activities.

Project Execution

Project execution is the act of performing the tasks or work associated with the project. The project plan, containing elements described in the planning process, becomes the road map for the project. With a well-written plan, the project team should be able to work the plan and appropriately respond to events that signal an off-course project. During execution, periodic reports and communication should take place as well as celebration of project accomplishments.

Project Closure

During project closure, the team should review lessons learned, that is, what went wrong and what went well within the project. This information is captured for sharing with other current and future projects. Effectiveness data are collected and evaluated during closing as well as identification of future enhancements.

The appropriate application of project management skills, tools, and techniques increases the probability of project success. The extensive planning process should cumulate in a project plan that creates a common understanding of the project and its goals. The project plan becomes an active road map for the project.

Ann Church, PMP, MS-PM, CQA, CQIA(ASQ), MT(ASCP)SBB, Project Manager, American Red Cross Blood Services, BHQ-IRL Operational Support, Philadelphia, Pennsylvania.

ISBT 128 for the IRL

ISBT 128 is an international information standard for blood, tissue, and cellular therapy products. It defines how information can be encoded for

transmission via different delivery mechanisms, including linear bar codes (Code 128 symbology), two-dimensional bar codes, radio frequency identification tags, or electronic messaging. It is currently in use in many countries with more countries implementing it each year. The AABB will require U.S. blood banks to implement the system for blood labeling by May 2008.

Data that can be encoded using the *ISBT 128* information standard include the unique donation identification number, ABO and Rh, product code, donation type, expiration date, HLA test results (genomic or serologic), RBC antigens, platelet antigens, CMV, IgA, HbS, patient identification number, patient date of birth, catalog and lot numbers of supplies, and staff identification codes. Specifics of how the information is encoded, as well as reference tables, can be found in the document available from ICCBBA titled *ISBT 128 Standard, Technical Specification*. Databases supporting the encoded information are maintained by ICCBBA, Inc., and are available on its Web site (www.iccbba.org). Specifics on labeling in the United States may be found in the *U.S. Consensus Standard*.

Of particular interest to immunohematology reference laboratories is the ability of *ISBT 128* to encode a RBC phenotype obtained by testing for antigens such as: A1, C, c, E, e, C^w, VS/V, K, k, Kp^a, Js^a, M, N, S, s, U, P1, Lu^a, Le^a, Le^b, Fy^a, Fy^b, Jk^a, Jk^b, Do^a, Do^b, Di^a, Mi^a as well as a long list of less commonly typed antigens. By encoding information for transfer via bar codes or electronic messaging, greater process control and accuracy can be achieved. In addition, automated transmission allows clear, unambiguous information to be transferred across language barriers, supporting the international sharing of rare blood, tissue, and cellular therapy products.

ISBT 128 is not merely a labeling system. By understanding and using its potential, secure information transfer for blood, tissue, and cellular therapy products is possible. This should lead to an increased level of safety for patients.

Pat Distler, MS, MT(ASCP)SBB, Technical Director, ICCBBA, Inc., York, Pennsylvania.

Lean but Not Mean: Doing More with Less in the IRL

Continuous process improvement can be a valuable approach to achieve and sustain customer satisfaction as well as process excellence. There are many methodologies and tools for process

Table 1. Examples of waste in the IRL

Source of waste	IRL example
Overproduction	Antigen typing of donor units for stock i.e., without a customer order
Inventory	Storage of excessive amounts of test tubes or other supplies, such as blank forms
Defects	Incomplete documentation on testing worksheets, incorrect test result, delayed result
Over-processing	Academic "for fun" testing that is performed routinely, testing protocols beyond industry standard of practice, antibody reconfirmation, ABO and D testing performed after initial sample testing
Waiting	Time that elapses from sample notification to sample receipt, from sample receipt to initiation of testing
People underutilized	Highly trained staff performing filing or other clerical tasks, not including staff in problem solving or improvements efforts
Motion	Excessive up and down activity to obtain reagents and supplies during testing, cell washers located away from work stations, reagents stored in remote sites
Transportation	Movement of sample from patient to testing site, supplies from warehouse to laboratory, blood products from IRL to distribution department

improvement, one of which is Lean. Though many definitions exist for Lean, it can be described as a comprehensive evaluation of operations to identify and eliminate waste, decrease variation, and increase efficiency. Central to the Lean approach is to use the voice of the customer and evaluate processes on the basis of what the customer views as valuable. Table 1 lists sources of waste that are relevant to the immunohematology reference laboratory (IRL).

Lean has a "tool box" of tools that can be applied when evaluating and improving processes. Value stream mapping involves "walking" the process from beginning to end with intense observation and interviewing, as needed, to fully understand the process. Cycle times and measurement of wait are also included. The value stream map is evaluated for waste and a desired state or "to be" map is drawn. Gaps between the "as is" and "to be" are identified and prioritized for improvement efforts. The application of 5S can yield benefits: sort (clear out clutter), set in order (place remaining supplies or items in alignment with process flow), shine (clean the work area), standardize, and sustain. Other Lean tools include streamlined physical layout, standardized work, batch-size reduction, workplace teams, kanban, or pull system (perform work only when customer order is placed), and point-of-use storage.

Though Lean has many benefits and opportunities, it can fall prey to challenges that serve as obstacles to its successful use. These challenges include the human element; staff with a pervasive batch mentality, desire for autonomy, resistance to change, and perspective that “we are different; we save lives.” Other challenges include lack of staff training in the Lean tools and teamwork, not enough attention to voice-of-the-customer, and conflicting needs among different customer groups (FDA, AABB, patients, physicians, quality department, donors, etc). Last, but not least, of the obstacles is that Lean may not be a good fit for every organization. If appropriately chosen and applied, Lean can add value to the IRL through removal of waste, creating increased efficiency that allows for opportunities to enhance and expand service.

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D.R.U.G.S. in the Workplace

Drug-induced immune hemolytic anemia (DIIHA) is an uncommon finding. To confirm its diagnosis, drug testing is performed to identify drug-dependent antibody (DDA).

Drugs most often implicated in DIIHA today are second- and third-generation cephalosporins. Cefotetan leads the list in reports by Arndt and Garratty as well as in testing performed in this author's laboratory.¹ There are many other drugs known to cause DIIHA. Important in determining the testing needed to detect these drug-dependent antibodies is having knowledge of the drug's characteristics in laboratory testing. Some drugs, like the cephalosporins and penicillin group, bind tightly to the RBC membrane. Most others require that the drug be present in a soluble form. Many nonsteroidal anti-inflammatory drugs require drug metabolites for detection of DDA.

Initial serologic testing normally shows the DAT to be positive. Strength of reactivity is reported from strong (3–4+) positive because of IgG binding to weak positive because of complement binding only. Work performed in this author's laboratory has shown that the DAT is most often strongly positive (2–4+) regardless of the drug. Most often, the DAT is positive because of IgG and C3, less often because of IgG only, and least often because of only complement coating

the RBCs. The eluate is classically negative because drug is not present in the test mixture. However, there are several reports of eluates being disproportionately weaker ($\leq 2+$) as compared with the strength of the DAT (3–4+). Serum is also reported to be negative in routine antibody detection tests for the same reason. However, drug-independent “autoantibody” or drug present in the patient's circulation may cause a positive antibody detection test without drug.

Arndt and Garratty propose dividing DDAs into two categories, those that react with drugs bound firmly to RBCs, called the “drug adsorption mechanism” and those that react with drugs that do not bind firmly, known as the “immune complex mechanism.”¹

A new classification is proposed when referring to DDAs on the basis of testing methods: those that react with drug-treated RBCs or those reacting in presence of drug. This classification is suggested to eliminate confusion and controversy in using mechanisms of drug-dependent antibody binding to categorize these antibodies.

A careful drug history is important in the face of significant RBC hemolysis in a patient. DIIHA should be considered when there is serologic evidence of warm autoimmune hemolytic anemia with a strong positive DAT and positive IAT, or of cold agglutinin syndrome with a strong positive DAT because of C3 and serologic testing shows a positive antibody detection test at immediate spin. A thorough investigation for DDA requires knowledge of the characteristics of the putative drug to confirm the presence of DDA.

1. Arndt PA, Leger RM, Garratty G. Serology of antibodies to second- and third-generation cephalosporins associated with immune hemolytic anemia and/or positive direct antiglobulin tests. *Transfusion* 1999;39:1239-46.

Susan T. Johnson, MSTM, MT(ASCP)SBB, Manager, Immunohematology Services, BloodCenter of Wisconsin, Milwaukee, Wisconsin.

Coagulation for Blood Bankers

Most blood bankers understand ABO blood groups and how to match a unit. Words like Kell, Kidd, RhIG, and transfusion reactions are commonplace. They are familiar with transfusion medicine and when to dispense platelets, FFP, and products that can help trauma victims or hemophiliacs. With all of that knowledge, why do they need to add another topic to

their plate? They are certainly busy enough! Well, when the blood bank is busy, so is the coagulation department! So what happens when the two departments meet and what do they have in common? There are many concepts in each department that can enhance the understanding of testing and benefit the most common denominator—patient care.

Hemostasis

Hemostasis is a system of checks and balances. It compromises the vascular system, platelets, and a series of enzymatic reactions that affect the coagulation factors. When the coagulation system is activated inappropriately, an individual will experience a bleed or a thrombotic event.

Primary hemostasis in coagulation deals with platelets. These small disc-shaped cells do not contain a nucleus; their activity is controlled by their granules. Platelets are the primary response to an injury. They undergo a shape change from a disc to a spiny sphere. They then adhere to the site of the vessel injury. Many factors are required in this process, including fibrinogen and von Willebrand factor (vW). This is considered primary aggregation and is reversible. The final phase is a release reaction whereby platelets release their contents of dense and alpha granules. This is called secondary aggregation and is irreversible.

Secondary hemostasis involves a series of enzymatic reactions that activate the coagulation factors, resulting in the formation of a fibrin clot. This complex reaction includes a system of inhibitors and activators and uses a complex mixture of relatively unstable proteins that are difficult to purify as well as phospholipids and calcium ions.

The Coagulation Laboratory

The coagulation laboratory evaluates secondary hemostasis by assessing the in vitro coagulation cascade by performing the screening tests: the prothrombin time (PT) and the activated partial thromboplastin time (APTT). This cascade does not reflect clotting physiologically; however it does play a role in the laboratory evaluation of a potential coagulation disorder.

The PT and the APTT provide a tremendous amount of information to the physician. They can be performed quickly and accurately. Abnormalities of the test results can assist the clinician in determining preoperative status and bleeding disorders, and in monitoring anticoagulation therapy.

Prothrombin Time

The PT test evaluates factors in the extrinsic pathway. It uses citrate anticoagulated plasma, and after the addition of an optimum concentration of calcium and an excess of thromboplastin, clot detection is measured by an automated device. The result is reported in seconds. The PT is exclusive for Factor VII, but assesses other deficiencies of factors II, V, and X, which are found in the common pathway. Therefore, if a patient presents with a prolonged PT and there is no other clinical abnormality or medication, the patient is most likely Factor VII deficient.

This test also looks at the monitoring of warfarin therapy; excessive dosage of this anticoagulant is the most likely reason for a prolonged result. Monitoring anticoagulation has a variable and unpredictable response. As a result, if the level is inadequate, the patient may experience thrombosis and if the level is excessive, the patient may bleed. So how does this affect the blood bank? Warfarin inhibits the carboxylation of the glutamate residues of the vitamin K-dependent factors (II, VII, IX, X, proteins C, and S), rendering them nonfunctional and impairing fibrin formation. Their loss of function is half-life-dependent. Factor VII has the shortest half-life, 4 hours, while Factor II has the longest, 2 days. For example, if a factor level is at 100 percent and it has a half-life of 4 hours, the activity of this factor will be at 50 percent after 4 hours. Understanding this becomes important when looking at replacement therapy as this will impair fibrin formation. Warfarin has a half-life of 35 hours. It can be administered for life and bleeding is a potential risk because of the influences of diet, vitamin K ingestion, body mass, and liver function. Eighty drugs interfere with coumadin and blood levels are only therapeutic 65 to 80 percent of the time. In addition, the results of the test are affected by the instrument and reagent system used in the laboratory. A system, the International Normalized Ratio (INR), has been developed to help standardize the monitoring so that treatment can be more precise. It uses a formula that combines the patient PT, the mean of the normal range, and the sensitivity of the reagent as determined by the manufacturer.

The therapeutic range of the INR is 2.0 to 3.0 for prophylaxis and treatment of thrombosis, a pulmonary embolism, or a myocardial infarction. A high dose range of 2.5 to 3.5 is used for treatment of a mechanical heart valve. (Table 1) So how does this information

Table 1. Guidelines for patient management: oral anticoagulation, target range INR is 2 to 3

INR value	Clinical risk	Patient management
3.1 to 3.9	No bleeding	Day 1: subtract 5-10% of total weekly dose (TWD)
4.0 to 5.0	No bleeding	Day 1: no warfarin, weekly reduce TWD by 10-20%
5.1 to 9.0	No bleeding but at risk for bleeding	Hold warfarin, monitor INR until it reaches upper limit of therapeutic range Weekly: reduce TWD by 20-50% Recheck INR in 72 hours
> 9.0	Significant risk for bleeding	Hold warfarin, give vitamin K, admit patient to hospital Monitor INR until it reaches upper therapeutic limit, reinstitute warfarin Recheck INR until stable
>3.0	With bleeding	Hold warfarin, give vitamin K by IV, give plasma Get hematology consult Test INR 6-12 hours

Guidelines from New York-Presbyterian pharmacy.

affect blood bankers? The AABB guidelines for using FFP are:

1. Bleeding or planned invasive or surgical procedure and 1 or more of the following:
 - a. PT greater than 1.5 times the mean of the normal range or greater than 17 seconds
 - b. APTT greater than 1.5 times the mean of the normal range or greater than 49 seconds
 - c. Deficiency of factor II, V, VII, X, or XI
 - d. Massive transfusion of more than 10 units
 - e. Disseminated intravascular coagulation
 - f. Thrombotic thrombocytopenic purpura or hemolytic-uremic syndrome

Activated Partial Thromboplastin Time

The APTT evaluates deficiencies of intrinsic factors VIII, IX, XI, and XII. The methodology involves the addition of a contact activator (e.g., celite, kaolin, microsilicate, or ellagic acid) and plasma. This mixture is incubated at 37°C, usually for 5 minutes. Thromboplastin preparation is added and mixed. CaCl₂ is added and the result is measured in seconds.

This test is also used to monitor heparin therapy. Heparin is an acidic mucopolysaccharide that inhibits all of the active serine proteases (IIa, Xa, IXa, XIa, and XIIa). It has a strong negative charge and a circulating half-life of only a few hours. It is stable for 24 hours in a 5% dextrose solution. Elimination is largely by the kidney so that heparin must be used cautiously in patients with impaired glomerular filtration. Heparin is best administered intravenously, intermittently, or,

better, as continuous infusion in a dosage of 400 to 500 units/kg body weight/day divided into every 6-hour dose so that 100 to 125 units/kg body weight are given each 6 hours. (APTT target range of 60 to 85 seconds or APTT ratio that is equivalent to a heparin level by antifactor Xa assay of 0.3 to 0.7 units/mL). Because of the biological variability that occurs with heparin, there has been no ability to standardize testing. Heparin is greatly affected by weight, is not well absorbed by the gastrointestinal tract, and is affected by liver function as well as by the concentration of antithrombin that is required for binding. There is no dose-response relationship, meaning that each patient will react differently to a dose.

The Common Pathway

The common pathway is the part of the cascade where the intrinsic and extrinsic pathways merge and factors I, II, V, and X are measured. However, it is important to know that the PT and the APTT will not detect qualitative or quantitative platelet disorders or a Factor XIII deficiency. Factor XIII is fibrin stabilizing factor; this is responsible for stabilizing a soluble fibrin monomer to form an insoluble fibrin clot. If a patient is Factor XIII deficient, the patient will form a clot but will not be able to stabilize the clot and bleeding will occur later.

Inhibitors

Inhibitors are soluble plasma proteins that act as natural anticoagulants; they prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control: the protease inhibitors and the protein C pathway. The protease inhibitors inhibit coagulation factors; they include antithrombin III, heparin cofactor, tissue factor pathway inhibitor, and alpha 2 protease inhibitor. The protein C pathway causes inactivation of activated cofactors; these include protein C, its cofactor of protein S, as well as activated protein C.

It is important for the coagulation laboratory to communicate to the blood bank the type of reagent that is used for coagulation studies and how it performs. When a lot of reagent is received from a manufacturer, it is assumed that a normal result obtained on running the PT or APTT will correspond to a normal amount of factor level reflecting about 50 percent of a factor level. Patients will do well with 30 to 40 percent of a factor level. Therefore, a normal PT and APTT should minimally be able to reflect a 30 to 40 percent factor level. These screening test results are

Table 2. Relationship of factor level to APTT results

Pooled Normal Plasma (%)	APTT (seconds)*
100	32.4
75	33.0
50	34.0
25	34.5 [†]
12	36.0

*normal range 24.3 to 35.4 sec.

[†]normal APTT; only 25% of factor; patient will bleed.

used by physicians to determine if a patient may bleed in surgery. However, reagents can vary in the amount and concentration of phospholipids. It is possible to obtain a lot of reagents from a manufacturer that is insensitive to certain factors. For example, a patient sample is tested preoperatively with an APTT result of 34.5 seconds, the upper limit of the normal range is 35.4 seconds; therefore, the patient result is within the normal range. The patient has a normal PT. The information from these tests tells us that all factors in the intrinsic, extrinsic, and common pathways appear to be normal. The patient is cleared for surgery. During the surgery the patient bleeds. There were no platelet problems. The problem is that the reagent used to test the APTT has a poor sensitivity to Factor IX, resulting in the inability of the APTT to detect an abnormal Factor IX level. It is important to understand how the reagent performs, as illustrated in Table 2.

Having a basic understanding of how coagulation testing works and its relation to blood bank outcomes can improve the understanding of patient results by both departments.

Donna D. Castellone, MS, MT(ASCP)SH, New York-Presbyterian Hospital—Weill Cornell Medical Center, New York City, New York.

Replacement Bodies

The vacancies of medical technologists (MT) and clinical laboratory scientists (CLS) in 2000 were frightening to everyone; many presentations and initiatives were implemented. The vacancy rate for MT and CLS staff members was 14 percent in 2000 but, since then, it has dropped to 4.3 percent according to

the 2003 wage and vacancy survey by the American Society for Clinical Pathologists. The need for replacement bodies in blood banking has been a concern as the number of specialist in blood banking (SBB) programs has diminished drastically since 1984. The average age of MT and CLS staff members has been estimated to be 51. The baby boomers are due to retire and they will take with them the knowledge and love of antibody identification. The question remains: What is the blood bank community doing to educate and encourage MT and CLS graduates to specialize in blood bank technology? The SBB programs have reviewed this question and some have decided to try a distance format for educating students. Currently there are six programs offering distance education in blood banking. The average number of people sitting for the SBB ASCP exam is 125 each year, with less than 40 percent passing. For graduates of a Commission on Accreditation of Allied Health Education Programs (CAAHEP), the pass rate increases to 75 percent for those passing the ASCP SBB exam. It was estimated that 9000 MT and CLS graduates will be needed in the year 2010. Currently, about 2000 people are taking the MT ASCP examination each year. There will be a shortage. For the first time ever, the workforce includes four generations: those over 60 are working alongside baby boomers as well as with Generation X and Generation Y individuals.

What can be done for a better future? At the national level, organizations need to support education and provide unity for the profession. At the hospital and program level, more attention needs to be paid to the needs of the Generation Y employees by looking at flexible scheduling, clearly defined employee roles, and rewarding achievements. As individuals, we need to encourage young people to join the field, to be mentors, and to be active in our organizations. Shortages in MT and CLS staff members seem to be looming on the horizon. New ways must be explored to educate and to keep employees interested in the field.

Janet Vincent, MS, SBB(ASCP), University of Texas Medical Branch, Galveston, Texas.

IN MEMORIAM

Scott Murphy, MD 1936–2006



Scott Murphy, an internationally respected authority on platelet transfusion medicine and platelet preservation, died on April 13, 2006, in Philadelphia, Pennsylvania, after a long battle with lymphoma.

Dr. Murphy, a Philadelphia native, received his bachelor of arts degree from Yale University as a member of Phi Beta Kappa and his medical degree from Columbia University. He had held a faculty appointment at Jefferson Medical College of Thomas Jefferson University and was adjunct professor of medicine at the University of Pennsylvania School of Medicine.

He also had held many prestigious hospital administrative positions including director of Hematology-Oncology Division, Presbyterian University of Pennsylvania Medical Center; acting director of the Department of Medicine, Presbyterian University of Pennsylvania Medical Center; and member and associate director of Clinical Programs, Cardeza Foundation of Hematologic Research, Jefferson Medical College of Thomas Jefferson University Hospital.

In 1994, Dr. Murphy became chief medical officer of the American Red Cross (ARC) Blood Services, Penn-Jersey Region. He continued his platelet research at the ARC for more than 10 years, as well as assuming his duties as medical officer.

He served on many committees, including the Ad Hoc Committee on Blood Component Therapy and Annual Meeting Program Committee of the AABB; chairman of the Biomedical Excellence for Safer Transfusion Collaborative (BEST); and the Scientific Council of the ARC. He assumed medical editorship of the ARC journal, *Immunohematology*, first as guest medical editor in 2004 and then as senior medical editor through 2006.

Dr. Murphy studied platelet storage and survival for more than 30 years and was recognized as a worldwide expert. He authored more than 90 original papers, chapters, editorials, and reviews. He was asked to speak all over the world and received many awards for his work; in 1998 he received the most prestigious award given by the AABB, the Karl Landsteiner Award. He also received the Charles R. Drew Award from the ARC in 2005.

Dr. Murphy was a unique individual in many ways and he will be remembered for much more than his scientific contributions. Those fortunate enough to have known him will always have the memory of a man who loved his family, always had a great smile and an elegant bow tie, and knew good food and wine. He will be greatly missed for more reasons than these.

IN MEMORIAM

L. Ruth Guy, PhD 1913–2006

Ruth Guy died on May 3, 2006, in Dallas, Texas. She was born in Kemp, Texas, graduated from Kemp High School, and received her undergraduate degree in 1934, her masters' degree in 1949 from Baylor University, and her doctoral degree from Stanford University in 1953. She returned to Dallas in 1953 where she began research at Woodlawn Hospital and teaching at Parkland Hospital School of Nursing, now known as University of Texas (UT) Southwestern. She served as associate director of Parkland Blood Bank for 25 years. Dr. Guy and Dr. E. E. Muirhead founded the School of Medical Technology, which became part of UT Southwestern; Dr. Guy became the first chairman of the Department of Medical Technology. Subsequently, a Specialist in Blood Bank Technology program was formed. Graduates of both programs contributed funds to establish the L. Ruth Guy Professional Development Award to subsidize the continuing education of an outstanding student. Dr. Guy trained hundreds of students in these programs. The Parkland Hospital Board of Managers recognized her scientific contributions, including more than 40 original papers, self-instructional manuals for medical students, workshops, local, state, national, and international lectures, and her teaching skills, by presenting her with a formal Resolution of Appreciation.

Credited with using the workshop method for educating laboratorians to current methodologies and concepts, she urged the AABB to do the same. In 1966, she presented the blood component workshop, which was a great success. The workshop concept caught on in many organizations. The AABB presented Dr. Guy with the prestigious John Elliot Award in 1973 for her work and the American Society of Clinical Pathologists (ASCP) presented her with the Recognition Award in 1978. They also presented her with a unique recognition, an Honorary Fellowship in the ASCP.

Dr. Guy was a Renaissance woman. She was an award-winning painter, using oil and watercolors, a gardener, a member of the Abilene women's polo team, and a milliner of note. She also had been on the Advisory Board of the Dallas Big Sisters, and president of the Business and Professional Women's Club of Dallas, from which she received the Award of Excellence and was named Woman of the Year. She was also chairman of Business Women in Arts, on the Board of the Repertory Theater, and a member of the Royal Haven Baptist Church, just to mention a few. She was listed in American Women of Science in 1955, in Who's Who of American Women in 1968, and in The Two Thousand Women of Achievement in 1970.

Professionally, she served on the editorial boards of the American Journal of Clinical Pathology and Laboratory Medicine; was president and served on committees of the South Central Association of Blood Banks, and was chairman of many committees and on the Board of Directors of the AABB.

She was not only a unique person but an asset to the field of transfusion medicine.

SPECIAL ANNOUNCEMENTS

September 14 Annual Symposium

The 25th annual symposium of the National Institutes of Health, Department of Transfusion Medicine: Immunohematology and Blood Transfusion will be held on September 14, 2006. This event will be cohosted by the Greater Chesapeake and Potomac Region of the American Red Cross and is free of charge; advance registration is encouraged. For more information, **contact** Karen Byrne, NIH/CC/DTM, Building 10/Room 1C711, 10Center Drive MSC 1184, Bethesda, MD 20892-1184, or KByrne@mail.cc.nih.gov or visit the Web site at <http://www.cc.nih.gov/dtm> >education.

March 23-25 AABB Immunohematology Reference Laboratory (IRL) Conference 2007

The AABB Immunohematology Reference Laboratory (IRL) Conference 2007 will be held March 23 through 25, 2007, at the Hyatt Regency in Albuquerque, New Mexico. Continuing education credits will be provided. Registration will begin in January 2007. For more information, **contact** the AABB Department of Meetings and Programs at (301) 215-6480 or, beginning in October 2006, visit the Web site at <http://www.aabb.org> >meetings and events >national and regional conferences.

IMPORTANT NOTICE ABOUT MANUSCRIPTS

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Please **e-mail** all manuscripts for consideration to Marge Manigly at mmanigly@usa.redcross.org

Phone, Fax, and Internet Information: If you have any questions concerning *Immunohematology*, *Journal of Blood Group Serology and Education*, or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org

CLASSIFIED AD

DEPARTMENT OF CLINICAL LABORATORY SCIENCES
SCHOOL OF ALLIED HEALTH PROFESSIONS
VIRGINIA COMMONWEALTH UNIVERSITY

Faculty Position

The Department of Clinical Laboratory Sciences at Virginia Commonwealth University invites applications for a full-time, 12 month, tenure-track faculty position. The Department, located on the MCV Campus of VCU, is one of nine departments in the School of Allied Health Professions. VCU is a large urban, research-extensive institution with a richly diverse university community and commitment to multicultural opportunities. The Department offers both B.S. and M.S. degree programs in Clinical Laboratory Sciences and provides the CLS specialty track in the Ph.D. program in Health Related Sciences.

The successful candidate will be responsible for teaching clinical immunology and immunohematology (blood banking) courses on campus and on line at the undergraduate and graduate levels, interacting with clinical faculty at affiliated clinical sites, and student mentoring. Also expected are scholarly activities and research, university service responsibilities, and professional activities.

Applicants must have a master's degree (Ph.D. preferred), national certification as a generalist in the clinical laboratory, clinical or college teaching experience, excellent interpersonal and written and oral communication skills, and demonstrated scholarly productivity. Preference will be given to applicants with specialist certification in blood banking and a record of active participation in professional societies.

Salary and rank will be commensurate with education and experience.

Review of applications will begin immediately and continue until the position is filled. Send a letter of interest, curriculum vita, and the names of three references to: William Korzun, Ph.D., Department of Clinical Laboratory Sciences, Virginia Commonwealth University, P O Box 980583, Richmond, VA 23298-0583.

"Virginia Commonwealth University is an equal opportunity/affirmative action employer. Women, minorities and persons with disabilities are encouraged to apply."

Free Classified Ads and Announcements

Immunohematology will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) **without charge**. Deadlines for receipt of these items are as follows:

Deadlines

- 1st week in January for the March issue
- 1st week in April for the June issue
- 1st week in July for the September issue
- 1st week in October for the December issue

E-mail or fax these items to Cindy Flickinger, Managing Editor, at (215) 451-2538 or flickingerc@usa.redcross.org.

ANNOUNCEMENTS

Monoclonal antibodies available at no charge:

The New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for donor screening and for typing RBCs with a positive DAT. These include anti-A1, -M, -s, -U, -D, -Rh17, -K, -k, -Kp^a, -Js^b, -Fy3, Wr^b, -Xg^a, -CD99, -Do^b, -H, -Ge2, -CD55, -Ok^a, -I, and anti-CD59. Most of the antibodies are murine IgG and require the use of anti-mouse IgG for detection (Anti-K, k, -Kp^a, and -Fy^a). Some are directly agglutinating (Anti-M, -Wr^b and -Rh17) and one has been humanized into the IgM isoform (Anti-Js^b). The antibodies are available at no charge to anyone who requests them. Please visit our Web site for a complete list of available monoclonal antibodies and the procedure for obtaining them.

For additional information, contact: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021 / e-mail: ghalverson@nybloodcenter.org or visit the website at <http://www.nybloodcenter.org> >research >immunochemistry >current list of monoclonal antibodies available.

Manuscripts: The editorial staff of *Immunohematology* welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. **Deadlines** for receipt of manuscripts for consideration for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. For instructions for scientific articles, case reports, and review articles, see "Instructions for Authors" in every issue of *Immunohematology* or on the Web. **Include fax and phone numbers and e-mail address with your manuscript.**

ANNOUNCEMENTS CONT'D

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The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:

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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

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SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Type the manuscript on white bond paper (8.5" × 11") and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. 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—see 7 under Preparation
8. Figures—see 8 under Preparation

Preparation of manuscripts

1. Title page
 - A. Full title of manuscript with only first letter of first word capitalized (bold title)
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 - C. Running title of ≤ 40 characters, including spaces
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2. Abstract
 - A. One paragraph, no longer than 300 words
 - B. Purpose, methods, findings, and conclusions of study
3. Key words—list under abstract
4. Text (serial pages)

Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.

 - A. Introduction

Purpose and rationale for study, including pertinent background references.
 - B. Case Report (if study calls for one)

Clinical and/or hematologic data and background serology.
 - 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

Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.

5. Acknowledgments

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- B. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
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8. Figures

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9. Author information

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1. Heading—To the Editor:
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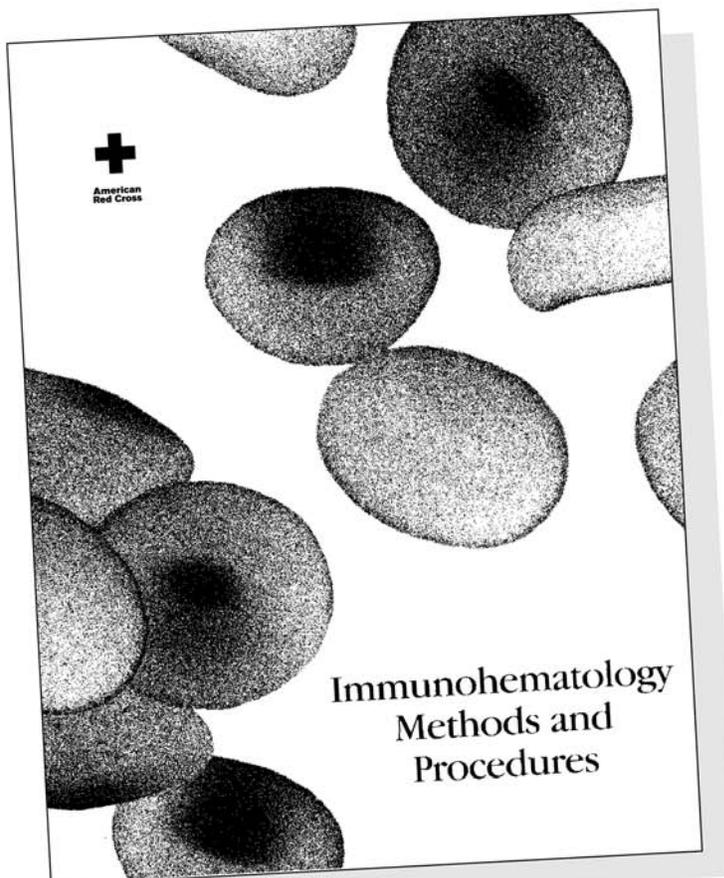
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