Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 17, NUMBER 1, 2001

American Red Cross

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Immunohematology is published quarterly (March, June, September, and December) by the American Red Cross, National Headquarters, Washington, DC 20006.
The contents are cited in the EBASE/Excerpta Medica and Elsevier BIOBASE/Current Awareness in Biological Sciences (CABS) databases.

The subscription price is
$30.00 (U.S.) and $35.00 (foreign) per year.

Subscriptions, Change of Address, and Extra Copies:
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Or call (215) 451–4902
Web site: redcross.org/immunohematology

ISSN 0894-203X

Red blood cell antigen changes in malignancy: case report and review

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Red blood cell (RBC) antigens represent inherited traits and as such, their expression should be constant throughout the life of an individual. We describe a patient in whom the expression of the Rh D and C antigens was lost due to the development of chronic myelogenous leukemia (CML). For this patient, this represented more than a blood bank curiosity but was of critical importance in determining further treatment of the leukemia.

The mechanisms behind changes in RBC antigens due to malignancy are reviewed for a number of antigens, antigen systems, and antigen collections. Previous case reports of RBC antigen changes due to malignancy are summarized.

Key Words: leukemia, erythrocytes, antigens, mutation, ABO blood group system, Rh blood group system, MNS blood group system, Lewis blood group system, I blood group collection, chromosome abnormalities

Case Report

A 48-year-old Caucasian woman presented to a referring clinic with a 3-month history of increasing malaise and poor energy. She had experienced recent bruising and abdominal pain. Her past medical history revealed a well adult with prior surgeries including total abdominal hysterectomy and tonsillectomy/adenoidectomy and no prior hospitalizations for medical illnesses. She was on no routine medications. She denied exposure to known marrow toxins. Her family history revealed her to be one of a set of identical twins. Significant physical exam findings included multiple bruises on the extremities and a palpable spleen. Hemogram showed a white blood cell count of 225,200/mL (normal 4,800 to 10,800/mL), hematocrit of 36.8 percent (normal 37 to 47%), hemoglobin of 12.8 g/dL (normal 12 to 16 g/dL), and a platelet count of 468,000/mL (normal 130,000 to 400,000/mL). White cell differential showed 65 percent neutrophils (normal 40 to 70%), 3 percent metamyelocytes (normal 0%), 15 percent myelocytes (normal 0%), 7 percent lymphocytes (normal 24 to 44%), 1 percent monocytes (normal 3 to 7%), 4 percent basophils (normal 0 to 1%), 4% eosinophils (normal 0 to 3%), and 1 percent blasts (normal 0%). A bone marrow biopsy and aspirate were obtained and demonstrated a hypercellular (100%) bone marrow with histologic and flow cytometric features consistent with CML. Cytogenetic analysis revealed the presence of the Philadelphia chromosome (t [9;22]), consistent with CML. No further abnormalities, including abnormalities of chromosome 1, were noted on karyotype analysis. Subsequent molecular studies from peripheral blood confirmed the presence of BCR/ABL fusion product. The patient was treated with hydroxyurea for normalization of peripheral blood counts.

Following initial presentation and management, the patient was referred to our center to be evaluated for syngeneic hematopoietic stem cell transplantation as definitive therapy for her diagnosis. The patient's physical examination and pretransplant organ function studies were normal. Results from physical examination and donor evaluation studies of the patient's sibling were also normal. The twins physically appeared to be identical. Molecular typing of the sisters for HLA revealed them to be identical (6 out of 6 match at the *HLA-A, HLA-B*, and *HLA-DR* loci). Additional molecular studies were undertaken to prove that the sisters were identical twins. These consisted of comparing four variable number of tandem repeats (VNTRs) and nine short tandem repeats (STR) loci. The two were identical at all 13 loci tested. However, ABO and Rh typing by serologic methods revealed that the patient typed as A, D– whereas her sister typed as A, D+. In order to resolve this apparent discrepancy, additional testing was performed. A new specimen was obtained from each sister that confirmed the previous ABO and Rh typing. Weak D testing with appropriate controls was

performed on the patient and was negative. An extended RBC phenotype was performed and is given below:

Patient: D–, C– E–, c+, e+, M+, N+, S+, s–, K–, k+, Fy(a+b+), Jk(a+b+)

Sister: D+, C+, E–, c+, e+, M+, N+, S+, s–, K–, k+, Fy(a+b+), Jk(a+b+)

Additional history was obtained from the patient and from review of outside medical records. As opposed to her current D– typing, the patient had typed as D+ at the birth of her second child in 1982 and also at four whole blood donations between 1984 and 1989. Therefore, further testing was initiated. Additional studies included molecular genotyping for the *RHD* gene via polymerase chain reaction. This revealed that both the patient and her sister were D+ by molecular methods (amplification of *RHD* exons 7 and 10). Finally, adsorption and elution studies using anti-D and anti-C demonstrated that the patient's RBCs could adsorb and elute both anti-D and anti-C. Taking into consideration the results of all tests and historical events recorded, we concluded that the patient and her sister were identical twins.

There are significant clinical implications surrounding the determination of identical twin status. Hematopoietic stem cell transplant (HSCT) for chronic phase (CP) CML with syngeneic versus allogeneic donor stem cells has implications on the implementation of graft-versus-host disease (GVHD) prophylaxis as well as expected outcome from therapy. No GVHD prophylaxis is warranted in the syngeneic setting. Clearly, this is not the case in allogeneic transplants in which the presence of GVHD posttransplant is readily recognized as a cause of transplant-related toxicity and mortality. Syngeneic transplantation with an effective conditioning regimen has a high probability of curing patients with CML in the CP. That said, data analyzed and published by the International Bone Marrow Transplant Registry showed identical twin transplants in CML to be associated with increased relapse risk when compared with HLA-identical sibling transplants.[1] However, they reported leukemia-free survival to be similar after twin and HLA-identical sibling transplants. This is due to the increased relapse potential in identical twin transplants being offset by decreased treatment-related mortality. Our patient remains free of hematologic, morphologic, and cytogenetic evidence of disease 100 days following syngeneic HSCT. Molecular studies of bone marrow aspirate for the presence of the BCR/ABL fusion product remain positive.

Changes in Red Blood Cell Antigens With Malignancy

Either loss or diminished expression of RBC antigens have been reported to occur in a number of malignancies (both hematologic and solid; see Table 1). The first report,[2] and those which make up the majority of reports,[3–6] have involved alterations in the expression of ABO blood group antigens. In addition to the ABO blood group system, multiple other antigens and antigen systems/collections have been reported to be affected by malignancy (see Table 1).

These changes in blood group antigens can result from a number of mechanisms; the mechanism involved depends on the antigen involved as well as on the disease. For hematopoietic diseases, the loss of expression predominantly results from a mutation within a stem cell that affects antigen production. If the affected clone gives rise to a significant proportion of the RBCs, it may result in the appearance of a mixed population of antigen-negative and antigen-positive RBCs. If the clone has given rise to all or the majority of the RBCs present, then complete loss of the antigen occurs.

ABO system

Loss or weakening of ABO antigens presents as a discrepancy in the forward and reverse typing of patients. Because ABO typing is routinely performed in pretransfusion testing, ABO antigens are the most frequently reported blood group antigen change identified in malignancy. In order to detect changes in other systems or antigens, one must either have known the patient's previous phenotype and observed a change or detect a return to their original phenotype during the course of treatment.

The production of ABO antigens results from the interactions of two glycosyl transferases. The first enzyme, *H* transferase, adds a fucose to the terminal galactose of precursor substance. The H substance generated is then acted on by the *A* and/or *B* transferases that add an N-acetylgalactosamine or a galactose, respectively.

In leukemias and other hematopoietic disorders, two possible mechanisms exist to explain the weakening of ABO antigens. In the first, the *A* and/or *B* transferase are inactivated. The result is weakening or loss of the A and/or B antigens with a concurrent increase in H antigen

Table 1. Red blood cell antigens reported to be altered by malignancy

Antigen, antigen system, or antigen collection	Effect of malignancy	Chromosomal location	Malignancies reported to be associated with changes
ABO	Decreased A and/or B with increased H	*A* and *B* transferase gene – *9q34*	AML CML
	Decreased A and/or B with decreased H	Fucosyl transferase *H* gene – *19q13*	MDS Hodgkin's disease
	"Apparent" loss of A and/or B		Gastric carcinoma Pancreatic carcinoma Ovarian carcinoma Colon carcinoma Cholangiocarcinoma
Rh	Decrease/loss of D Decrease/loss of E Decrease/loss of C Combinations of the above	*1p36*	AML CML Myeloid metaplasia Polycythemia vera Myelofibrosis Chronic myelomonocytic leukemia
Lewis	Loss of Lewis antigens	Lewis gene – *19p13* Secretor gene – *19q13*	AML CML Gastric carcinoma
Ii	Decreased I and increased i Increased i with normal I	Unknown	AML CML ALL CLL
MNSs	Loss of s	*4q28*	CML
LW	Loss of LW antigens	*19p13*	Hodgkin's disease Non-Hodgkin's lymphoma
Colton	Loss of Colton antigens	*7p14*	MDS with monosomy 7
AnWj (Anton)	Loss of AnWj	Unknown	Hodgkin's disease Non-Hodgkin's lymphoma
Cromer	Loss of Cromer antigens	*1q32*	PNH MDS
Cartwright	Loss of Cartwright antigens	*7q22*	PNH MDS
Dombrock	Loss of Dombrock antigens	*12p13*	PNH MDS
JMH (John Milton Hagen)	Loss of JMH	*15q23*	PNH MDS
Tn antigen	Expression of Tn antigen	_____	AML Myelofibrosis MDS

AML: acute myelogenous leukemia; CML: chronic myelogenous leukemia; ALL: acute lymphocytic leukemia; CLL: chronic lymphocytic leukemia; MDS: myelodysplastic syndrome; PNH: paroxysmal nocturnal hemoglobinuria

because H antigen is no longer converted to A and B antigen, a finding that has seen by a number of authors.[5,7–10] It has been suggested that, at least in CML, such a change may result from inactivation of the *A* and/or *B* transferase genes on chromosome 9 through the generation of the 9;22 chromosomal translocation (Philadelphia chromosome).[11] The gene encoding the *A* and *B* transferases is located at 9q34, the area in which the break point for the chromosomal translocation occurs. As a result, the translocation could disrupt the gene, thereby preventing expression of the transferase. Although this is a possibility, ABO changes are more commonly seen in acute myelogenous leukemia (AML), where this chromosomal translocation is uncommon.[9]

One study of 12 patients with acute myelogenous leukemia (AML) and weakening of the ABO antigens suggested that the *ABO* gene inactivation was not random. In four of four patients studied, only the maternally derived *A* or *B* gene was affected. This suggested the presence of genomic imprinting, defined as the differential expression of a gene as determined by whether it is inherited from the mother or father, in determining which gene was inactivated. The authors of this report realized that additional study was needed, given the small number of patients examined.[12]

The second possible mechanism for the loss of ABO antigens involves the inactivation of the *H* transferase encoded at 19q13. The loss of *H* transferase would result in decreased H substance and a resulting decrease in *A* and/or *B* substance, again identified in a number of patients.[7,9–13]

Loss of ABO antigens is of more than academic interest. In a number of cases, the loss or weakening of ABO antigens has been detected prior to the diagnosis of the underlying hematopoietic malignancy, frequently in the setting of long-standing myelodysplasia.[10,14,15] It has been suggested that the identification of a loss of ABO antigens should result in the search for an underlying hematopoietic malignancy. Finally, changes in ABO antigens have also mirrored the course of the malignancy with return of the original blood type upon remission and reappearance of antigen-negative cells with recurrence.[6–16]

In the case of solid tumors such as pancreatic, gastric, colonic, ovarian, and biliary carcinomas, an apparent loss of ABO antigens also can be seen.[16] The term "apparent" is used because unlike in hematopoietic malignancies, the number of *A*, *B,* and *H* antigens on the RBCs is not altered. Instead, the tumors secrete large amounts of soluble *A* and/or *B* substance. The soluble blood group substance that neutralizes the typing reagents resulting in the apparent loss of A and B antigens can be overcome by washing the RBCs thoroughly prior to forward typing in order to remove the plasma with its soluble blood group substance.[17]

This finding is usually detected as a discrepancy between the forward and reverse typing of the patient.

Rh system

The loss or weakening of the D antigen, after ABO, is the second most commonly reported change in blood group antigens. Again, this may be because the presence or absence of D is routinely determined and therefore changes are noted. In addition to the loss of D,[18–24] loss or weakening of other Rh system antigens including C and E have been reported.[25–28] Frequently, this has occurred with concurrent loss or weakening of D.[25,27,28] Patients have presented either with a complete loss of selected Rh system antigens[20,23–25,28] or with the development of a mixed population of antigen-positive and antigen-negative cells (frequently referred to as mosaicism).[18,19,21,22,26,27]

The antigens of the Rh system are multi-pass proteins encoded by two genes located at 1p36. The *RHD* gene encodes the antigen D whereas the *RHCE* gene encodes the antigens C, c, E, and e. Rh negative individuals either lack the *RHD* gene or have mutations within the gene that prevent D antigen production. The mechanism of the loss or weakening of D and the other Rh antigens is thought to be disruption or mutation of these genes. In three reported cases of loss of the D antigen, chromosomal abnormalities involving either the entire chromosome 1[26] or the short arm of chromosome 1[20,23] were seen on cytogenetic analysis. These were thought to be responsible for the loss of antigen expression through deletion or disruption of the *RHD* gene. In another report,[24] cytogenetic abnormalities were not identified but sequencing of the *RHD* gene revealed a single base pair deletion within the gene. This deletion resulted in a frameshift and a premature stop codon, eliminating production of the D antigen.[24]

As with changes in ABO antigens, changes in Rh antigens have been reported to mirror the course of the disease.[21,23,25] When patients enter remission, their original Rh phenotype returns, whereas antigen-negative cells reappear during relapse.

In one reported case, the disappearance of the D antigen was associated with the subsequent development of anti-D.[20] This patient suffered from myeloid metaplasia, a chronic myeloproliferative disorder, and was noted to have lost expression of the D antigen at age 37; he had been typed as D+ at age 33. Twenty years later, at age 57, he was found to still be D– but had developed anti-D as well as anti-C. The authors postulated that because of the prolonged course of the illness, the patient lost tolerance to the D antigen.[20]

Lewis system

Lewis antigens are not intrinsic to the RBC surface but are adsorbed onto the surface of RBCs from the surrounding plasma. Lewis antigens are produced by the interactions of two fucosyltransferases. Le^a is produced when the fucosyltransferase encoded by the Lewis gene (*Le*) at 19q13 adds a fucose to type 1 chain. In the presence of the secretor gene (*Se*), a fucose is added to the terminal galactose of the type 1 chain to generate type 1 H substance. This substance can then be acted upon by the Lewis gene (*Le*) to produce Le^b.

As Lewis antigens represent extrinsically acquired RBC antigens, it would not be expected that hematologic malignancies would affect the expression of Lewis antigens. Cases of loss of Lewis antigens with leukemia have been reported.[3,29] The mechanism behind this loss is unknown but the authors of these reports postulated that the loss of the ABO antigens in these patients may have interfered with the binding of soluble Lewis substance to the red blood cell surface.[3,29]

Abnormalities of RBC Lewis antigens adsorbed onto RBCs also have been reported with gastric carcinomas. An increased frequency of Le(a–b–) phenotype has been seen in gastric cancer patients (16% versus 6.3% of healthy controls).[30] It was therefore thought that the Le(a–b–) phenotype represented a risk factor for the development of gastric carcinoma. Csato et al.[30] examined the saliva of Le(a–b–) gastric cancer patients and healthy controls for the presence of Le^a antigen and discovered 55.3 percent of the Le(a–b–) gastric cancer patients secreted Le^a antigen in their saliva versus 4.3 percent of healthy controls. This indicated that half of the Le(a–b–) gastric cancer patients possessed the *Le* gene and therefore should have at least Le^a substance on their RBCs, depending upon their *Se* gene status.[30]

Ii collection

I and i antigens are located on the subterminal portions of the oligosaccharides that are converted to H and subsequently A or B antigens. The chromosomal location of the genes encoding the production of these oligosaccharide antigens is unknown. The I antigen is found in 99.9 percent of adults but is not found on cord RBCs. Conversely, i is present on cord RBCs but is poorly expressed in adults. During the first 2 years of

life, the amount of I present on RBCs increases while the amount of i decreases in a reciprocal fashion.

Changes in the level of I and i antigens, both increases and decreases, have been reported in association with leukemia. Schmidt et al.[31] reported decreases in I antigen among 30 percent of patients with CML, AML, chronic lymphocytic leukemia (CLL), and acute lymphocytic leukemia (ALL). Others have also reported a decrease in I antigen.[3] Jenkins et al.[32] and Kolins et al.[29] reported not only a decrease in I in patients with leukemia, but also a reciprocal increase in i. The mechanism behind these changes is uncertain but may represent a response to hematopoietic stress,[32] as an increase in i with a stable expression of I has been reported to occur in some nonneoplastic diseases.[33] Alternately, it has been suggested that loss of specific transferases may occur or that failure to form normal antigen configurations of ABH, Lewis, and I antigens may result in antigen loss.[3,29,32] Jenkins et al.[32] found weakening of the A antigen in addition to decreased I antigen and increased i antigen. In the cases reported by Kolins et al.[3,29] in 1978 and 1980, ABH and Lewis antigens were also lost.

MNS system

MNS are antigens located on glycophorins A and B, which are single-pass transmembrane glycoproteins. The genes encoding these antigens, *GYPA* and *GYPB*, are located at 4q28.

A single report has appeared where the weakening of s antigen was noted in a patient with CML.[21] The patient presented with CML and was noted to demonstrate mixed field typing for D antigen. Extended red blood cell antigen typing showed the patient to be M+, N–, S+, and s–. Subsequent evaluation of the MNSs phenotypes of the patient, his wife, and their children suggested that the patient should be s+. Microscopic examination of the patient's RBCs using different anti-s reagents demonstrated mixed-field agglutination. Adsorption and elution studies similarly demonstrated the presence of the s antigen.[21] A Philadelphia chromosome was noted in this patient but abnormalities of chromosome 4, the location of the gene coding for this antigen, were not seen.

Landsteiner-Wiener (LW) system

LW system antigens are glycoproteins similar to adhesion molecules and are encoded by a gene located at 19p13. They are intimately associated with Rh system antigens and are a part of the complex formed by Rh system antigens on the RBC membrane.

Two reports of loss of LW antigens and concurrent development of anti-LW in association with hematologic malignancies have appeared. The first report involved a patient with mixed cellularity Hodgkin's disease. At the time of presentation with his illness, he was found to have anti-LW and his RBCs were LW–. Approximately 1 year later, he was found to be LW+ and his anti-LW was no longer detectable.[34] The second report involved a patient with a T-cell non-Hodgkin's lymphoma. Again, the patient was noted to have an antibody that was identified as anti-LW^a. RBC typing revealed her RBCs to be LW(a–). Following chemotherapy and remission of her lymphoma, the patient was noted to have lost her anti-LW^a and to type as LW(a+). Subsequently, her lymphoma recurred and she was again noted to be LW(a–) with anti-LW^a. Again, remission was obtained, her antibody disappeared, and the antigen reappeared.[35]

The mechanism behind the decreased expression of LW antigens is unknown. In addition to the cases reported above, the loss of LW antigens with the development of anti-LW also has been seen during pregnancy[36] and in association with autoimmune diseases.[37]

Colton system

Colton system antigens are found on the membrane portion of the RBC water transporter, CHIP-1. They are encoded by a gene located at 7p14.

Loss of the Colton system antigen Co^a has been reported in the setting of myelodysplastic syndrome with monosomy 7, a common chromosomal abnormality in this disorder. The association was initially identified in two of five patients with monosomy 7.[38] Because Co^a is a high-incidence antigen, the absence of expression in these patients suggested an association. Additional patients were subsequently identified.[39,40]

The mechanism behind the loss of Co^a antigen is unknown. It is difficult to explain how loss of only one copy of a gene could result in loss of antigen expression given the fact that the patients still possess another copy on the other chromosome 7. Possibilities could include mutations in the other Colton gene or genomic imprinting with silencing of the other gene. Even more difficult to explain is the finding of Pasquali et al.[40] that only patients with monosomy 7 who had not received transfusions prior to testing lacked Co^a antigen. The authors postulated that either the transfusion of Co(a+)

RBCs prevented recognition of RBCs weakly expressing Co^a or that the transfusions somehow resulted in the expression of the antigen on the patient's RBCs.

AnWj (Anton)

AnWj (Anton) is a high-incidence antigen and is the receptor for *Haemophilus influenzae*. The location of the gene encoding AnWj is unknown but the antigen is carried on CD44 proteoglycan.[41]

A single case of loss of the AnWj antigen and concurrent development of anti-AnWj has been reported. The patient was a male with Hodgkin's disease. At presentation, an antibody was identified in his serum that reacted with all RBCs. Subsequently, this was identified as anti-AnWj and the patient was found to be AnWj–. Six months after the initiation of therapy, the patient went into remission. At that time, anti-AnWj was no longer detectable and his RBCs typed as AnWj+.[42]

Cromer, Yt, and Dombrock systems, JMH antigen

Cromer, Yt, and Dombrock system antigens, as well as the high-incidence antigen John Milton Hagen (JMH), are encoded by different genes on different chromosomes. Cromer antigens are located on the complement regulatory molecule CD55, whereas Yt antigens are located on RBC acetylcholinesterase. The functions of the molecules carrying Dombrock system antigens and the JMH antigen are unknown. While representing a heterogeneous group of antigens, they do share one common feature: they are all attached to the RBC membrane by glycosylphosphatidylinositol (GPI) anchors.

Paroxysmal nocturnal hemoglobinuria (PNH) is a disorder characterized by hemoglobinuria, thrombosis, and bacterial or fungal infections. In 25 percent of cases, it can evolve into aplastic anemia and in 5 to 10 percent of cases, it can progress to AML.[43] The biochemical defect in PNH is a stem cell mutation leading to the loss of the GPI anchors with resulting loss of proteins requiring this for membrane attachment. As a result, antigens of the Cromer, Yt, and Dombrock systems as well as the JMH antigen are lost from RBCs in PNH. In fact, the loss of Cromer antigens is intimately involved in the hemolysis seen in this disorder. Cromer antigens are epitopes on CD 55, also called decay-accelerating factor. CD55 and CD59, membrane inhibitor of reactive lysis, are responsible for inactivating complement that accumulates on RBCs. The loss of these molecules results in complement build up and complement-mediated cell lysis of the RBCs.[43]

In addition to occurring in PNH and aplastic anemia, loss of GPI-anchored proteins also has been seen in 23 percent of myelodysplastic patients.[44] These patients also will demonstrate loss of Cromer, Yt, and Dombrock system antigens as well as the JMH antigen. Identification of these patients is important because many will respond to immunosuppressive therapy, which is a therapy used for aplastic anemia but one not commonly used for myelodysplasia.[44]

Tn antigen

Tn antigen is a cryptic antigen that is present in the tetrasaccharide side chains attached to glycophorins A and B of the RBC membrane. Its expression results from the loss of the enzyme 3-*ß*-D-galactosyltransferase (T transferase) due to somatic mutation within the bone marrow stem cells.[45] All normal adult sera contain antibodies that recognize Tn, resulting in polyagglutination of RBCs expressing the antigen. In addition, Tn antigen is similar to A antigen and can react with anti-A and anti-A,B.[46] As in the case of loss of A and B antigens, the result is discrepant forward and reverse typings. Tn antigen activation can be identified by its pattern of reactivity with a variety of lectins. This can be used to distinguish Tn expression from other causes of polyagglutinability. Tn activated cells are agglutinated by *Dolichos biflorus*, *Glycine max*, *Salvia sclarea*, and *Salvia horminum* but not *Arachis hypogaea*.[46]

Tn antigen expression has been associated with leukemia,[47–49] myelodysplasia,[50] and myelofibrosis.[51] In a study examining bone marrow and peripheral blood of normal individuals and patients suffering a variety of hematologic disease, Roxby et al.[49] found no evidence of Tn activation in 35 normal subjects and expression in 5 of 725 patients with hematologic disease. Of these five, only two were detectable by polyagglutination alone. Both of these patients had AML and both exhibited increases in the number of RBCs expressing Tn antigen as their leukemias progressed. In both patients, all RBCs expressed Tn antigen at the time of death due to their disease.[49] The remaining patients expressed low levels of Tn-positive RBCs detectable only with immunohistochemical staining.[49] It has been suggested by some authors that clones expressing Tn antigen may have a growth advantage and that this may explain in some cases why there is a progressive increase in the number of Tn-expressing RBCs.[48]

Summary

Changes in RBC antigen phenotype rarely occur. They are most frequently seen in association with hematologic malignancies but can be seen in association with solid tumors as well. The identification of these changes represents more than just an academic exercise. Changes have been identified prior to the diagnosis of the responsible underlying malignancy and have heralded relapse of the malignancy. In addition, the realization that Rh D had been lost in our patient and that the patient and her sister were identical twins avoided additional therapy that would have resulted in a significantly increased risk to the patient of morbidity and mortality. For these reasons, it is important for blood bank professionals to be aware of the existence of this phenomenon.

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Discrepancies in Rh(D) typing of sensitized red blood cells using monoclonal/polyclonal anti-D reagents: case report and review

B.J. Padget and J.L. Hannon

Instructions included with monoclonal Rh(D) typing reagents do not require routine use of an Rh control as immunoglobulin-coated red blood cells (RBCs) rarely yield falsely positive results with low protein reagents. However, the American Association of Blood Banks (AABB) *Technical Manual* recommends a concurrent control be performed on patients' RBCs that type as group AB, D+. Proficiency testing surveys presented sensitized AB, D– RBCs, which resulted in a positive direct antiglobulin test and, in some samples, spontaneous agglutination in saline. One intent of the surveys was to monitor the accuracy of the reported Rh(D) type. On an initial survey, 19 of 115 (16.5%) participants reported the RBCs as D+. Of these laboratories, 63.2 percent (12/19) had used a monoclonal/polyclonal blend anti-D reagent. On a subsequent survey, after educational material had been distributed, only five of 113 (4.4%) participants reported the Rh type as D+. Two of these five laboratories had used a monoclonal/polyclonal blend anti-D reagent. As RBCs coated with immunoglobulin may give unreliable results with Rh typing reagents, laboratories should follow the guidelines of the AABB *Technical Manual*. An appropriate control should be performed whenever RBCs from patients type as AB, D+.
***Immunohematology* 2001;17:10–13.**

Key Words: Rh(D) typing, sensitized red blood cells, monoclonal anti-D

Since the first description of the Rh system by Landsteiner et al.[1] in 1940, the D antigen has been recognized as a significant factor in transfusion. Both the *Standards for Blood Banks and Transfusion Services*[2] of the American Association of Blood Banks (AABB) and the *Standards for Transfusion Medicine*[3] of the Canadian Society for Transfusion Medicine require that potential blood transfusion recipients be tested for Rh type using anti-D reagent. Both standards also state that a control system appropriate to the anti-D reagent in use shall be included with all tests for D typing.

Red blood cells (RBCs) may become coated in vivo with immunoglobulin and/or complement in a variety of situations. The binding of protein to RBCs is recognized in vitro as a positive direct antiglobulin test (DAT), which may present a complex serologic situation. Techniques used for testing sensitized RBCs must ensure accurate RBC antigen typing to allow selection of suitable blood for transfusion.

Traditionally, Rh(D) typing of RBCs with anti-D suspended in a high protein (22% albumin) medium has been accompanied by the use of an appropriate control as required by pertinent standards. However, the advent of monoclonal typing reagents resulted in the deletion of this practice in many institutions. Some monoclonal D typing reagents are prepared from human monoclonal IgM anti-D blended with human serum containing polyclonal IgG anti-D.[4–5] More recently, manufacturers have begun to produce anti-D reagent by blending the secretions of two human/murine heterohybridomas, thereby manufacturing a monoclonal/monoclonal blend reagent with IgM and IgG components.[6] The diluent for the reagent may contain from 3 to 8% bovine serum albumin and, consequently, the reagent is regarded as a low protein mixture.

Instructions included with monoclonal D typing reagents do not require the routine use of an Rh control, based on the assumption that immunoglobulin-coated RBCs rarely yield falsely positive results with low protein reagents.[4–6] However, the AABB *Technical Manual* states that, "For red cell specimens that show agglutination in all tubes (i.e., give the reactions of group AB, D-positive), a concurrent control must be performed on the patients' cells."[7] Package inserts from monoclonal anti-D reagents include the possibility of spontaneous agglutination when RBCs are heavily coated with immunoglobulin, in the presence of strong

cold autoagglutinins, or with a protein imbalance causing rouleaux. However, although one manufacturer does state that a control test should be performed when both anti-A and anti-B show definite or doubtful agglutination,[4] another only suggested that inclusion of a control may be desirable.[5]

Study Design

An external proficiency testing program distributes survey challenges to 117 hospital laboratories in two Canadian provinces. Two challenges are sent bimonthly. Each challenge consists of a simulated patient's RBCs and plasma and RBCs from two donors. The testing level of the participants ranges from laboratories performing basic testing only (ABO and Rh(D) typing, antibody screen, and crossmatch) to immunohematology reference laboratories. Survey challenges monitor preanalytic sample evaluation, testing methods, results, and postanalytic follow-up. Participants are expected to process the survey samples as they would patient's samples. The results submitted by participants are evaluated on the following basis:

- Test results
- Interpretation of results
- Use of appropriate procedures
- Clerical accuracy

Results from five referee laboratories are assessed prior to evaluation of participant responses to ensure that sample quality has not deteriorated during the shipping process.

This article describes the results of two similar survey challenges. Survey 99–05 was tested by 115 laboratories in May 1999. A subsequent survey (Survey 00–06) was tested by 113 of the original 115 participants as a follow-up approximately 1 year later.

Both challenges presented RBCs from a type AB, D– patient. In Survey 99–05, the RBCs were sensitized with anti-e. In Survey 00–06, anti-c as well as anti-e was used for sensitization of the RBCs. The RBCs exhibited a positive DAT, with varying amounts of IgG, IgM, and complement (C3d) detectable in the distributed samples. The RBCs in some samples became "over-sensitized" and demonstrated spontaneous agglutination in saline because of extended time in shipping and storage prior to testing.

The preshipping test results of the RBCs are presented in Table 1. Although all testing performed on proficiency challenges is evaluated and graded, surveys are constructed to monitor specific aspects of laboratory testing. One aim of Surveys 99–05 and 00–06 was designed to monitor the accuracy of Rh(D) typing in the presence of RBC sensitization.

Table 1. Preshipping testing results

Survey	Anti-B	Anti-A	Anti-A,B	Slide & tube anti-D	Rh control
99–05	++++	++++	++++	++	++
00–06	++++	++++	++++	w+	w+

Survey	Monoclonal* anti-D	6% albumin control	Saline anti-D	Saline control	DAT	Saline control
99–05	+	+	w+	w+	++	w+
00–06	+	w+	w+	w+	+	w+

*Monoclonal/polyclonal blend

Results

The initial testing results found in Surveys 99–05 and 00–06 and the interpretation of the Rh(D) type are summarized in Table 2.

Table 2. Initial anti-D test results and Rh interpretation

Test method	Anti-D test result		Rh(D) type reported		
	Positive	Negative	Positive	Negative	Inconclusive
Survey 99–05					
Monoclonal anti-D*	48	3	12	3	36
Slide and tube anti-D	60†	2	7	2	53
Gel column agglutination	0	2	0	2	0
Total	108	7	19	7	89
Survey 00–06					
Monoclonal anti-D*	49	8	2	7	48
Slide and tube anti-D	52†	2	3	3	48
Gel column agglutination	0	2	0	2	0
Total	101	12	5	12	96

*Monoclonal/polyclonal blend

†Rh control: 99-05: 58 positive, 2 negative; 00-06: 50 positive, 2 negative

Survey 99–05

Fifty-one participants performed the initial Rh(D) typing on the RBCs using a monoclonal/polyclonal blend anti-D reagent. Forty-eight of these participants (94.1%) detected a positive reaction in the test. Only nine facilities performed an appropriate control for this reagent (6–8% albumin and/or autologous serum); all of these controls were positive. Sixty-two participants tested the RBCs using a slide and tube reagent, with 60 (96.8%) detecting a positive reaction with the anti-D. Two of these laboratories reported the control as negative, a result assessed as an error. Seven participants correctly typed the RBCs as D– on initial testing; three used monoclonal/polyclonal blend anti-D, two used slide and tube anti-D, and two performed gel column agglutination testing.

Following an initial D+ test result, 36 of 48 (75%) participants using a monoclonal/polyclonal blend reagent and 53 of 60 (88.3%) using a slide and tube anti-D indicated that the Rh(D) type of the patient was inconclusive and further testing would be required prior to reporting the Rh(D) type. Fifty laboratories performed additional testing. A summary of this testing is included in Table 3. Ten of these laboratories obtained an accurate D– blood typing.

Table 3. Additional test results

	Survey 99-05 test result		Survey 00-06 test result	
Test Method	**Positive**	**Negative**	**Positive**	**Negative**
Monoclonal anti-D*	21	1	16	0
Slide and tube anti-D	10	0	11	1
Saline anti-D	4	0	4	1
Chloroquine-treated RBCs	2	3	1	1
DTT-treated RBCs	0	0	0	2
EDTA glycine-acid-treated RBCs	0	5	0	6
Saline-washed RBCs	3	1	5	0
Total	40	10	37	11
No additional testing performed	65		65	
Total	115		113	

*Monoclonal/polyclonal blend

Nineteen of the 115 (16.5%) participants testing Survey 99–05 received major error assessments for incorrectly reporting the patient's Rh type as D+. Twelve of the 19 (63.2%) laboratories had used a monoclonal/polyclonal blend anti-D typing reagent without an appropriate control and did not recognize the possibility of a falsely positive result. This constituted 23.5 percent of the laboratories using a monoclonal reagent. The other seven participants who reported an incorrect Rh type had used a slide and tube anti-D reagent with a parallel control. Five of these laboratories failed to recognize the significance of the positive control result and, as previously mentioned, two participants erroneously reported a negative control result.

Educational Intervention

The survey report distributed for Survey 99–05 included educational materials addressing the problems that may be encountered with the Rh typing of sensitized RBCs. The AABB *Technical Manual* guideline that a concurrent control of 6–8% albumin must be performed on all RBCs typing as AB, D+ was cited.

Survey 00–06

Fifty-seven participants tested the simulated patient's RBCs using a monoclonal/polyclonal blend anti-D reagent. This demonstrated an increased use of this class of reagent of approximately 5 percent of total laboratories as compared with the previous survey. Forty-nine of these laboratories (86.0%) detected a positive reaction in the test. Twenty-four of the 57 facilities (42.1%) performed a 6–8% albumin and/or autologous serum control and detected a positive result. In the previous survey, only nine of 51 (17.7%) participants using monoclonal/polyclonal blend anti-D included an appropriate control. Fifty-four facilities used a slide and tube reagent for Rh typing, with 50 (92.6%) detecting a positive reaction in both the anti-D and Rh control tests. Two laboratories found a negative control despite a positive anti-D test, a result assessed as an error. Twelve participants found a valid negative typing on initial testing and it was assumed that these aliquots of the RBC sample were not as strongly sensitized as the others.

Forty-seven of the 49 (95.9%) participants detecting an initial D+ result and one detecting an initial negative result using a monoclonal/polyclonal blend reagent indicated that the patient's Rh type was inconclusive and further testing would be required prior to reporting a valid type. This demonstrated a marked improvement over the rate of 75 percent in Survey 99–05. Forty-eight of the 50 (96%) laboratories detecting positive D and control results with a slide and tube reagent made similar observations. Forty-eight laboratories performed additional testing, with 11 obtaining an accurate D– type (see Table 3).

Only five of 113 (4.4%) participants incorrectly reported the patient's Rh type as D+. This showed significant improvement over the rate of 16.5 percent in Survey 99–05. Of these five laboratories, two used a monoclonal/polyclonal blend reagent and three used a slide and tube anti-D.

Conclusion

RBCs that are sensitized with immunoglobulin may give unreliable results with anti-D typing reagents. Traditionally, the use of an Rh control reagent has identified this serologic abnormality and alerted the laboratory practitioner to the possibility of falsely positive Rh typing.

The advent of monoclonal anti-D reagents prompted many laboratories to abandon the use of an Rh control as the reagents were classified as low protein and manufacturers did not require the routine use of a control.

The submitted results of two proficiency testing surveys demonstrated that the policy of not including an Rh control must be modified in the case of type AB RBCs. In this situation, no negative control test is present to determine if nonspecific agglutination of the RBCs has occurred. On an initial survey, 16.5 percent of all participants and 23.5 percent of laboratories using monoclonal/polyclonal blend anti-D reagents reported the patient's Rh type as positive. These errors were attributed to failure to perform an appropriate control, thereby not recognizing the possibility of a falsely positive result. On a subsequent survey, which followed educational material addressing this issue, only 4.4 percent of total participants and 3.5 percent of facilities testing with monoclonal/polyclonal blend anti-D committed a similar error.

The problems encountered with the Rh (D) typing of RBCs similar to those distributed in the proficiency surveys may not occur with the use of monoclonal/monoclonal blend anti-D rather than monoclonal/polyclonal blend reagents. However, as the diluent of the monoclonal/monoclonal blend reagents may contain up to 7% bovine albumin, appropriate controls must be included when using these reagents.

The results of these proficiency testing surveys emphasize that laboratories should follow the recommendation of the AABB *Technical Manual* and include an appropriate control on patient's RBCs that type as AB, D+. This testing will alert laboratory practitioners to a possible falsely positive result and assist in the determination of a valid blood type.

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Confirmation of positive antibody screens by solid-phase red cell adherence assay using a tube technique method with polyethylene glycol enhancement

R.R. Gammon M. Lake, N. Velasquez, and A. Prichard

Our blood bank routinely screens donors for antibodies using a solid-phase red cell adherence (SPRCA) assay. Positive results are then confirmed using a tube technique with polyethylene glycol (PEG) enhancement due to reported higher specificity than with SPRCA. Over a 5-month period, 49,084 donor serum or plasma samples were tested using the SPRCA assay. Further identification of positive samples was performed using a PEG enhancement method. Testing was performed with strict adherence to the manufacturers' inserts. Of 49,084 samples, 313 (0.64%) were positive by the SPRCA assay. Of these, 99 (31.6%) samples remained positive when tested with PEG enhancement. The remaining 214 (68.4%) were negative, giving specificity for the SPRCA assay of 99.6 percent (48,985/49,199). We report a high specificity for antibody screening using the SPRCA assay. However, it is cost effective to perform a confirmatory tube test with PEG enhancement because 214 SPRCA assay samples were interpreted as having a negative antibody screen, thus allowing the release of valuable blood components for transfusion. ***Immunohematology* 2001;17:14–16.**

Key Words: solid-phase red cell adherence, antibody screens, polyethylene glycol enhancement

We routinely screen donor samples for antibodies using a solid-phase red cell adherence (SPRCA) assay. Recently, increased specificity has been reported for antibody screening using a tube technique with polyethylene glycol (PEG) enhancement.[1] We describe our experience of creating a testing algorithm in which positive SPRCA assays are confirmed by a tube technique method with PEG enhancement.

Materials and Methods

Sample collection

Samples were obtained from 49,084 volunteer blood donors from January 1, 1999 through May 31, 1999. Specimens were collected either as serum or as EDTA-, ACD-, CPD-, CPDA-1- or CP2D-anticoagulated plasma.

SPRCA assay

The SPRCA assay (Capture-R® Ready-Screen® [Pooled Cells]; Immucor, Norcross, GA) utilizes membranes of red blood cells (RBCs) bound to the surface of polystyrene microtitration strip wells. All Capture-R® reagents and samples are brought to room temperature (18 to 30^0 C) before testing. Two drops (100 ± 10 µL) of Capture-R® LISS are added to each of the microtitration wells. One drop (50 ± 5 µL) of serum or plasma is then added to each corresponding well. Positive and negative Capture-R® controls are tested in a similar manner. The wells are then incubated in a dry heat incubator at 37^0 C ± 2^0 C for 20 to 65 minutes. During the incubation period, the membrane antigens in the wells capture the RBC-specific IgG antibodies from the donor serum or plasma.

To remove unbound residual immunoglobulins, each plate is washed (CSW 100 Plate Washer, Immucor). One drop (50 ± 5 µL) of Capture-R® indicator cells is added to each well, which are then immediately centrifuged (2 minutes; 450 to 600 x g). This is to bring the indicator RBCs in contact with antibodies bound to the reagent RBC membranes. The plate is then read (IBG reader, Immucor).

In a positive test, migration of indicator RBCs cells to the bottom of the wells is impeded as anti-IgG–IgG complexes are formed on the surface of the immobilized reagent layer. As a consequence of

antibody bridging, the indicator cells adhere to screening cells as a second immobilized layer. In a negative test, indicator cells are not impeded in their migration and pellet to the bottom of the wells as tightly packed RBC buttons.[2]

All first-time SPRCA assay positive donor specimens are sent to a designated reference laboratory for antibody screening using tube testing with PEG enhancement.

Tube technique with PEG enhancement

Two drops of the donor's serum are added to two properly labeled 12 x 75 mm tubes. One drop from each of the commercially prepared reagent screening cells (Immucor) is added to the appropriate tube. The tubes were centrifuged (15 seconds; 900 to 1000 x g) and examined for hemolysis. The cells are resuspended by gentle agitation and examined macroscopically for agglutination. Results are recorded.

Two drops of PEG (Gamma PeG™; Gamma Biologicals, Houston, TX) are then added to each tube. All tubes are thoroughly mixed and incubated at 37^0 C $\pm$ 1^0 C for 10 to 15 minutes (incubation could be extended to 30 minutes). The tubes were examined for hemolysis and results recorded.

The tubes are then immediately washed at least x 3 in saline (0.9%). Two drops of anti-human globulin (anti-IgG) serum are added to each tube. The tubes are centrifuged (15 seconds; 900 to 1000 x g), examined for agglutination, and results recorded. One drop of Coombs control cells is added to all negative test results. Tubes are then centrifuged (15 seconds; 900 to 1000 x g) and examined for agglutination.[3]

If the results are negative, those units with a significant amount of plasma, including platelets, fresh frozen plasma, cryoprecipitate, and cryoprecipitate-reduced plasma, could be released for transfusion.

Component Preparation/Disposition

At our blood collection center, RBCs as well as components containing a significant amount of plasma (e.g., platelets, fresh frozen plasma, cryoprecipitate, and cryoprecipitate-reduced plasma) are prepared before antibody screening is complete. If the antibody screen is positive by SPRCA and confirmed by PEG, RBCs are labeled as containing "atypical antibodies," and it is up to the individual transfusion services to decide if they are willing to accept these components. RBCs for pediatric patients (CPDA-1 anticoagulant) containing antibodies are not accepted for transfusion by the hospitals that our blood collection center services. All components containing a significant amount of plasma are discarded if PEG confirms the antibody screen.

Cost Analysis

We determined the combination of components prepared at our blood collection center that contained sizable quantities of plasma that could be prepared from whole blood donations at our blood center to be (1) platelets and fresh frozen plasma or (2) cryoprecipitate and cryoprecipitate-reduced plasma.

Potential revenue recovery was calculated as follows:

> Potential revenue recovery = (number of units tested negative by tube technique with PEG enhancement x average charge of unit to hospital) − total expenditure for tube testing with PEG enhancement (includes those samples both positive and negative by PEG).

The reference laboratory is located in the same building as the blood bank, so there is no additional expense incurred for transport of specimens.

Results

SPRCA assay results were compared with those of tube technique with PEG enhancement.

Of the 49,084 samples tested, 313 (0.64%) were positive by the SPRCA assay. Of the 313 samples positive by SPRCA assay, 99 (31.6%) remained positive when tested with PEG enhancement. The remaining 214 (68.4%) were negative. Therefore, at our institution, we report specificity for the SPRCA assay of 99.6% (48,985/49,199).

Considering the two combinations of plasma-containing components that may be prepared from whole blood donations at our blood collection center, the recovery of revenue as a result of this testing algorithm over a 5-month period would be $11,759.00 for platelets and fresh frozen plasma, and $12,026.50 for cryoprecipitate and cryoprecipitate-reduced plasma (Table 1).

Table 1. SPRCA-positive, PEG-negative plasma-containing components prepared from whole blood and released for transfusion

Combination	Components	Number of Units	Total Revenue*
1	Platelets and	214	$7811.00
	fresh frozen plasma	214	$7704.00
2	Cryoprecipitate and	214	$6066.90
	cryoprecipitate-reduced plasma	214	$9715.60

**Note*: 313 tests were performed with PEG enhancement at a total cost of $3756.00.

Discussion

Alternatives to tube testing have come into widespread use in the blood banking industry in recent years.[4-7] In 1986, Lapierre patented the gel test,[8] and SPRCA assays were developed in the 1980s.[9] Benefits of these methodologies include reproducibility, the possibility of automation, and ease of interpretation.[4-10]

A recent comparison study reported higher specificity with the traditional tube testing with PEG enhancement when compared with SPRCA assays.[1]

At our blood bank, we have adopted an algorithm in which all donor specimens having a positive antibody screen for the first time by SPRCA assay are sent to our reference laboratory for tube testing with PEG enhancement. If the results are negative, all components containing a significant amount of plasma (e.g., platelets, fresh frozen plasma, cryoprecipitate, and cryoprecipitate-reduced plasma) are released for transfusion. We have found that other blood centers have adopted this testing algorithm as their standard operating procedure.

Over a 5-month period, 214 donor specimens with a positive antibody screen by SPRCA were confirmed negative using the tube technique with PEG enhancement. Considering the two combinations of plasma-containing components that may be prepared from whole blood donations at our blood center, platelets and fresh frozen plasma as well as cryoprecipitate and cryoprecipitate-reduced plasma, the recovery of revenue from these components released for transfusion as a result of this testing algorithm would be substantial.

We feel that although the confirmatory antibody testing results is an additional up-front expenditure, it is cost effective if performed in a timely fashion so as not to delay the release of valuable inventory for transfusion.

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Evaluation of a new solid-phase immunoassay for alloantibody detection using bromelin-treated and untreated red blood cells

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The enzyme test is used to detect certain antibodies or facilitate antibody identification. This study compares antibody reactivity with bromelin-treated red blood cells (RBCs) and untreated RBCs using a newly developed solid-phase immunoassay. The reactivity of irregular antibodies was tested by a magnetic-mixed passive hemagglutination assay (M-MPHA). In addition, antibody reactivity was tested with dried stroma of bromelin-treated RBCs and untreated RBCs (M-MPHA-Dry). Rh antibodies were detected with enzyme-treated intact RBCs and untreated RBCs by M-MPHA. The slight increase in reactivity using M-MPHA was not seen using dried RBC stroma (M-MPHA-Dry). All donor-derived IgG alloantibodies, which were detected by either a conventional tube enzyme test or an indirect antiglobulin test, were detected by M-MPHA without using enzyme-treated RBCs. Both M-MPHA and M-MPHA-Dry can be used for antibody detection without using enzyme-treated RBCs and are also useful for antibody identification. ***Immunohematology* 2001;17:17–21.**

Key Words: solid-phase immunoassay, M-MPHA, enzyme-treated red blood cells, bromelin, irregular antibodies

Serologic pretransfusion testing is changing from conventional tube tests to new systems (e.g., microcolumn agglutination test[1] and solid-phase immunoassay).[2] Furthermore, automation of pretransfusion testing is possible using these new systems. One of these new systems, solid-phase immunoassay, has a tendency to show high sensitivity, but nonspecific positive reactions can occur.[3]

Our newly developed solid-phase immunoassay system, named magnetic-mixed passive hemagglutination assay (M-MPHA), is an advanced technique involving mixed-passive-hemagglutination (MPHA).[4] M-MPHA uses anti-human-IgG-coated indicator magnetic particles for detecting IgG antibodies on intact red blood cells (RBCs)[5] or on dried RBC stroma,[6] which adhere to a microplate well. This technique has high sensitivity but may show a few nonspecific positive reactions. It is also suitable for automation, because centrifugation is not required for a reactivity pattern.[7,8] In the conventional tube test, certain antibodies are detected better by an enzyme test than an indirect antiglobulin test (IAT).[9] In this study, we evaluated the use of enzyme-treated RBCs and RBC (dried) stroma for our M-MPHA.

Materials and Methods

RBCs

Selected commercially available group O RBCs (Ortho-Clinical Diagnostics, Raritan, NJ) were used.

Antibodies

Commercially available human polyclonal antibodies (Anti-D, -E, -e, -C, -c, -Fy^a, -Fy^b, -Jk^a, -Jk^b, -K, -k, -S, and -s) were obtained from Ortho-Clinical Diagnostics and Organon Teknika (Boxtel, Holland), and an anti-D (ICSH/ISBT, 91/562 reference material) also was used. Ninety-six donor-derived antibody positive sera, 20 donor sera without antibody, and 6 bromelin falsely positive sera were obtained from irregular antibody screening tests at the Tokyo Metropolitan Blood Center. These samples were stored at –20° C until used.

Reagents

Bromelin solution (ID-Diluent1) was obtained from DiaMed, AG Morat, Switzerland, and Casein (hammarsten grade) was obtained from Wako Ltd., Osaka, Japan. Polyspecific anti-human-globulin was obtained from Ortho-Clinical Diagnostics.

Table 1. The influence of bromelin protease activity on IgG antibody reactivity

Antibodies	Bromelin activity (units)											
	15	7.5	3.75	1.88	0.94	0.47	0.23	0.12	0.06	0.03	0.02	0
Anti-D	1	1	1	1	1	1	1	1	1	1	1	1
Anti-Fy^a	–	–	–	–	–	–	–	1	2	2	2	2
Anty-Fy^b	–	–	–	–	–	–	–	–	–	1	1	2
Anti-Le^a	1	1	1	1	1	1	1	W	W	–	–	–
Anti-M	–	–	–	–	–	–	–	2	2	2	2	2
Anti-S	W	W	W	W	W	1	1	1	1	W	W	W
Anti-s	1	1	1	1	1	1	1	1	1	1	1	1
Anti-K	1	1	1	1	1	1	1	1	1	1	1	1
Anti-k	1	1	1	1	1	1	1	1	1	1	1	1
Anti-Jk^b	1	1	1	1	1	1	1	1	1	1	1	1
Bromelin falsely positive sera (6 samples)	–	–	–	–	–	–	–	–	–	–	–	–
Negative sera (20 samples)	–	–	–	–	–	–	–	–	–	–	–	–

2 = Strong positive; 1 = Positive; W = Weak positive; – = Negative

Bromelin enzyme activity assay

The protease activities of bromelin were assayed by the modified method of Hagihara et al.[10] For comparative purposes, enzyme activities were expressed in units (U) per 50 µL.[11-13]

M-MPHA

Preparation of anti-human-IgG antibody sensitized indicator particles and lectin-coated plates for M-MPHA and the assay procedure were performed using our previously published methods.[5]

Preparation of dried stroma plates and other equipment and the assay procedure were performed by the method of Tamai and Mazda.[6]

Enzyme treatment of RBCs for M-MPHA.

For the purpose of identifying the optimal bromelin activity, 50 µL of twofold doubling dilution series of bromelin (0 to 50 U) in phosphate-buffered saline solution were added to RBC-coated plates and incubated for 10 minutes at room temperature, and then washed x 3 with normal saline. Prediluted commercial reagent antibodies anti-D, -Fy^a, -Fy^b, -Jk^b, -S, -s, -K, and -k and donor derived anti-Le^a, -M, and negative control sera, were tested by M-MPHA. Furthermore, six bromelin falsely positive sera, which were also positive by the conventional tube test, and 20 negative sera from donors were tested by the above methods. Ninety-six antibody-positive sera were tested by M-MPHA using bromelin-treated and untreated RBCs.

Results

Enzyme concentration and antibody reactivity

Optimal bromelin protease activity for M-MPHA was approximately 7.5 U. The influence of bromelin protease activity on IgG antibody reactivity is shown in Table1. The reactivities of anti-Fy^a and -M antibodies disappeared at 0.23 U and anti-S reactivity was reduced at 0.94 U. Anti-D, -s, -K, -k, and -Jk^b antibodies showed almost the same reactivity, with 0 to 15 U of bromelin treatment. All of the tested bromelin falsely positive sera were negative from 0 to 15 U (Table 1). Twenty healthy donor sera were negative up to 15 U but were falsely positive at 30 U (data not shown). Consequently, the optimal enzyme concentration for M-MPHA was determined to be 7.5 U.

Comparison of maximum titers

The reactivity of reagent antibodies and donor-derived antibodies, comparing enzyme-treated and nontreated RBCs, are shown in Tables 2–1 and 2–2. As expected, reactivity of anti-Fy^a, -Fy^b, -M, -S, and -s antibodies was reduced or eliminated vs. enzyme-treated RBCs. On the other hand, the maximum titer of other antibodies, such as Rh antibodies, was slightly increased (almost one titer grade) on enzyme-treated intact RBCs as compared with nontreated intact RBCs, but this increase was not seen on dried stroma. Maximum titers of anti-D (ISBT/ICSH reference serum) vs. enzyme-treated intact RBCs, nontreated intact RBCs, dried enzyme-treated stroma, and nontreated stroma were 256,128, 256, and 256, respectively (Table 2–2).

Table 2–1. The reactivity of reagent antibodies on bromelin-treated RBCs and untreated RBCs (maximum titer)

Antibodies	Untreated RBCs	Bromelin-treated RBCs
Anti-D	64	64
Anti-E	320	320
Anti-C	160	320
Anti-c	640	640
Anti-e	160	320
Anti-Fy^a	320	No reaction
Anti-Fy^b	160	No reaction
Anti-S	128	32
Anti-s	256	128
Anti-K	128	128
Anti-k	128	512
Anti-Jk^b	512	512

Table 2–2. The reactivity of donor-derived antibodies on bromelin-treated RBCs and untreated RBCs (maximum titer)

	M-MPHA		M-MPHA-Dry	
Antibodies	Untreated RBCs	Bromelin-treated RBCs	Dried stroma of untreated RBCs	Dried stroma of of bromelin-treated RBCs
Anti-D	128	128	128	128
Anti-D	1	4	2	2
Anti-D	8	32	32	32
Anti-D	256	512	512	512
Anti-D	32	64	16	32
Anti-D	8	16	8	16
Anti-D	32	64	64	64
Anti-D	128	256	256	256
(ISBT/ICSH)				
Anti-E	32	64	128	128
Anti-E	128	128	256	256
Anti-c	16	32	32	64
Anti-c	32	64	64	64
Anti-c	2	16	32	64
Anti-D+C	16	32	32	64
Anti-D+C	128	256	256	512
Anti-M	32	No reaction	32	No reaction
Anti-Le^a	16	32	32	32
Anti-K	128	256	128	128
Anti-K	32	64	128	128
Anti-Fy^a	8	No reaction	32	No reaction
Anti-Fy^a	64	No reaction	64	No reaction
Anti-C+s	8	16	64	64

Detection of antibody-positive sera

The reactivities of 96 donor-derived antibody-positive sera are shown in Table 3. All seventy-three antibodies considered clinically significant (Rh, Duffy, Kidd, Kell, Jr^a, and k) were detected by M-MPHA without using enzyme-treated RBCs. In particular, bromelin one-stage-only positive antibodies (5 anti-E, 1 anti-c+e, anti-K, and anti-k antibodies), were also detected without using enzyme-treated RBCs. Of the 23 antibodies that are usually clinically insignificant (anti-Le^a, -Le^b, -Le^a+Le^b, -M, -P$_1$), only two (one anti-M and one anti-Le^b) which were IAT positive by the conventional tube test, were detected by M-MPHA. The twenty donor control sera were negative with all of these tests.

Discussion

Treatment of RBCs with proteases such as ficin, papain, and bromelin causes removal of certain membrane-associated proteins. Their removal may cause steric hindrance for certain antibodies[14] or reduce the surface electric charges of RBCs, thereby increasing the

Table 3. Comparison of the detection of 96 donor-derived antibody-positive sera vs. bromelin-treated RBCs and untreated RBCs

			M-MPHA		M-MPHA-Dry	
Antibodies	No. of Tests	Nature of the conventional tube test	Non-treated RBCs	Bromelin-treated RBCs	Dried stroma of non-treated RBCs	Dried stroma of bromelin-treated RBCs
Anti-D	11	IAT + Enz	11	11	11	11
Anti-E	5	IAT	5	5	5	5
Anti-E	20	IAT+ Enz	20	20	20	20
Anti-E	5	Enz	5	5	5	5
Anti-c+e	1	IAT + Enz	1	1	1	1
Anti-c+e	1	Enz	1	1	1	1
Anti-E+c	1	IAT + Enz	1	1	1	1
Anti-D+C	1	IAT	1	1	1	1
Anti-D+C	4	IAT + Enz	4	4	4	4
Anti-c	3	IAT+ Enz	3	3	3	3
Anti-C	1	IAT+ Enz	1	1	1	1
Anti-Fy^b	2	IAT	2	0	2	0
Anti-Fy^a	1	IAT	1	0	1	0
Anti-Jr^a	8	IAT	8	8	8	8
Anti-E+Le^a	1	IAT + Enz	1	1	1	1
Anti-Jk^a	1	IAT	1	1	1	1
Anti-K	3	IAT	3	3	3	3
Anti-K	1	Enz	1	1	1	1
Anti-k	2	IAT+ Enz	2	2	2	2
Anti-k	1	Enz	1	1	1	1
Anti-Le^a+Le^b	1	IAT + Enz	0	0	0	0
Anti-Le^a+Le^b	8	Enz	0	0	0	0
Anti-Le^a	5	Enz	0	0	0	0
Anti-Le^b	1	IAT	1	1	1	1
Anti-Le^b	1	IAT + Enz	0	0	0	0
Anti-Le^b	4	Enz	0	0	0	0
Anti-M	2	IAT	1	0	2	0
Anti-P$_1$	1	Enz	0	0	0	1

IAT = Indirect antiglobulin test positive only; Enz = Bromelin one-stage test positive only; IAT + Enz = IAT and bromelin one-stage test positive

reactivities of certain antibodies, especially Rh antibodies. Furthermore, enzyme testing is often used to facilitate antibody identification by denaturing certain antigens. However, it is well known that enzyme tests have many disadvantages. Standardization of enzyme treatment (e.g., enzyme concentration, incubation time) is very important.[11,15] If RBCs are overtreated with protease, many nonspecific reactions will occur.

In this study, we used enzyme-treated RBCs for our newly developed M-MPHA. RBCs were treated with various enzyme concentrations. Fy^a, Fy^b, and M antigens were denatured by very low enzyme concentrations and incubation times (0.23 units, 10 minutes.). As to Rh antibody reactivity, Rh antibodies exhibited only a minimal increase with enzyme treatment. Sallender et al.[16–17] and Shanwell et al.[18] already reported antibody detection with enzyme-treated RBCs using a solid-phase test. In their reports, Rh antibodies of patients or pregnant women were more often detected with enzyme-treated RBCs, but they concluded that the difference was minimal as was the case with our findings. However, in our study, when dried stroma was used (M-MPHA-Dry), there was no difference for most of the Rh antibody titers between enzyme-treated stroma and nontreated stroma (Table 2–2). We consider the explanation for this finding was the sensitivity of M-MPHA-Dry. Although the sensitivity of M-MPHA, which uses intact RBCs, is lower than that of M-MPHA-Dry, it is a fact that the maximum titer of anti-D antibody (ISBT/ICSH reference) of M-MPHA, which uses intact RBCs, was 128 (Table 2–2). This is almost equal to that of the conventional tube enzyme test.[15]

Various methods can be used for RBC enzyme treatment. Enzyme-treated reagent RBCs were allowed to adhere to microplate wells and then dried for M-MPHA-Dry. It is possible to do enzyme treatment either before or after RBC adherence. Furthermore, it is possible to add enzyme solution to the sample dilution buffer, just as in a one-stage test (data not shown).

In our hands, all clinically significant antibodies detected by the conventional tube enzyme test alone were also detected by M-MPHA without using enzyme-treated RBCs. On the other hand, some Lewis antibodies were not detected when nontreated RBCs were used. These undetected antibodies appeared to be of minor clinical significance, as they had been detected with a conventional tube enzyme test alone. Furthermore, most of these antibodies were apparently IgM antibodies, as they were detected by IgM-M-MPHA (data not shown). IgM-M-MPHA is an IgM antibody detection system, which uses antihuman IgM antibody sensitized indicators instead of antihuman IgG antibody.[19] Issitt et al.[20] concluded that the highly sensitive indirect antiglobulin test should be used for routine antibody screening, because most "enzyme-only" antibodies appeared to be clinically insignificant.

In conclusion, enzyme-treated RBCs are not necessary for M-MPHA alloantibody detection, particularly for M-MPHA-Dry. Consequently, M-MPHA appears to be a suitable test for routine antibody screening, in terms of both cost and work load. M-MPHA can also be used to facilitate antibody identification, as Fy^a, Fy^b, and M antigens are easily denatured by enzyme treatment. In particular, use of dried stroma, which is stable for a long period, is likely to become an excellent tool for serologic testing.

Acknowledgment

We thank Mrs. Ito for technical assistance.

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Likelihood of D heterozygosity in Mestizo Mexicans and Mexican Americans

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Information on the gene frequencies of the Rh system in the Mexican or Mexican American population is currently not available in the medical literature, thus hindering management of pregnancies at risk for development of hemolytic disease of the newborn. Data from four recent large studies in the broader scientific literature of Mestizo Mexicans and Mexican Americans is reviewed. Gene frequencies are calculated from the pooled data. A table of gene frequencies in the Caucasian and African American population is provided for comparison. ***Immunohematology* 2001; 17:22–23.**

Key Words: hemolytic disease of the newborn, Rh system, Mexican D gene frequency

Estimation of the likelihood of heterozygosity for the D allele is useful in counseling couples at risk for hemolytic disease of the newborn due to anti-D. A high likelihood of paternal heterozygosity would prompt amniocentesis to determine fetal Rh typing using nucleotide amplification (polymerase chain reaction). A high likelihood for paternal homozygosity would require close clinical monitoring for fetal distress and possible therapeutic intervention. Many areas of the United States are currently experiencing a significant increase in the size of their Hispanic population, whereas other areas have had stable Hispanic populations for many years. Although tables estimating D zygosity are available for Caucasians, Asians, and African Americans, similar tables are not available for Mestizo Mexicans or Mexican Americans.

The term "Mestizo" is commonly used in Hispanic countries to describe persons of mixed European and American Indian ancestry. However, the Mestizo Mexican and Mexican American populations are actually a trihybrid mixture of Caucasian (41 to 71%), African (3 to 14%), and American Indian (27 to 55%) lineage.[1-4] A review of four recent large studies of Rh genotypes in the Mestizo population (one from Texas,[1] one from Arizona,[2] and two from Mexico City[3,4]) demonstrated comparable genotype frequencies (Table 1). A weighted gene frequency for the combined population (N = 2662) was used to create a zygosity table for all possible Rh positive phenotypes (Table 2). A comparison of the likelihood of zygosity for Rh positive phenotypes in Mestizo Mexicans and Mexican Americans, Caucasians, and African Americans[5] is given in Table 3.

Although this information will be useful when evaluating individuals of Mexican ancestry, caution must

Table 1. Rh allelic frequencies in Mestizo Mexican and Mexican Americans. Genotype nomenclature is given in Fisher-Race terminology (CDE) and their corresponding modified Wiener terminology (R/r). The Wiener terminology is commonly used by blood bankers.

Genotype Fisher-Race	Genotype Mod Wiener	Texas 948*	Arizona 730*	Mexico City 474*	Mexico City 510*	Weighted total 2662
DCE	R^Z	0.017	0.02	0.0206	0.101	0.035
DCe	R^1	0.438	0.443	0.4565	0.451	0.445
DcE	R^2	0.174	0.225	0.2126	0.2	0.200
Dce	R^0	0.065	0.042	0.0343	0.031	0.047
dCE	r^Y	0	——†	——†	0.0002	0.000
dCe	r'	0	0.013	0.0069	0.029	0.010
dcE	r''	0.003	——†	0.0032	0.001	0.002
dce	r	0.303	0.257	0.2659	0.185	0.261
Overall Rh negative		9.4%	7.3%	7.6%	4.6%	7.5%
Overall Rh positive		90.6%	92.7%	92.4%	95.4%	92.5%

*Numbers tested
†Not given

Table 2. Frequencies of Rh positive phenotypes and possible genotypes. The likelihood of heterozygosity for D is given for each phenotype.

Phenotype	Genotype Fisher-Race	Genotype Mod Wiener	Genotype frequency (%)	Liklihood of hetero-zygosity for D
CcDe	*CDe/cde*	R^1r	23.252	85
	CDe/cDe	R^1R^0	4.159	
	cDe/Cde	R^0r'	0.097	
CDe	*CDe/CDe*	R^1R^1	19.816	4.5
	CDe/Cde	R^1r'	0.921	
cDEe	*cDE/cde*	R^2r	10.439	85
	cDE/cDe	R^2R^0	1.867	
	cDe/cdE	R^0r''	0.017	
cDE	*cDE/cDE*	R^2R^2	3.994	1.8
	cDE/cde	R^2r	0.073	
CcDEe	*CDe/cDE*	R^1R^2	17.792	12
	CDE/cde	R^zr	1.805	
	cDE/Cde	R^2r'	0.414	
	CDE/cDe	R^zR^0	0.323	
	CDe/cdE	R^1r''	0.163	
	cDe/CdE	R^0r^y	0.000	
cDe	*cDe/cde*	R^0r	2.440	92
	cDe/cDe	R^0R^0	0.218	
CDE	*CDE/CDE*	R^zR^z	0.119	0.2
	CDE/CdE	R^zr^y	0.000	
CcDE	*CDE/cDE*	R^zR^2	1.381	1.0
	CDE/cdE	R^zr''	0.013	
	cDE/CdE	R^2r^y	0.002	
CDEe	*CDE/CDe*	R^zR^1	3.077	1.2
	CDE/Cde	R^zr'	0.036	
	CDe/CdE	R^1r^y	0.002	

Table 3. Comparative likelihood of heterozygosity for D (%) of Rh positive phenotypes in Mestizo Mexican and Mexican Americans, Caucasians, and African Americans*

Phenotype: antigens present	Mestizo Mexicans and Mexican Americans	Caucasians[5]	African Americans[5]
CcDe	85	90	41
CDe	4.5	9	19
cDEe	85	90	37
cDE	1.8	13	1
CcDEe	12	11	10
cDe	92	94	54

* Rare phenotypes such as CDE, CcDE, CDEe all have a likelihood of heterozygozity less than 2%. In other racial groups, the likelihood of being heterozygous for D is reduced because absence of D is so uncommon. [5]

be exercised when extrapolating this information to individuals from other Latin American countries, due to the possibility of differing ancestral admixtures. It is also important to note that, although probability of heterozygosity given in Tables 2 and 3 assumes that an individual with a certain phenotype is selected randomly from a population, if information is available regarding the phenotype of previous offspring of the parents, the calculation of the probability can be further refined.[6] In the future, it is anticipated that the use of gene amplification for determining RHD zygosity will improve our ability to counsel such patients.[7]

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BOOK REVIEW

Centralized Transfusion Systems: Models and Systems. Marcus B. Simpson, MD, ed. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2000. 174 pp. Domestic: member $59, nonmember $84. International: TBA. ISBN: 1–56395–107–X. To order: e-mail: sales@aabb.org or fax: (301) 951–7150.

Dr. Marcus B. Simpson, editor of this timely and practical paperback, observes that "pressures for cost reductions, increased efficiency, and improved resource management" have driven renewed interest by hospitals for centralized transfusion services (CTSs). Despite many recent conferences and lectures on the subject of CTSs, there is no comprehensive resource available that provides sufficient detail of the issues and requirements to assist hospital executives make responsible and informed decisions. Dr. Simpson invited authors who operate CTSs in Seattle, WA (Puget Sound Blood Center), Pittsburgh, PA (Institute for Transfusion Medicine and the Centralized Transfusion Service), and Tampa, FL (Florida Blood Services), as well as other experts in blood center administration, legal, regulatory, and computer operations, to fill that gap. The result is an up-to-date and highly informative volume that "rings true," because it was written by experts who either operate successful CTSs or deal with their issues on a daily basis.

Three of the eight chapters describe the operations of the Pittsburgh CTS. Dr. Darrell J. Triulzi describes clinical and quality perspectives, noting that improved care of patients can be achieved by extending high quality reference laboratory and transfusion medicine expertise, typically located only in large hospitals, to all participating hospitals regardless of their size. Patti Larson emphasizes the importance of software in developing a secure and effective centralized repository of blood types, serological data, and other patient- and product-related information. Linda F. Hahn outlines the structure of the Pittsburgh operation, including details of laboratory testing (work flow, turnaround times), inventory management, labor requirements (full-time equivalents), automation (IBG system, microtiter plates, solid-phase antibody screening, throughput), and transportation. According to Ms. Hahn, "This is a model that is transferable to any region or hospital network willing to make the effort to implement it."

Two chapters describe the Seattle system, which was founded by Dr. Richard Czajkowski in 1944 and is generally recognized to be the longest continuously operating CTS in the United States. Cynthia Murray provides an outstanding 36-page chapter that outlines the unique historical, geographic, and logistical features of the Seattle system and, also, identifies specific operational advantages for hospitals. Ms. Murray compares the number of tests performed per full-time equivalent at the CTS with benchmarking data from tertiary hospitals to illustrate the increased efficiency of CTSs. Linda S. Barnes covers the regulatory issues, identifying the pertinent accrediting agencies and outlining requirements of the U.S. Food and Drug Administration.

The Tampa Bay system is described by Ruth A. Zatik and Dr. Germán F. Leparc. They explain the unique situation of the Florida Blood Services, which is a regional blood center that provides pretransfusion compatibility testing to hospitals in a widely dispersed area through five regional testing laboratories in designated "hubs." Four of the laboratories are located inside a hospital and the fifth is in the blood center. The authors comment that the Tampa Bay system "contrasts with other blood center-based services that use the fully centralized approach to testing." Dr. Alfred J. Grindon and Pamela Leach of the American Red Cross guide readers in the process of moving from a positive awareness of the operational issues to the development of a specific business plan for a CTS. They suggest that an action plan should include a situation analysis, financial projections, formation of working committees and, perhaps, a Gantt chart or other planning tools to ensure reliable tracking. Legal issues are outlined in a chapter by Philip D. Schiff, General Counsel of the American Association of Blood Banks. Mr. Schiff explains that legal issues for establishing a CTS include all of the legal issues that face blood services, as well as the additional issues that face other health care-related commercial enterprises, i.e., hospitals. He cautions that his chapter is offered as a general check list of key issues and cannot address the specific state and local laws that may apply to each specific CTS.

This compact volume is full of the kind of practical information and guidance needed to evaluate successful CTS models and systems. The authors are the experts and the clarity of their presentations reflects their extensive hands-on knowledge and experience. If there is a weakness, it is the common weakness of all compact books: namely, you can't cover it all in only

174 pages. As Dr. Simpson acknowledges, there are CTSs in the United Kingdom, Europe, Latin America, Australia, and New Zealand. At a time when the American Association of Blood Banks is reaching out to serve a broad worldwide ("international") constituency, descriptions of CTSs operating outside the United States would have added an additional dimension. Overall, this is a welcome and overdue compilation of what is known about CTSs in the United States. I highly recommend it to all persons who are considering such services in their communities.

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COMMUNICATIONS

Letter From the Editors

It's our 17th year!

Let me extend the wishes of the editors and reviewers to the authors and readers for a very happy, healthy, and productive 2001.

There will be few changes in *Immunohematology* in 2001. It has been decided to publish the Instructions for Authors in every issue for the convenience of our authors. This change started with the last issue in 2000.

Both subscribers and casual viewers are using the *Immunohematology* Web site. It is a very comprehensive site and all information in the printed issue can be accessed on the Web site. In addition, you can send a letter to the editor, send an article, subscribe on a secure site, and do a literature search by word or words.

For those readers who remember the "good old days," *Immunohematology* is looking for interesting remembrances from the earlier days of blood banking for the column, "Those were the Days." I know that there are many stories probably told and retold daily that would be interesting and insightful for our readers.

The biggest problem and one that is experienced by many journals is the lack of articles. It is the policy of *Immunohematology* to publish articles concerning blood group serology, education, and computer technology. This includes articles on red cell, white cell, and platelet serology. There is a concerted effort by other journals to publish the same types of articles; therefore, the competition is becoming intense. When you have an interesting subject, you should write the article, and we hope you will consider *Immunohematology* as the journal of choice.

The editors of *Immunohematology* would like the 17th year of publication to be one of many more, not the last!

Delores Mallory
Editor-in-Chief

Mary McGinniss
Managing Editor

ERRATA

Vol. 16, No. 4, 2000; page 161

Letter to the Editors: *Re: Gel Technology for RhIG dosage*

We regret the misspelling of Dr. Stephen Apfelroth's last name on the Contents page and following the letter submitted by him for publication under Communications. In addition, the last sentence of Dr. Apfelroth's letter should read "It should also be noted that the AABB *Technical Manual* gives 30 mL of fetal whole blood volume as the recommended amount for coverage by **300 µg** of RhIG as opposed to 20 mL as used by the authors."

ANNOUNCEMENTS

Twelfth Annual Blood Bank Symposium. The Joint Committee for Blood Bank Education is pleased to announce a meeting titled "Twelfth Annual Blood Bank Symposium" on Saturday, April 21, 2001 at the New Yorker, located across from Penn Station in New York City. This meeting is a joint effort between the Blood Bank Supervisors Association, the Council of Hospital Blood Directors, and the New York Blood Center. **Contact**: George Mann, Chairman, The Brooklyn Hospital Center, 121 Dekalb Avenue, Brooklyn, New York, 11201; phone: (718) 250–8215.

Monoclonal anti-Js^b. A murine monoclonal anti-Js^b that reacts by the indirect antiglobulin test using anti-mouse globulin serum is available to anyone who asks. **Contact:** Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

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Notice to Readers: *Immunohematology, Journal of Blood Group Serology and Education,* is printed on acid-free paper.

IMPORTANT INFORMATION!

The National Reference Laboratory for Blood Group Serology of the American Red Cross, formerly located in Rockville, Maryland, has transferred all functions to the Musser Blood Center, Penn-Jersey Region of the American Red Cross, in Philadelphia, Pennsylvania. Please use the following address and phone numbers.

Address all mail for the American Rare Donor Program, Reference Laboratory, Quality Control/ Blood Components (Factor VIII assays), or *Immunohematology, Journal of Blood Group Serology and Education* to:

American Red Cross
P.O. Box 40325
Philadelphia, PA 19106

The phone numbers are as follows:

American Rare Donor Program:
(215) 451–4900 (24-hour number)

Reference Laboratory:
(215) 451–4901 (24-hour number)

Quality Control/Blood Components:
(215) 451–4903 (8 am to 5 pm, EST)

Immunohematology:
(215) 451–4902

Information:
(215) 451–4904

Fax:
(215) 451–2538

E-mail:
immuno@usa.redcross.org

The National Reference Laboratory for Neutrophil Serology remains at the North Central Region of the American Red Cross in St. Paul, Minnesota.

CLASSIFIED AD

Reference Laboratory Supervisor: A large, progressive AABB-accredited reference laboratory located in a metropolitan area seeks an experienced serologist to be responsible for daily operations, staff training, and compliance. This laboratory performs advanced problem solving, compatibility testing for problem cases, donor screening, routine pretransfusion testing, and platelet studies. The successful candidate will have SBB certification, minimum 3 years' experience in a reference laboratory, and excellent communication and customer service skills. Supervisory, transfusion medicine, and quality assurance experience preferred. Excellent benefits package. **Contact:** send resume to American Red Cross, Southern Region, Human Resources Dept., 1925 Monroe Dr., NE, Atlanta, GA 30324 or fax: (404) 253-5348.

ADVERTISEMENTS

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