

# Immunohematology

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JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 19, NUMBER 4, 2003



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# Immunohematology

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# Loss of enzyme-sensitive antigens due to the presence of leukocytes, neomycin sulfate, and LISS

R.W. VELLIQUETTE, P. HOWARD, H. MALYSKA, AND M.E. REID

Previous studies have shown that RBCs with residual WBCs stored in LISS and neomycin sulfate develop characteristics associated with enzyme-treated RBCs. During a mass screening program to antigen type donor RBCs, we observed that the Fy<sup>a</sup> antigens on a RBC sample from an in-house panel became non-detectable with anti-Fy<sup>a</sup> after incubation overnight in Diluent 2 from Micro Typing Systems, Inc. (MTS, Pompano Beach, FL). In response to this observation, we initiated an investigation to determine the cause. Tests were performed according to the manufacturer's instructions in MTS neutral gel cards or gel cards containing anti-IgG. We found that a reduction or loss of the Fy<sup>a</sup>, Fy<sup>b</sup>, and M antigens occurs when RBCs were prepared from samples containing residual WBCs (as a source of enzymes) and subsequently incubated in media containing neomycin sulfate and LISS. We showed that the effect did not occur in the absence of neomycin sulfate. RBC antigens can be altered in LISS if they have first been exposed to neomycin. We recommend restricting the use of RBCs suspended in MTS Diluent 2 to the day of dilution (as indicated in the package insert) if preparing reagent RBCs from sources that were not leukoreduced and were stored in the presence of neomycin. *Immunohematology* 2003;19:109-111.

**Key Words:** blood group antigens, enzyme-sensitive antigens, protease-sensitive, RBCs

RBCs stored in LISS in the presence of neomycin sulfate over time may (when WBCs are present) exhibit marked alterations in antigen reactivity that resemble protease modification of the RBC. Malyska et al.<sup>1</sup> showed that the antigenic changes occurring during RBC storages in neomycin-LISS are due to proteolytic modification of the RBC membrane by enzymes released from contaminating WBCs. Any of these three conditions alone or in dual combination do not lead to changes in antigen reactivity during storage; however, the changes do occur when all three are present concomitantly or, remarkably, sequentially. The storage time in neomycin-LISS required to produce the RBC antigen effect varied from experiment to experiment. Notably, however, reduced reactivity of Fy<sup>a</sup> and s

(which are protease-sensitive) occurred in some experiments after only 1 day of storage.<sup>1</sup> The authors noted that WBCs, particularly the phagocytic granulocytes, contain lysosomes that carry numerous proteolytic (both serine and thiol active) and hydrolytic enzymes capable of degrading a variety of extracellular proteins.<sup>1</sup> The hypothesis was made that proteases released from the WBCs are capable of cleaving certain proteins from the RBC membrane. This hypothesis is consistent with the knowledge that certain blood group antigens are sensitive to deliberate treatment of RBCs with enzymes.<sup>2,3</sup>

During a mass screening program to antigen type donor RBCs after the World Trade Center disaster, we observed that after overnight incubation in MTS Diluent 2 (Micro Typing Systems, Inc., MTS, Pompano Beach, FL), the Fy<sup>a</sup> antigen on a RBC sample used as a Fy(a+) control became non-detectable with anti-Fy<sup>a</sup>. This RBC sample had been stored in Alsever's solution for 4 days prior to suspension in MTS Diluent 2. In response to this observation, a new set of RBC suspensions were made from a freshly prepared in-house RBC panel to serve as controls for the monoclonal anti-Fy<sup>a</sup> (MIMA-19) used for the mass screening. Similarly, this cell suspension was stored overnight in MTS Diluent 2. The testing of this new sample 24 hours later showed slight loss, but not the same, dramatic loss, of Fy<sup>a</sup> antigen reactivity as initially observed 2 days previously with the initial RBC suspension used as a Fy(a+) control.

The purpose of this study was to determine the reason for the loss of antigen reactivity on RBCs stored in neomycin-LISS.

**Materials and Methods**

In-house stock reagent RBCs, one Fy(a+b-) and one Fy(a+b+), recovered from frozen storage in glycerol were washed with PBS at pH 7.4 and resuspended in Alsever's solution (Gamma Biologicals, Inc., Houston, TX) for different periods of time. Aliquots were washed with PBS and diluted to 0.8% in MTS Diluent 2. We tested these panel RBCs for Fy<sup>a</sup> reactivity with commercial human anti-Fy<sup>a</sup> (Gamma Biologicals, Inc.) and mouse monoclonal anti-Fy<sup>a</sup> (MIMA-19, NYBC, New York) immediately after preparation (day 1) and after 24-hour overnight storage (day 2). We combined 50 µL of 0.8% RBCs and 25 µL of anti-Fy<sup>a</sup> into MTS Anti-IgG (human and mouse) gel cards (MTS, Pompano Beach, FL), incubated the cards for 15 minutes at 37°C, and centrifuged them for 10 minutes in the MTS centrifuge. Similarly, we suspended Fy(a+b+), M+, N+ RBCs from a commercial panel (neomycin sulfate present) and Fy(a+b+), M+, N- RBCs from an EDTA sample (no neomycin sulfate present) to 0.8% in MTS Diluent 2. All RBCs were stored at 4°C and subsequently tested for Fy<sup>a</sup>, Fy<sup>b</sup>, and M antigens over a 27-day period. For Fy<sup>a</sup> and Fy<sup>b</sup> typing, 50 µL of 0.8% RBCs and 25 µL of commercial anti-Fy<sup>a</sup>, anti-Fy<sup>b</sup> (DiaMed AG, Cressier sur Morat, Switzerland), and mouse monoclonal anti-Fy<sup>a</sup> were added to the respective MTS gel cards, incubated for 15 minutes at 37°C, and centrifuged for 10 minutes in the MTS centrifuge. For M typing, 50 µL of 0.8% RBCs was added to a DiaMed anti-M (monoclonal) gel card (DiaMed AG) and centrifuged for 10 minutes in the MTS centrifuge.

**Results**

When RBCs from two examples each of Fy(a+b-), Fy(a+b+), and Fy(a-b-), were incubated in MTS Diluent 2 overnight at 4°C, a slight reduction of antigen expression was obtained with commercial anti-Fy<sup>a</sup> (Table 1). We noted that the only difference between these RBCs and those used in the initial screen (which lost their Fy<sup>a</sup> antigen expression after the same treatment and overnight incubation) was that they had not been stored in Alsever's solution for 4 days.

We then suspended two Fy(a+b+) M+ RBC samples, one from a commercial panel and one

**Table 1.** Testing of RBCs recovered from frozen storage (glycerol) and suspended in MTS Diluent 2 (no neomycin sulfate)

NYBC in-house panel RBCs	Monoclonal MIMA-19* anti-mouse IgG (goat)		Commercial anti-Fy <sup>a</sup> anti-human IgG (rabbit)	
	Day 1 <sup>†</sup>	Day 2 <sup>‡</sup>	Day 1	Day 2
Fy(a+b-) #1	4+	4+	3+	2+
Fy(a+b-) #2	4+	4+	3+	2+
Fy(a+b+) #1	4+	4+	3+	2+
Fy(a+b+) #2	4+	4+	2+	2+
Fy(a-b-) #1	0	0	0	0
Fy(a-b-) #2	0	0	0	0

\*Anti-Fy<sup>a</sup>  
<sup>†</sup>Day of recovery  
<sup>‡</sup>After overnight incubation at 4°C

collected in EDTA, in MTS Diluent 2 and incubated them at 4°C for several days. Aliquots were tested at days 1, 6, 13, 20, and 27 with anti-Fy<sup>a</sup> (commercial human and murine monoclonal), with anti-Fy<sup>b</sup> (commercial human), and with anti-M (commercial rabbit). RBCs from the commercial panel lost both Fy<sup>a</sup> and Fy<sup>b</sup> antigens by, respectively, day 13 and day 20, and also exhibited a weakening of the M antigen by day 20. RBCs collected in an EDTA tube showed no weakening of the antigens tested (Table 2).

**Table 2.** Testing of 0.8% RBC suspensions in MTS Diluent 2 with and without neomycin sulfate

Days Stored	Fy(a+b+) M+ N+ commercial panel RBCs (neomycin sulfate)				EDTA Fy(a+b+) M+ N- RBCs (no neomycin sulfate)			
	Anti-Fy <sup>a</sup> Human*	Anti-Fy <sup>a</sup> Mab <sup>†</sup>	Anti-Fy <sup>b</sup> Human	Anti-M Rabbit	Anti-Fy <sup>a</sup> Human	Anti-Fy <sup>a</sup> Mab	Anti-Fy <sup>b</sup> Human	Anti-M Rabbit
1	2+	3+	2+	3+	2+	3+	2+	3+
6	2+	3+	2+	3+	2+	3+	2+	3+
13	2+	0	0	3+	2+	3+	2+	3+
20	0	0	0	2+	2+	3+	2+	3+
27	0	0	0	1+	2+	3+	2+	3+

\*Commercial human polyclonal antibody  
<sup>†</sup>Monoclonal antibody (MIMA-19)

**Conclusion**

Our results are consistent with the premise that storage of RBCs in LISS-neomycin sulfate-containing media releases exogenous proteases from WBCs, which modify the RBC membrane.<sup>1</sup> A reduction or loss of the Fy<sup>a</sup>, Fy<sup>b</sup>, and M antigens occurred when RBCs were prepared from samples containing residual WBCs (as a source of enzymes) and were subsequently incubated in media containing neomycin sulfate and LISS. Remarkably, the loss of antigen reactivity occurred even when neomycin sulfate and LISS were not present at the same time, i.e., antigen loss occurred even after RBCs were washed free of neomycin sulfate and later

incubated with LISS. Just as remarkable, we observed the effect with RBCs from a commercial panel, which is prepared from leukoreduced blood. The initial loss of Fy<sup>a</sup> antigen occurred more rapidly than in our subsequent experiment, probably due to the higher concentration of WBCs present in the absence of leukoreduction. The RBCs collected in EDTA did not show a weakening of protease-sensitive antigens, probably because chelation of calcium and magnesium inactivated any proteolytic enzyme present.

This detrimental effect of neomycin-LISS storage on enzyme-sensitive antigens is noteworthy for those who prepare reagent RBCs. We recommend that RBCs suspended in any LISS not containing EDTA be used for no more than the day of dilution. Our study also highlights the importance of appropriate controls.

### Acknowledgments

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# False reactivity in GTI Pak Plus<sup>®</sup> ELISA kits due to the presence of anti-mouse antibody in patients' samples

M.F. LEACH AND J.P. AUBUCHON

The development of commercially available ELISA kits (GTI, Inc., Waukesha, WI) that use antigens adhered to microtiter plate wells by the use of mouse monoclonal antibodies made it possible for hospital transfusion service laboratories to test for platelet- and/or HLA-specific antibodies without reliance on reference laboratories. However, human anti-mouse antibodies (HAMAs) may cause false reactions in ELISAs. We designed a study to determine the impact of HAMAs on these ELISAs. Samples from 210 patients were evaluated from January 1995 to April 2002; 79 (38%) were found to be positive for HLA- and/or platelet-specific antibodies. Thirty (38%) of these positive samples, as well as ten negative samples that served as controls, underwent HAMA neutralization/inhibition procedures before being retested by ELISA. One (10%) of the control samples was reactive after treatment. When the samples that were positive in routine testing were treated to neutralize/inhibit HAMAs, reactivity was unchanged in 20 (67%); reactivity was eliminated in eight (27%) of the samples tested. All of the specimens that showed a reduction or elimination of their reactivity after neutralization/inhibition had an initial optical density (OD) ratio  $< 3.0$  whereas those that remained unchanged in reactivity had an OD ratio  $> 7.0$  ( $p < 0.05$ ). Reactivity present only in the treated samples was observed in three (10%) of the positive samples tested; one was additionally reactive with HLA antigen only and two with glycoprotein Ia/IIa. The presence of HAMAs should be considered when antibodies against more than one platelet-specific glycoprotein are detected and if the optical density ratio is  $< 3.0$ . *Immunohematology* 2003;19:112-116.

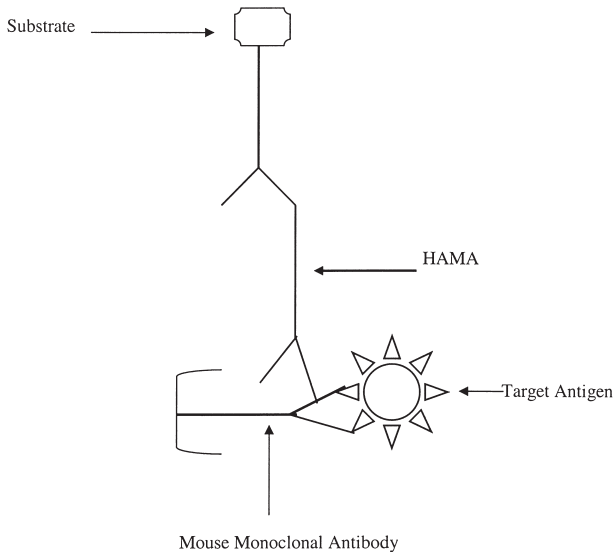
**Key Words:** human anti-mouse antibodies, ELISA, platelet- and HLA-specific antibodies

ELISAs are used in many laboratory procedures. With the development of ELISA test kits (GTI, Inc., Waukesha, WI) this technique became available to hospital transfusion service laboratories, adding a much-needed means of analyzing patients' samples without dependence on external reference laboratories. Tests using these ELISA kits include assays for HLA- and/or platelet-specific antibodies, platelet factor 4 analysis for heparin-induced platelet antibodies, and screening for the presence of HLA class

I antibodies. The GTI Pak Plus<sup>®</sup> ELISA test kit is used in our transfusion service laboratory to evaluate samples from patients who are refractory to platelet transfusion. When indicated by medical condition or history, prenatal samples are also evaluated for the presence of platelet-specific antibodies known to cause neonatal alloimmune thrombocytopenia (NAIT). In this test kit the platelet-specific glycoproteins and HLA class I antigens are immobilized on the microtiter plate by the use of mouse monoclonal antibodies specific for the platelet glycoproteins or HLA class I antigen.

The use of nonhuman monoclonal antibodies in the preparation of ELISA test kits has been shown to lead to erroneous test results in analyses for troponin, human chorionic gonadotropin (hCG), CA 125, and other tumor marker assays.<sup>1-5</sup> As mouse monoclonal antibodies are used in the manufacturing of the Pak Plus<sup>®</sup> ELISA test kit, samples that contain human anti-mouse antibodies (HAMAs) could cause similarly inaccurate test results. As the use of monoclonal antibodies for diagnostic procedures and as therapy for various diseases is increasing, it is possible for more patients to develop these antibodies. The development of HAMAs has also been reported to occur when no known antigenic exposure has occurred.<sup>6</sup> It has also been reported that HAMAs have the capability of crossing the placenta, as they have been identified in both maternal and fetal samples.<sup>7</sup> HAMAs may cause falsely positive or falsely negative results in ELISAs. Klee et al.<sup>6</sup> found that 10-40 percent of patients had nonspecific antibodies to murine immunoglobulins. As described by them, three types of HAMAs can be developed, each having the ability to cause specific

problems in ELISA testing. The first type of antibody is directed against antigenic components found on the Fc portion of the murine protein molecule. These antibodies may be nonspecific, having no known antigenic stimulus. HAMAs may also be anti-idiotypic or anti-anti-idiotypic,<sup>6,8</sup> both of which can cause false reactions in ELISA testing. The anti-idiotypic antibodies may bind to the labeled antibodies at or near the binding site for the target antigen, thus blocking the binding of the antibodies in the test kits to the specific antigen. The anti-anti-idiotypic antibodies may bind to the original antigen and compete with the diagnostic antibody for binding sites.<sup>6</sup> If nonspecific HAMAs have developed, falsely high results occur by the binding of the HAMA to both the mouse monoclonal antibody bound to the antigen and the antibody added in the test process (Fig. 1). Falsely low results can occur when the anti-idiotypic HAMAs bind directly to the diagnostic antibody, in a way that prevents the detection of the patient's antibody, which was bound to the target antigen (Fig. 2).



**Fig. 1.** Falsely positive results due to HAMAs.

HAMAs are surprisingly common. In 1994, Dillman et al.<sup>9</sup> reported the results of a study in which 61 cancer patients had each received a single injection of radiolabeled murine monoclonal antibodies; 41 percent of the patients in the study developed HAMAs within 2 weeks of the injection. In the same year, Legouffe et al.<sup>10</sup> reported that HAMAs developed 7-10 days after patients received a monoclonal antibody to IL-6. These studies, in conjunction with the development of HAMAs as a result of an unknown stimulus, indicate that a high percentage of patients who may undergo

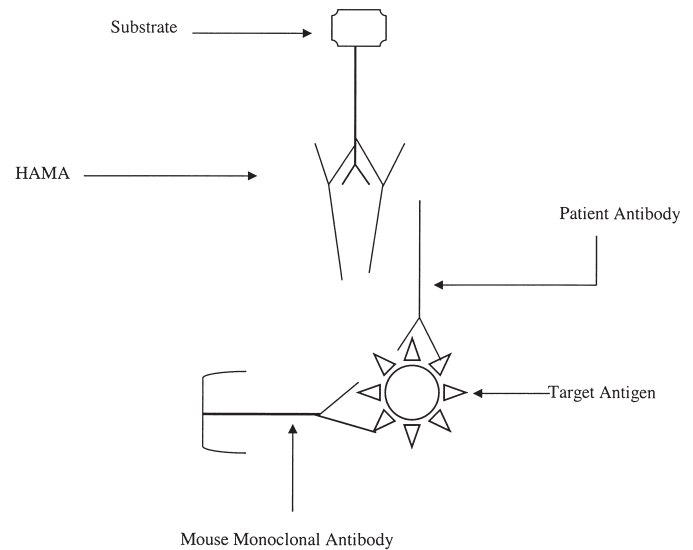
ELISA testing for detection of platelet- and/or HLA-specific antibodies may have HAMAs that could cause false test results. As both falsely positive and falsely negative results can occur in ELISA testing for platelet- and/or HLA-specific antibodies, it is important to have knowledge regarding the incidence of HAMAs in the patient population.

We initiated this study to determine the effect of HAMAs on these test results and the impact of such antibodies on the treatment of patients.

## Materials and Methods

### Study design

All patients' samples tested by ELISA for the presence of HLA- and/or platelet-specific antibodies between January 1995 and April 2002 were considered for inclusion in the study. Thirty positive samples, as well as ten negative samples, were selected randomly for HAMA neutralization/inhibition procedures. These samples were selected based only on the availability of



**Fig. 2.** Falsely negative results due to HAMAs.

sufficient sample to perform all the required testing. Positive samples, frozen for future use, underwent procedures to neutralize/inhibit any HAMAs that might be present; ELISA testing was repeated using these treated samples. An ELISA using the untreated sample was also repeated to detect any changes in reactivity that had occurred through storage.

The set of samples found negative after routine testing served as negative controls and underwent neutralization/inhibition procedures, followed by repeat ELISAs. All negative control samples were fresh.

## Technique

All ELISA tests were performed using manufacturer's directions and the reagents supplied in the test kit.

### *Neutralization/inhibition*

Samples underwent neutralization for possible HAMAs by the addition of 100  $\mu$ L of purified mouse immunoglobulin (kindly provided by GTI, Inc.) to the sample diluent. After this step, the sample was allowed to sit at room temperature for 10–15 minutes, then ELISA testing was performed using routine testing methods. To determine whether reactivity apparently neutralized by the addition of mouse immunoglobulin occurred due to neutralization and not to further dilution, the untreated samples were also tested after the addition of an equal volume of 5% bovine albumin. If mouse immunoglobulin was not available for use, Non-Specific Antibody Blocking Tubes (NABT, Scantibodies Laboratory, Inc., Santee, CA) were used for the neutralization process. The NABT contain immunoglobulins to which the nonspecific antibodies in the patients' samples bind. As these nonspecific antibodies, such as HAMAs, are bound to the immunoglobulins, they are then blocked from interfering in antibody detection assays. The NABT are for use only in antibody assays; similar blocking tubes, Heterophilic Blocking Tubes (HBT, Scantibodies Laboratory), are available for use in antigen detection assays. If the NABT were used in the neutralization/inhibition process, 500  $\mu$ L of sample was added to a tube, mixed well, and allowed to incubate at room temperature for 60 minutes; a negative control sample was treated in the same manner. After the incubation, an ELISA was repeated using the treated sample. To determine whether the reagent used in the neutralization/inhibition process caused different test results, samples were tested using both NABT and mouse immunoglobulin. The samples tested included those known to contain only HLA antibodies, those known to contain antibodies to HPA-1a, and those showing neutralization/inhibition after treatment with either mouse immunoglobulin or NABT. As no difference between the two methods of treatment was observed in this study conducted with a small number of samples, either was found suitable for use with this assay.

## Results

During the study period, 210 patients' samples were analyzed for the presence of platelet- and/or HLA-specific antibodies by ELISA. Negative results were obtained in 131 (62%) of the samples evaluated, with 79 (38%) found to be positive. Thirty (38%) of the samples found positive and ten samples giving negative results (controls) underwent neutralization/inhibition, followed by repeat ELISA testing. Of the ten samples tested as negative controls, nine (90%) were also negative after neutralization procedures. One (10%) of these negative control samples was found to be positive for HLA antibodies only when the treated sample was tested. Lymphocytotoxicity testing performed on the untreated sample was also found to be positive. The results of lymphocytotoxicity testing showed the presence of 41 percent reactivity, with HLA-specific antibodies directed against the A30, A31, A33, A29, and A11 antigens being detected.

When the positive samples were tested after neutralization/inhibition, reactivity was unchanged in 20 (67%) of the samples tested (Table 1). Eight of 20 positive samples (27%) showed reactivity that was neutralized by mouse immunoglobulin but still present when an equal amount of 5% bovine albumin was added (Table 2). It is important to note that no ELISA reactivity directed at HLA- or HPA-specific antigens was found to be attributable to HAMAs. Three (10%) of the

**Table 1.** Reactivity unchanged after neutralization

Reactivity	Number of Samples
HLA only	5
HLA and HPA-1a	2
HLA and HPA-5b	2
HPA-1a	1
HPA-5a	1
HLA and IIb/IIIa	4
IIb/IIIa	1
Ia/IIa	2
All wells reactive	2

**Table 2.** Reactivity neutralized by mouse immunoglobulin

Reactivity	Number of Samples
IIb/IIIa	3
Ia/IIa	2
Ib/IX	1
Ia/IIa; IV	1
All platelet glycoproteins	1

positive samples tested showed new reactivity that was present only in the samples that had undergone the neutralization/inhibition procedure. Of these three samples, one was reactive only with HLA antigen and two were reactive only with glycoprotein Ia/IIa.

We calculated the optical density (OD) ratios observed in initial testing for those samples that showed no changes in reactivity after neutralization in comparison with those samples in which reactivity was neutralized. This ratio was determined by comparison of the ODs obtained for the test and control wells. If reactivity of a positive sample was not affected by the neutralization process, the OD ratio was found to be  $7.08 \pm 8.82$  (2.47 to 22.83). The OD ratio for the reactivity that was neutralized by the addition of mouse immunoglobulin was found to be  $2.27 \pm 0.25$  (2.00 to 2.79;  $p < 0.05$ ).

## Discussion

This study showed that the presence of HAMAs influences the results of testing for the presence of HLA- and/or platelet-specific antibodies when the GTI ELISA test kit is used. Even though the reporting of falsely positive test results could influence patient care decisions, the ability of HAMAs to inhibit reactivity might be considered a more significant finding. If neutralization/inhibition procedures were not performed, falsely negative results would be reported, and thus important information would not be available for use in selection of appropriate units for transfusion.

A study of 26 nonneutralized serum samples using the Pak Plus<sup>®</sup> test kits, the platelet immunofluorescence test, and the monoclonal antibody immobilization of platelet antigen (MAIPA) procedure failed to detect eight antibody specificities in seven samples (26%); falsely positive reactions were observed in four (15%) samples.<sup>11</sup> These results were obtained only with the Pak Plus<sup>®</sup> test kit. As our study showed that the presence of HAMAs caused falsely positive reactions in 27 percent and falsely negative results in 10 percent of the samples tested, the presence of HAMAs could possibly be the cause of some of the discrepancies reported in the earlier study.<sup>11</sup>

As a result of our study, we consider the possibility of HAMAs in samples that are tested by ELISA if antibodies against more than one platelet-specific glycoprotein are detected on routine testing. If

antibodies against multiple platelet glycoproteins were truly present, the patient would either have multiple platelet-specific antibodies, lack a specific platelet glycoprotein (leading to the development of the corresponding antibodies), or have a diagnosis of idiopathic thrombocytopenic purpura (ITP). When such reactivity is observed, it is important to further evaluate the samples to determine whether these reactivities are due to HAMAs or to antibodies actually produced by the patient. The presence of HAMAs should also be considered if the OD ratio of a positive sample is found to be less than 3.0. If the presence of HAMAs is suspected due to the fulfillment of one or both of these criteria, testing is repeated on samples in which HAMAs have been neutralized/inhibited.

As the presence of HAMAs was associated with falsely negative results in 10 percent of the apparently negative samples, the possibility of falsely negative results in patients' samples should be considered if HLA- and/or platelet-specific antibodies are suspected from the patient's history. In these cases, testing after neutralization/inhibition procedures should also be performed.

The use of the ELISA test kits for detection of both HLA- and platelet-specific antibodies can provide valuable information that can be used in patient treatment, in cases of suspected NAIT, or in the selection of appropriate platelet units for thrombocytopenic patients. It is important to consider the possible presence of falsely positive results whenever weakly positive test results are found. Falsely negative results should also be considered if the presence of antibodies is suspected, based on response to transfusion or patient history. In either case, neutralization/inhibition and repeat ELISA testing should be performed and the results evaluated for the presence of HAMAs that may have interfered with the original test results. The use of neutralization/inhibition procedures allows laboratories to eliminate falsely positive and falsely negative reactions and thus leads to more accurate results that can be important in the provision of care for the patients being evaluated. When the effect of these HAMAs on test results is considered, transfusion service laboratories should evaluate the possibility of routinely treating samples prior to initial testing to avoid the reporting of false test results and also to avoid the time and cost involved in repeat testing of implicated samples.

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# Equivalence of spray-dried K<sub>2</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and liquid K<sub>3</sub>EDTA anticoagulated blood samples for routine blood center or transfusion service testing

S. LEATHEM, N.D. ZANTEK, M. KEMPER, L. KORTE, A. LANGE BERG, AND S.G. SANDLER

We compared the results of routine blood tests for 102 blood donors' samples and 100 patients' samples collected in spray-dried K<sub>2</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and liquid K<sub>3</sub>EDTA blood collection tubes to evaluate the impact of changes in formulation of the anticoagulant (K<sub>2</sub>EDTA vs. K<sub>3</sub>EDTA), its application (liquid vs. spray-dried), and tube material (glass vs. plastic). Methods for ABO/D testing, antibody screening, and antibody identification included direct hemagglutination/microplate (Olympus® PK 7200) and gel column methods (Ortho ID-Micro Typing System™/Gel Test™). Additional studies on blood donors' samples included time delayed antigen testing and antibody identification and half-draw/half-evacuated collections. Also, we compared the results of routine ABO/D testing and antibody screening for 50 patients' samples collected in spray-dried K<sub>2</sub>EDTA and spray-dried K<sub>3</sub>EDTA and for an additional 50 patients' samples collected in spray-dried K<sub>2</sub>EDTA tubes from two different manufacturers. All patients' samples were tested in parallel by solid phase/microplate method (Immucor® ABS 2000) and the standard manual tube method. All test results for routine blood bank tests on donors' and patients' samples were concordant, demonstrating the equivalence of spray-dried K<sub>2</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and liquid K<sub>3</sub>EDTA blood collection tubes for routine donor center or transfusion service testing. *Immunohematology* 2003;19:117–121.

**Key words:** blood typing, antibody screening, blood donors, EDTA, blood collection tubes

Manufacturers of blood sample collection tubes have increased the options for anticoagulants that can be used to collect blood samples for routine testing in blood centers and transfusion services. Blood sample tubes are now commercially marketed containing dipotassium or tripotassium ethylenediaminetetraacetic acid (K<sub>2</sub>EDTA or K<sub>3</sub>EDTA) anticoagulant in liquid or spray-dried formulation, and in glass or plastic

tubes.<sup>1,2</sup> EDTA in blood collection tubes may induce platelet agglutination and falsely low platelet counts (EDTA-dependent pseudothrombocytopenia),<sup>3–11</sup> requiring specific validation of K<sub>2</sub>EDTA- and K<sub>3</sub>EDTA-anticoagulated blood samples for various hematology analyzers.<sup>12–15</sup> Also, EDTA in blood collection tubes has been reported to cause agglutination of RBCs tested in blood banks,<sup>16–20</sup> to inhibit the detection of anti-A<sub>1</sub> when reagent RBCs were suspended in EDTA,<sup>21</sup> and to cause prolonged in vitro hemolysis in samples collected from a patient after a delayed hemolytic reaction.<sup>22</sup> Therefore, it is important that new EDTA-anticoagulated blood collection tubes be validated for their suitability for routine blood bank testing. The following report describes an evaluation of four EDTA anticoagulant blood sample tubes that are currently available to hospitals and blood centers in the United States. The results of this study provide general guidance for using these blood collection tubes, but they do not substitute for the validations that each testing facility should conduct using diagnostic reagents, analyzers, and technical methods that are specific for that facility.

## Materials and Methods

### *Blood samples from blood donors*

Three blood samples were collected from each blood donor, using a Vacutainer™ K<sub>3</sub>EDTA (liquid

K<sub>3</sub>EDTA, 7.0 mL 13 × 100 mm glass tube) (Becton Dickinson, Franklin Lakes, NJ), a Vacuette® EDTA K3 (spray-dried K<sub>3</sub>EDTA, 6.0 mL, 13 × 100 mm plastic tube) (Greiner Bio-One, Monroe, NC), and a Vacuette® EDTA K2 (spray-dried K<sub>2</sub>EDTA, 6.0 mL, 13 × 100 mm plastic tube) (Greiner Bio-One). Blood donors' samples were collected at two sites (BloodSource Center for Blood Research, Sacramento, CA, and Gulf Coast Regional Blood Center, Houston, TX), under each site's institutional review board's approval and informed consent. The categories and numbers of blood donors' samples from sites one and two are specified in Tables 1 and 2.

**Table 1.** Donor center/site 1

Categories of blood donors' samples (number)
Apparently healthy donors (full-draw tubes) (50) Subset: apparently healthy donors for antigen phenotyping (10) Subset: apparently healthy donors for delayed antigen testing (0, 15, or 19 days) (10)
Known antibody-positive blood donors (full-draw tubes) (15) Subset: known antibody-positive individuals (half-draw/half-evacuated tubes) (10) Subset: known antibody-positive individuals (full- and half-draw/half-evacuated tubes for delayed testing) (10)

**Table 2.** Donor center/site 2

Categories of blood donors' samples (number)
Apparently healthy donors (52) Subset: apparently healthy donors for antigen phenotyping (10)
Apparently healthy donors with known red cell antibodies (10)

### Blood samples from patients

Blood samples from patients in selected disease categories were collected at Georgetown University Hospital/Medstar Health, Washington, DC (site 3). Patients were recruited for the study when their physicians ordered routine compatibility tests. To minimize the volume of additional blood collected from study patients, only 3.0 mL blood collection tubes were used and the study was designed so only one additional blood tube was collected from each patient. In an initial study, two blood sample tubes were collected from each of 50 patients, one sample for the physician-ordered compatibility testing using the hospital's standard Vacutainer™ Plus K<sub>2</sub>EDTA (spray-dried K<sub>2</sub>EDTA, 3.0 mL plastic tube, Becton Dickinson) and a second sample using a Vacuette® EDTA K3 (spray-dried K<sub>3</sub>EDTA, 3.0 mL plastic tube, Greiner Bio-One). In a second study, two blood sample tubes were collected from each of 50 additional patients, one sample for the physician-ordered compatibility testing using the

hospital's standard Vacutainer™ Plus K<sub>2</sub>EDTA (spray-dried K<sub>2</sub>EDTA, 3.0 mL plastic tube, Becton Dickinson) and a second sample using a Vacuette® EDTA K2 (spray-dried K<sub>2</sub>EDTA, 3.0 mL plastic tube, Greiner Bio-One). Georgetown University's Institutional Review Board approved the blood collection protocol and each participant volunteered by signing an informed consent form. The categories of diseases for the two groups of 50 patients were the same (Table 3).

**Table 3.** Hospital/site 3

Categories of disease for each 50-patient study group (number)
Multi-transfused [Hb SS (2), thalassemia (1) and other patients with antibodies (2)] (5)
Cardiology (5)
Leukemia (5)
Bone marrow transplant (5)
Liver disease (5)
General surgery (10)
General medicine (15)

### Test methods

#### Direct Hemagglutination (Microplate Method)

Donor center/site 1 performed routine donor testing (ABO/D) using the Olympus® PK 7200 Automated Microplate System (Olympus® America, Inc., Melville, NY). In addition, the antibody screening and identification, antigen phenotyping, and DATs were performed using manual methods.

#### Gel Column Method

Donor center/site 2 performed routine donor testing (ABO/D, antibody screening and identification, antigen phenotyping, and DATs) as above, using the Ortho ID-Micro Typing System™ (ID-MTS) Gel Test™ (Ortho-Clinical Diagnostics, Raritan, NJ).

#### Solid Phase and Microplate Methods

Hospital/site 3 performed routine compatibility testing (ABO/D and antibody screening) using the Immucor® ABS 2000 Blood Typing System (Immucor®, Norcross, GA) in parallel with a manual method (ABO/D and DAT) and a standard LISS tube method (antibody screening and identification).

#### DATs

Using the standard method, blood centers performed DATs on blood samples, from the 102 blood donors, that had been collected in the three EDTA-containing collection tubes described above. In

addition, a panel of five simulated DAT-positive samples were prepared and tested at donor center/site 1 using samples collected in the three EDTA-containing collection tubes. Anti-Fy<sup>a</sup>-coated RBCs were prepared according to the method described in the FDA Center for Biologics Evaluation and Research Guidance Document "Recommended Methods for Anti-Human Globulin Evaluation" (March 1992). The dilutions were selected to represent a range of positive reactivity. The samples were tested on day 0 (date of preparation) and repeated on days 7 and 14.

## Results

### *Blood donors' samples*

#### ABO/D Testing

ABO/D typing was performed in triplicate using blood samples from 102 apparently healthy blood donors, including 25 known antibody-positive donors, at donor centers/sites 1 and 2, by automated microplate method (50 donors) and by gel column agglutination method (52 donors). All test results were concordant.

#### Antigen Phenotyping

RBC phenotyping was performed at donor centers/sites 1 and 2 in triplicate on blood samples from 20 apparently healthy blood donors. The samples were phenotyped for selected antigens in the Rh, Kell, Duffy, Kidd, and MNS blood group systems (Table 4). All phenotype results were concordant, whether the samples were collected in liquid K<sub>3</sub>EDTA, spray-dried K<sub>3</sub>EDTA, or spray-dried K<sub>2</sub>EDTA.

### *Antibody screening and identification*

#### Full-Draw Tubes

Antibody screening was performed in triplicate on samples from 102 apparently healthy blood donors, including 25 donors with known blood group antibodies, using blood samples that contained the manufacturers' recommended volume (full draw). All positive antibody screening samples were followed up with antibody identification. All results were concordant. No antibodies were detected in the 100 apparently healthy donor samples. Paired samples from the 25 antibody-positive donors gave positive screens and concordant antibody identifications, as follows: anti-K (6 donors), anti-M (2 donors), anti-D (8 donors), anti-E (4 donors), anti-Fy<sup>a</sup> (2 donors), an anti-Jk<sup>a</sup>, anti-DC (3 donors), anti-C (2 donors), an anti-Sc1, and a previous anti-D, now negative. Some samples

**Table 4.** Results of phenotyping RBCs from unselected blood donors collected in liquid K<sub>3</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and spray-dried K<sub>2</sub>EDTA

Reagent antiserum	Donor center/site 1: Olympus® PK 7200 automated microplate system		Blood center/site 2: Ortho ID micro typing system™	
	Antigen detected	Antigen not detected	Antigen detected	Antigen not detected
C	6	4	5	5
E	1	9	2	8
c	8	2	NT*	NT
e	9	1	NT	NT
K	0	10	1	9
k	NT	NT	10	0
Fy <sup>a</sup>	7	3	6	4
Fy <sup>b</sup>	9	1	5	5
Jk <sup>a</sup>	8	2	9	1
Jk <sup>b</sup>	5	5	7	3
S	7	3	4	6
s	10	0	10	0
M	7	3	NT	NT
N	8	2	NT	NT

\*NT = Not tested

contained multiple antibodies. In some comparisons, there was a 1+ difference, but these minimal differences did not change interpretations of test results.

#### Half-Draw Tubes

In addition, ABO/D typings, antibody screenings, and antibody identifications were performed in triplicate on a subset of ten of the known antibody-positive blood donors at donor center/site 1, using half-draw/half-evacuated spray-dried K<sub>3</sub>EDTA and K<sub>2</sub>EDTA plastic tubes and full-draw liquid K<sub>3</sub>EDTA glass tubes. All results were concordant with each other and the full-draw results. In some of the comparisons, there was a 1+ difference in reactivity, but these minimal differences did not change interpretations of test results.

#### Delay in Testing

Ten of the antigen phenotyping samples and ten of the known antibody-positive samples (including both full- and half-draw/half-evacuated tubes) were stored at 2°-8°C in the original collection tubes after initial testing. Testing was repeated at 15 to 19 days after collection. The antigen phenotyping samples were only repeated for antigen phenotyping testing. These results were concordant at day 19. The known antibody-positive blood donor samples were retested

for ABO/D typing and antibody screening and identification. Concordant results were obtained between the full- and half-draw/half-evacuated plastic, spray-dried K<sub>3</sub>EDTA and K<sub>2</sub>EDTA collection tubes and the full-draw glass, liquid K<sub>3</sub>EDTA tubes at day 14. However, in some of the comparisons, there was a 1+ difference in reactivity. This variation is within the expected reproducibility of a subjective grading system. A decrease in grading results was observed in some samples between day 0 and the last day of testing (day 15 or day 19). This is also not unexpected, considering the age of the samples.

#### DAT

There were no DAT-positive results among the 102 blood donors in three types of collection tubes. In addition, the results of DATs were concordant for all simulated DAT-positive samples collected in spray-dried K<sub>2</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and liquid K<sub>3</sub>EDTA blood collection tubes on days 0, 7, and 14. For some samples, there was a 1+ difference in reactions, but these minimal differences did not change interpretations of test results.

#### *Compatibility testing—patients' samples*

The results of testing paired blood samples from the 100 patients for ABO/D and antibody screening were all concordant. Paired samples from 10 patients with alloantibodies gave positive screens and concordant antibody identifications, as follows: anti-C + anti-E + anti-Fy<sup>a</sup>, anti-K (2 patients), anti-c, anti-E (3 patients), anti-C + anti-E, anti-Js<sup>b</sup>, and anti-E + anti-Jk<sup>a</sup>. One patient was positive on antibody screening by the ABS 2000 but negative by manual testing and negative on antibody identification testing; this sample probably contained a LISS interfering substance.

## **Discussion**

The results of this study demonstrate the equivalence of spray-dried K<sub>2</sub>EDTA, spray-dried K<sub>3</sub>EDTA, and liquid K<sub>3</sub>EDTA blood collection tubes for blood samples intended for routine blood bank testing. The finding of concordant test results using highly sensitive solid phase and gel column methods, as well as standard tube methods, supports the premise that the changes in formulation of EDTA (K<sub>2</sub>EDTA vs. K<sub>3</sub>EDTA), its application (liquid vs. spray-dried), or tube material (glass vs. plastic), do not impact on blood group antibody-antigen interactions. The finding of concordant results for samples collected in plastic versus glass sample tubes demonstrates that neither ABO antibodies nor a wide range of other common blood group antibodies are adsorbed on the plastic surface during storage. There was no evidence that substances leached from the inner surface of plastic tubes and interfered with hemagglutination reactions.

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# Assessment of the relative number of copies of the gene encoding human neutrophil antigen-2a (HNA-2a), *CD177*, and a homologous pseudogene by quantitative real-time PCR

K. DITTMAR, J-B. LIM, L. CARUCCIO, M. BETTINOTTI, AND D. STRONCEK

Human neutrophil antigen-2a (HNA-2a; NB1) is located on the 58–64 kD NB1 glycoprotein (GP) and is encoded by the gene *CD177*. Searches of human genome databases have revealed that a pseudogene highly homologous to exons 4–9 of *CD177* is located adjacent to *CD177* on chromosome 19. The purpose of this study was to document the presence of the pseudogene and determine whether the polymorphic expression of NB1 GP is due to *CD177* gene deletions and duplications. Genomic DNA was isolated from leukocytes of 12 subjects. The number of copies of exon 2 of *CD177*, an exon that is unique to this gene, and the number of copies of exon 9, an exon that is found in both *CD177* and the pseudogene, was assessed with quantitative real-time PCR. The ratio of the number of copies of sequences homologous to *CD177* exon 9 to the number of copies of exon 2 was 1.5 or greater in 7 of the 12 subjects, suggesting that both *CD177* and the homologous pseudogene were present. The ratio of exon 9 to exon 2 in the other 5 subjects ranged from 1 to 1.25, suggesting that the pseudogene was not present in these subjects. However, results of assays were variable and we could not exclude the possibility that all subjects carried the pseudogene. These studies confirmed the presence of the pseudogene homologous to *CD177*, but quantitative real-time PCR was not precise enough to detect *CD177* duplications or deletions. *Immunohematology* 2003;19:122–126.

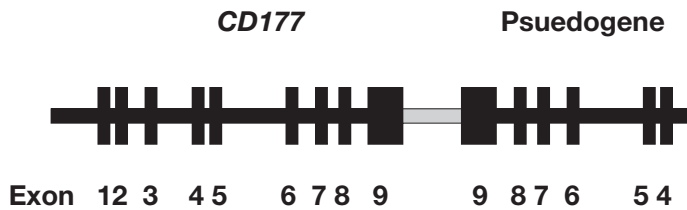
**Key Words:** HNA-2a, *CD177* gene, pseudogene, real-time PCR

Human neutrophil antigen-2a (HNA-2a) is located on NB1 glycoprotein (GP) and is encoded by *CD177*.<sup>1–4</sup> The antigen frequency of HNA-2a is 93 percent to 97 percent and HNA-2a is unique in that it is expressed on subpopulations of neutrophils.<sup>5–7</sup> *CD177* belongs to the Ly-6 superfamily. Members of this gene superfamily are characterized by conserved 70- to 80-amino acid domains containing ten cysteine residues. However,

genes in this family encode proteins with diverse functions and members of this family show relatively little nucleotide identity. Other genes in this family that are expressed by blood cells include urokinase-type plasminogen activator receptor (*uPAR*, *CD87*), which is found on leukocytes, and reactive inhibitor of lysis receptor (*CD59*), which is found on both RBCs and leukocytes.

The function of the NB1 GP encoded by *CD177* is not known, but *CD177* mRNA levels are increased in neutrophils from patients with polycythemia vera and from people receiving granulocyte-colony stimulating factor.<sup>8,9</sup> Antibodies to HNA-2a can cause neonatal alloimmune neutropenia, autoimmune neutropenia, TRALI, or delayed recovery of neutrophil counts after marrow transplantation.<sup>10</sup>

*CD177* is located on chromosome 19q13.2 and has 9 exons.<sup>11</sup> Searches of the human genome databases suggest that adjacent to *CD177* is a pseudogene that is highly homologous to exons 4 through 9 of *CD177* (Fig. 1).<sup>11</sup> Pseudogenes have sequences related to functional genes, but are unable to code for proteins due to deficiencies that affect translation or transcription. Because of the close proximity and high homology of *CD177* and the pseudogene, human genome database data did not allow the determination of the exact structure of chromosome 19 in the region of *CD177* and the pseudogene. The purpose of this study was to obtain experimental data that



**Fig. 1.** The proposed structure of *CD177* and a homologous pseudogene in chromosome 19q13.2. The structure of chromosome 19q13.2 was determined using GenBank and Joint Genome Institute databases. The GenBank data in this region of chromosome 19 were incomplete, creating a sequence gap. Public Joint Genome Institute databases allowed Bettinotti and colleagues to predict this structure of 19q13.2.<sup>11</sup>

documented the presence of the pseudogene homologous to *CD177* and, if *CD177* gene duplications, deletions, or both occur, determine whether they are responsible in part for the polymorphic expression of HNA-2a and NB1 glycoprotein.

Quantitative real-time PCR uses PCR and a fluorescent probe designed to be incorporated in the DNA being amplified.<sup>12</sup> In this assay, relative increases in fluorescent emissions are monitored during PCR, using an analytical thermal cycler. Quantitative real-time PCR is being used to quantitate small amounts of DNA or mRNA.

Neutrophil HNA-1 antigens are located on neutrophil Fc-gamma receptor IIIb (FcγRIIIb) and are encoded by *FCGR3B*.<sup>10</sup> The polymorphic expression of FcγRIIIb is in part due to duplications and deletions of *FCGR3B*. Quantitative real-time PCR has been used to document *FCGR3B* gene duplications<sup>13</sup> and we thought that a similar assay could be used to document the presence of the pseudogene homologous to *CD177*. We used quantitative real-time PCR to compare the numbers of copies of *CD177* and the *CD177* pseudogene.

**Methods and Materials**

*Study design*

The number of copies of the *CD177* gene and of the *CD177* pseudogene was assessed among several people, using quantitative real-time PCR. Since the number of copies of genomic *CD177* and of the *CD177* pseudogene was being measured, DNA was isolated from peripheral blood leukocytes. Quantitative real-time PCR was used to measure copies of genomic DNA

that was homologous to a part of *CD177* that was shared by both *CD177* and the homologous pseudogene, exon 9, and a part of *CD177* that was not shared by the pseudogene, exon 2. The ratio of the number of copies of the *CD177* exon 9 to *CD177* exon 2 was compared. If each chromosome 19 in a subject contains one copy of the *CD177* gene and one copy of the pseudogene, then the ratio of the number of copies of exon 9 to exon 2 is 2.0 (Table 1). If a pseudogene is present, but *CD177* is not, then the ratio of exon 9 to exon 2 is infinity. If *CD177* is present, but the pseudogene is not, then the ratio is 1.0. If a *CD177* gene duplication is present, the ratio is 1.5. The ratio will be less than 1.5 if multiple duplications are present. If a *CD177* pseudogene duplication is present, then the ratio of exon 9 to exon 2 is 3.0. The ratio is greater than 3.0 if multiple duplications are present (Table 1).

**Table 1.** Expected outcomes of comparisons of copies of genomic sequence homologous to exons 2 and 9 of *CD177* in people with various genotypes

Genotype*	Copies of each in chromosome 19				(Exon 9 copies)/ (Exon 2 copies)
	Gene	Pseudogene	Exon 2	Exon 9	
1 <i>CD177</i> + 1 <i>CD177</i> pseudogene	1	1	1	2	2.0
<i>CD177</i> deletion	0	1	0	1	infinity
<i>CD177</i> duplication	2	1	2	3	1.5
<i>CD177</i> pseudogene deletion	1	0	1	1	1.0
<i>CD177</i> pseudogene duplication	1	2	1	3	3.0

\*Assuming that the subjects are homozygous

*Quantitative real-time PCR*

Leukocyte DNA was isolated from 1.0 mL of whole blood collected in ACD using a kit according to the manufacturer's instructions (Puregene, Gentra Systems, Minneapolis, MN). One microliter leukocyte DNA at a concentration of 100 µg/mL was used as a template to measure genomic sequences homologous to exons 2 and 9 of *CD177* by quantitative real-time PCR using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer of Foster City, CA). Quantitative real-time PCR results were reported as the number of copies of exon 9 divided by the number of copies of exon 2.

Primers and TaqMan probes were designed to produce amplicons < 150 bp, enhancing the efficiency of PCR amplification (Biosource International, Camarillo, CA) (Table 2). TaqMan probes were labeled at the 5' end with the reporter dye molecule FAM (6-carboxyfluorescein; emission λ<sub>max</sub> = 518 nm) and at the 3' end with the quencher dye molecule TAMRA (6

**Table 2.** Primers and probes used to analyze by quantitative real-time PCR genomic sequences homologous to exons 2 and 9 of *CD177*

Exon	Type	Sequence	Amplicon Size (bp)
2	Forward primer-ex 2	5'GGCAATGGACCCCTAAGAACA 3'	74
	Reverse primer-ex 2	5'GCTCTCAATGAGCATCAACGTG 3'	
	Probe-ex 2	5'CAGCTGCGACAGCGGCTTGG 3'	
9	Forward primer-ex 9	5'GCCCCAACCTTCCAGCTTCTT 3'	79
	Reverse primer-ex 9	5'GCTGCACATCACGCTTCTCAC 3'	
	Probe ex 9	5'TTGAACCACACCAGACAAATCGGG 3'	

carboxytetramethylrhodamine; emission  $\lambda_{\max} = 582$  nm) (Table 2). The *CD177* coding region was isolated and used as a standard curve. The coding region of *CD177* was amplified using sequence-specific primers from human fetal liver total RNA (Stratagene, La Jolla, CA) and the amplicon was cloned using a Topo TA cloning kit (Invitrogen Corporation, Huntsville, AL). DNA from several of the clones was analyzed for the correct size insert by digestion with Eco RI and analyzed further by sequencing using sequence-specific primers and a cycle-sequencing kit (Big Dye Terminator, Perkin-Elmer Applied Biosystems, Inc., Foster City, CA). The sequencing reaction products were purified using the DyeEx Spin Kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions and the reactions were analyzed on a genetic analyzer (ABI Prism 377, Perkin-Elmer). The *CD177* coding sequence was purified and quantified by spectrophotometry (OD260). The number of DNA copies was calculated using the molecular weight of each gene amplicon. Serial dilutions of the amplified gene at known concentrations were tested by quantitative real-time PCR.

Quantitative real-time PCR reactions of DNA specimens and standards were conducted in a total volume of 25  $\mu$ L with 1  $\times$  TaqMan Master Mix (Perkin-Elmer) and primers and probes at optimized concentrations (primers 10,000 nM and probes 12,000 nM). Thermal cycler parameters were 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles involving denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. Real-time monitoring of fluorescent emission from the cleavage of sequence-specific probes by the nuclease activity of Taq polymerase allowed definition of the threshold cycle number during the exponential phase of amplification.

Standard curves of the threshold cycle number versus the log of the number of copies of genes were generated for exon 2 and exon 9. The reactions were found to have excellent PCR amplification efficiency

(90–100%; 100% indicates that after each cycle the amount of template is doubled) as determined by the slope of the standard curves. Linear regression analysis showed that  $r^2$  for all standard curves was  $> 0.98$ .

For each sample of DNA tested the threshold cycle number was measured with exon 2 primers and probes and exon 9 primers and probes. The standard curves were used to extrapolate the number of copies of exon 2 and exon 9. All PCR assays were performed in quadruplicate and reported as the average.

## Results

### Comparison of *CD177* and *CD177* pseudogene copy numbers

In 12 subjects quantitative real-time PCR was used to compare the number of copies of genomic sequences that were homologous to exons 2 and 9 of *CD177*. In all subjects the number of copies of exon 9 was greater than or approximately equal to the number of copies of exon 2. The ratio of copies of exon 9 to copies of exon 2 varied from 0.98 for subject 11 to 3.33 for subject three (Table 3). In seven of 12 subjects the number of copies of exon 9 was greater than the number of copies of exon 2: exon 9 to exon 2 ratios of 1.45 or greater. These results suggest that both *CD177* and the pseudogene were present in these seven subjects. In the other five subjects, the number of copies of exon 9 was approximately equal to the number of copies of exon 2: exon 9 to exon 2 ratios from 0.98 to 1.24. These results suggest that for these five subjects, *CD177* was present, but the homologous pseudogene was not.

**Table 3.** Comparison of the ratio of the numbers of genomic sequences homologous to exons 2 and 9 of *CD177* as determined by quantitative real-time PCR

Donor	Copies Exon 9/Copies Exon 2	Interpretation
1	1.13	No pseudogene
2	1.24	No pseudogene
3	3.33	Gene and pseudogene
4	2.54	Gene and pseudogene
5	2.38	Gene and pseudogene
6	1.20	No pseudogene
7	1.45	Gene and pseudogene
8	1.86	Gene and pseudogene
9	2.56	Gene and pseudogene
10	2.20	Gene and pseudogene
11	0.98	No pseudogene
12	1.12	No pseudogene

### Reproducibility of results

To compare the reproducibility of the quantitative real-time PCR assay in five subjects, comparisons of genomic sequences homologous to *CD177* sequences in exon 2 and exon 9 were performed on two separate occasions. In three of the five subjects the results of the first and second tests were very similar, but in two subjects the ratio of the number of copies of exon 9 to exon 2 varied markedly (Fig. 2).

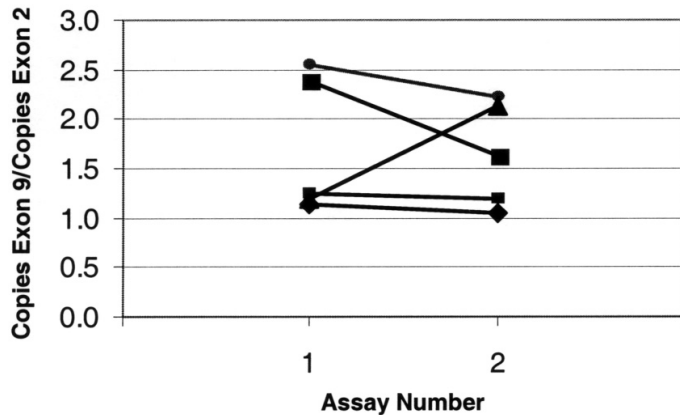


Fig. 2. The results of repeat analysis of the ratio of genomic sequences homologous to exon 9 and exon 2 of *CD177*. In five subjects the ratio of the quantities of sequences in leukocyte DNA homologous to exon 9 and exon 2 of *CD177* was measured on two separate occasions using quantitative real-time PCR.

### Discussion

These results provide experimental support of information in the human genome database that suggested that a pseudogene that is highly homologous to *CD177* is present in the human genome. Our experimental data suggest that both *CD177* and the homologous pseudogene were present in seven of 12 subjects tested. However, the large degree of variability inherent in the assay makes our results difficult to interpret and we cannot exclude the possibility that all subjects carry the pseudogene.

We had hoped to compare the size of the neutrophil population that expressed NB1 GP with a measurement of the number of copies of *CD177* relative to the number of copies of the pseudogene. However, the quantitative real-time PCR assay was not precise enough to determine the presence of duplications or deletions of *CD177* or the homologous pseudogene. Other investigators have reported that the reproducibility of the threshold cycle number measured by quantitative real-time PCR is 2 percent to 5 percent.<sup>14</sup> However, the threshold cycle number is linearly related to the log of the gene copy number. As

a result the variability of the number of gene copies is greater and can be as high as 12 percent. Since we were reporting one measure divided by another, the potential for variability is even greater. Our results are consistent with those of Gittinger and colleagues, who found variations in expected gene copy number and actual gene copy number were as great as 50 percent.<sup>15</sup>

Wolff and colleagues have used quantitative real-time PCR to compare neutrophil *CD177* mRNA copy numbers and the proportion of neutrophils expressing NB1 GP.<sup>9</sup> They found that people with a larger population of neutrophils expressing NB1 GP had greater quantities of *CD177* mRNA in their neutrophils, but the quantities of *CD177* mRNA varied more than 100-fold among individuals. In addition, Temerinac and colleagues have found that *CD177* mRNA levels are greater in neutrophils from people with polycythemia rubra vera and in some people with essential thrombocytosis.<sup>8</sup> However, in patients with polycythemia rubra vera *CD177* mRNA levels were severalfold greater than in healthy subjects.<sup>8,15,16</sup> These large variations in *CD177* mRNA levels make quantitative real-time PCR a useful tool in assessing *CD177* mRNA levels.

In conclusion, we confirmed that the genome carries a pseudogene homologous to *CD177*. While quantitative real-time PCR is important in studying and comparing *CD177* mRNA levels, it was not helpful in measuring and comparing genomic *CD177* copy numbers.

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# Neonatal alloimmune thrombocytopenia due to anti-HPA-5b (Br<sup>a</sup>)

S.A. CAMPBELL-LEE, D. DESANTIS-PARSONS, R. SUE SHIREY, AND T.S. KICKLER

Neonatal alloimmune thrombocytopenia (NAIT) results from maternal immunization against fetal platelet antigens and can occur during the first pregnancy. The most common complications of NAIT are neonatal thrombocytopenia, intracerebral hemorrhage, and fetal death. Most cases of NAIT in Caucasians are caused by anti-HPA-1a (PI<sup>A1</sup>). Anti-HPA-5b (Br<sup>a</sup>) accounts for only 4.3 percent of all NAIT cases. NAIT due to anti-HPA-5b is thought to be milder and have fewer complications than NAIT caused by anti-HPA-1a because of the lower number of HPA-5b antigenic sites per platelet. This report describes a severe case of NAIT due to anti-HPA-5b that was treated by intrauterine platelet transfusion. *Immunohematology* 2003;19:127–131.

**Key Words:** neonatal alloimmune thrombocytopenia (NAIT), HPA-5b, Br<sup>a</sup>

Platelets express class I human leukocyte antigens (HLA), ABH, P, Lewis, I, and platelet-specific antigens. Twenty-one platelet-specific antigens have been identified; they are referred to as human platelet antigens (HPA), with an identifying number in the order of discovery. As with the nomenclature for RBC blood group antigens, there are alternative terminologies for platelet-specific antigens (Table 1). For example, HPA-1a, the first platelet antigen identified, was originally designated Zw<sup>a</sup>, but is more commonly known as PI<sup>A1</sup>. HPA-1a or PI<sup>A1</sup> resides on glycoprotein (GP) IIIa and is present on the platelets of 98 percent of Caucasians. The antithetical antigen, HPA-1b (PI<sup>A2</sup>), is found in an estimated 27 percent of Caucasians. The HPA-5 system consists of two antigens, HPA-5a (Br<sup>b</sup>) and HPA-5b (Br<sup>a</sup>), located on platelet GPIa. In this instance, HPA-5a (Br<sup>b</sup>) is a high-frequency antigen found in 99 percent of Caucasians, while HPA-5b (Br<sup>a</sup>) is present on the platelets of approximately 20 percent of Caucasians.<sup>1</sup> The frequencies of platelet-specific antigens in African Americans are not well defined.

As with RBC blood group antigens, alloimmunization against foreign platelet antigens can occur.

**Table 1.** Human platelet-specific alloantigens. Reprinted with permission from Curtis BR, Gottschall JL, McFarland JG. Platelet immunology and alloimmunization. In: Simon T, Dzik W, Snyder E, Stowell C, Strauss R, eds. Rossi's Principles of transfusion medicine. 3rd ed. Philadelphia: Lippincott, 2002:203-17.

New nomenclature	Old nomenclature	Phenotype frequency(%)*
HPA-1a	PI <sup>A1</sup> (Zw <sup>a</sup> )	98
HPA-1b	PI <sup>A2</sup> (Zw <sup>b</sup> )	28
HPA-2a	Ko <sup>b</sup>	99
HPA-2b	Ko <sup>a</sup>	15
HPA-3a	Bak <sup>a</sup>	85
HPA-3b	Bak <sup>b</sup>	63
HPA-4a	Yuk <sup>b</sup> (Pen <sup>a</sup> )	99.9
HPA-4b	Yuk <sup>a</sup> (Pen <sup>b</sup> )	< 0.1
HPA-5a	Br <sup>b</sup>	99
HPA-5b	Br <sup>a</sup>	20
HPA-6wb	Ca <sup>a</sup> , Tu	< 1.0
HPA-7wb	Mo <sup>b</sup>	< 1.0
HPA-8wb	Sr <sup>a</sup>	< 0.1
HPA-9wb	Max <sup>a</sup>	< 1
HPA-10wb	La <sup>a</sup>	1
HPA-11wb	Gro <sup>a</sup>	< 0.5
HPA-12wb	Iy <sup>a</sup>	1
HPA-13wb	Sit <sup>a</sup>	< 1

\*Phenotype frequencies for Caucasians only. W denotes workshop—both alleles not defined yet.

Neonatal alloimmune thrombocytopenia (NAIT) results from maternal immunization against fetal platelet antigens. However, unlike its RBC counterpart, HDN, NAIT often affects first pregnancies. The incidence of NAIT has been estimated to be between 1 in 1000 and 1 in 2000 pregnancies. The majority of cases of NAIT are due to anti-HPA-1a. Anti-HPA-5b accounts for only 4.3 percent of all NAIT.<sup>2</sup> This report describes the case of a patient who had two fetal losses and a third pregnancy affected by NAIT due to anti-HPA-5b.

## Case Report

A 27-year-old African American female, group AB, D+, presented for fetal blood sampling at 28 weeks' gestation. The patient's medical history was significant for two fetal deaths in utero, both at 36 weeks' gestation, 6 and 4 years prior to this admission. Data related to the cause of death of either fetus were not available. As a result of her history, the patient was referred to the high-risk obstetrics clinic at our institution. At 11 weeks' gestation, platelet genotyping of the patient and father of the fetus had been performed. The patient was subsequently managed with administration of IVIG and serial ultrasound scans every 2 weeks to monitor the fetus. Periumbilical blood sampling (PUBS), with platelet transfusion to the fetus, was planned for 28 weeks' gestation.

The fetal genotype was determined to be homozygous for *HPA-1a*, and heterozygous for *HPA-5a/HPA-5b* (*Br<sup>a</sup>/Br<sup>b</sup>*). Follow-up ultrasound scans at 21 and 26 weeks were without abnormalities. At 28 weeks, fetal blood sampling was performed under ultrasound guidance via the hepatic vein. The initial fetal platelet count was 16,000/ $\mu$ L. Since the mother was unable to donate, a random apheresis platelet product was selected from the blood bank inventory. Therefore, the fetus was transfused with 15 mL of group A, D-, washed, concentrated, resuspended, CMV seronegative donor platelets, which were crossmatch compatible with the maternal plasma. Posttransfusion, the fetal heart rate was 130 beats per minute, and the fetus initially appeared to tolerate the procedure well. External fetal monitoring was initiated. However, 3 minutes later, fetal bradycardia (60 beats per minute) was noted and attempts were made to recover the fetal heart rate. The mother was taken for an emergency cesarean section.

An infant girl was delivered with Apgar scores of one at 1 minute, four at 5 minutes and seven at 10 minutes. The mother tolerated the delivery well, and was discharged on postoperative day 4. The infant girl was group B, D+. RBC antibody screen and DATs were negative. She was admitted to the pediatric intensive care unit, where her first platelet count was 166,000/ $\mu$ L, Hgb was 12.2 g/dL, and Hct was 36.1%. Accordingly, the infant was given IVIG. The platelet count reached a nadir of 129,000/ $\mu$ L on day of life (DOL) 2. Platelet transfusion was not required any time after delivery. On DOL 3, the platelet count rebounded to 146,000/ $\mu$ L. A head ultrasound on DOL 1 showed no intraventricular hemorrhage. Phototherapy was started

on DOL 2 secondary to a bilirubin level of 5.1 mg/dL, and was continued for 6 days. The infant received a total of five group O, HbS negative, irradiated, CMV seronegative RBC transfusions to treat anemia. She was also given iron sulfate at 4 mg/kg/day. On DOL 41, the infant's platelet count was 427,000/ $\mu$ L; she was therefore transferred to a pediatric rehabilitation hospital, and from there was discharged home.

## Materials and Methods

All serologic testing was performed using methods outlined in the AABB *Technical Manual*.<sup>3</sup>

Platelet genotyping was performed by the Blood Center of Southeastern Wisconsin Diagnostic Laboratories in Milwaukee, Wisconsin.

Platelet-specific antibodies were identified by testing the patient's plasma with a panel of isolated platelet glycoproteins, using an ELISA manufactured by GTI (Pak Plus™) in Brookfield, Wisconsin.

Platelet crossmatching was performed using a solid phase system (Capture™ P, Immucor, Inc., Norcross, GA). Donor platelets were affixed to microwells and incubated with the patient's plasma. After washing to remove unbound immunoglobulins, anti-IgG-coated indicator RBCs were added and the tests were centrifuged. The crossmatch was compatible if the indicator cells formed a solid, well-defined pellet at the bottom of the microwell. An incompatible crossmatch was indicated when the anti-IgG-coated indicator RBCs adhered to the immobilized platelets, forming a confluent monolayer.

## Results

The mother tested as group AB, D+ and the standard LISS antibody screening test was negative. Platelet genotyping results showed that the mother was homozygous for the *HPA-5a* (*Br<sup>b</sup>*) gene, and therefore lacked the platelet antigen *HPA-5b* (*Br<sup>a</sup>*). The father was heterozygous, *HPA-5a/HPA-5b* (*Br<sup>b</sup>/Br<sup>a</sup>*), indicating that there was a 50 percent chance that the fetus would be positive for the *HPA-5b* (*Br<sup>a</sup>*) antigen. Genotyping of amniocytes confirmed that the fetus was *HPA-5b* (*Br<sup>a</sup>*) positive, genotype *HPA-5a/HPA-5b* (*Br<sup>b</sup>/Br<sup>a</sup>*).

Tests with the mother's plasma showed the presence of platelet-specific antibodies reactive with platelet glycoprotein GPIa/IIa isolated from homozygous *HPA-5b* (*Br<sup>a</sup>*) donors, consistent with the presence of anti-*Br<sup>a</sup>* platelet antibodies. The mother's plasma did not react with platelet glycoprotein

GPIa/IIa isolated from homozygous *HPA-5a* (*Br<sup>b</sup>*) donors or with other platelet glycoproteins: GPIIb/IIIa, GPIb/IX, or GPIV. The patient also appeared to have HLA class I antibodies when tested with a pool of HLA antigens included in the panel studies.

To obtain platelets for transfusion to the fetus or neonate, the maternal plasma was crossmatched with ABO compatible, CMV seronegative platelet concentrates. Only three of ten (30%) platelet units were compatible, since the patient's plasma contained both platelet-specific anti-HPA-5b (*Br<sup>a</sup>*) and HLA antibodies.

## Discussion

The incidence of complications, namely intracerebral hemorrhage, neonatal thrombocytopenia, and fetal death, varies between NAIT due to anti-HPA-5b and that caused by anti-HPA-1a. Intracerebral hemorrhage is more common in NAIT associated with anti-HPA-1a than with anti-HPA-5b. An analysis of 39 cases of NAIT due to anti-HPA-5b revealed three cases (8%) of intracerebral hemorrhage compared with 20 cases (16%) in neonates with anti-HPA-1a NAIT.<sup>4</sup> There was no evidence of intracerebral hemorrhage during this pregnancy.

Neonatal thrombocytopenia also appears to be milder in cases of NAIT associated with anti-HPA-5b than in cases due to anti-HPA-1a. In the same series of 39 cases, 23 out of the 39 (59%) infants with anti-HPA-5b NAIT had no hemorrhagic symptoms, and 20 required no treatment. These findings are surprising when compared with the 17 out of 127 (13%) patients with anti-HPA-1a NAIT who were asymptomatic.

In our case, although quite thrombocytopenic in utero with a platelet count of 16,000/ $\mu$ L, this infant surprisingly required no further platelet transfusion after the PUBS procedure. The platelet count reached a nadir of 129,000/ $\mu$ L on DOL 3, but was 146,000/ $\mu$ L the next day, and by DOL 7 rebounded to 316,000/ $\mu$ L. On DOL 41 the platelet count was 427,000/ $\mu$ L (Fig. 1).

Anti-HPA-5b NAIT has been thought to be a milder disease than anti-HPA-1a NAIT because of the low number of HPA-5b antigenic sites per platelet. It had been reported previously that there were approximately 2000 HPA-5b antigen-binding sites present on the platelets of persons homozygous for *HPA-5b* and 1000 sites on those heterozygous for *HPA-5b*.<sup>5</sup> These numbers pale in comparison to the 20,000 to 50,000 HPA-1a antigen sites per platelet. The smaller number of binding sites makes identification of anti-HPA-5b with standard platelet ELISA tests

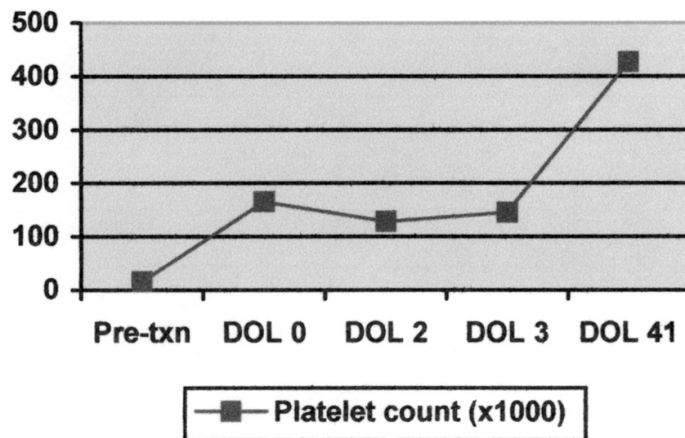


Fig. 1. Timeline of fetal platelet counts. DOL = day of life. Pre-Txn = platelet count prior to intrauterine transfusion at 28 weeks. DOL 0 = platelet count immediately after delivery.

inconsistent. GPIa is a major receptor for collagen on platelets, and defects in GPIa can lead to bleeding disorders. Polymorphisms in the *GPIa* gene, such as *807 C/T*, have been found to correlate with the number of GPIa molecules on the platelets of Caucasians. A separate polymorphism in the *GPIa* gene accounts for the HPA-5 antigen. The correlation between the HPA-5 polymorphism in the *GPIa* gene and the number of GPIa platelet surface molecules has been evaluated in 316 Caucasian individuals.<sup>6</sup> The number of GPIa molecules on platelets was found to be dependent on two *GPIa* polymorphisms, *807 C/T* and *HPA-5*. However, Panzer et al.<sup>7</sup> examined anti-HPA-5 NAIT and its potential relationship to the *807 C/T GPIa* polymorphism in 933 mother-child pairs, which included 79 *HPA-5a* homozygous mothers who gave birth to *HPA-5a/5b* children.<sup>7</sup> There was no association between the frequency of the *807 C/T* polymorphism and anti-HPA-5b antibody formation. In fact, in three cases of anti-HPA-5b antibody formation, despite increasing maternal antibody titers during pregnancy, normal platelet counts were seen at delivery. Notwithstanding, cases of anti-HPA-5b NAIT can have severe sequelae, and antibody titers have been examined as a means to predict those more likely to have severe complications. In a study of 21,354 pregnant women in Japan, 138 were identified with anti-HPA-5b antibodies.<sup>8</sup> Antibody titers were determined from serum samples obtained during the third trimester. A significant relationship was observed between an HPA-5b antibody titer > 64 and the development of thrombocytopenia (platelet count < 150,000/ $\mu$ L) in HPA-5b antigen-positive infants 1 day

and 3 days after birth. Antibody titers were not determined in our case.

The identification of the potential for NAIT (maternal-paternal incompatibility) and subsequent diagnosis in this case occurred only after the patient was referred to a high-risk obstetrics clinic. Screening for RBC alloantibodies is a mainstay of proper routine pregnancy management, but screening for anti-platelet alloantibodies is not routinely performed. Because of this practice, the overall incidence of NAIT is likely to be underestimated. Widespread routine screening of all pregnant women is limited by several factors, including current controversies in treatment options, the need to further develop testing strategies, and the potential for an increase in invasive testing that is not without risk.<sup>9</sup> Additionally, antigen frequencies for non-Caucasian populations are frequently unknown, and vastly different expressions in ethnic groups may result in increased risk for NAIT due to different HPA than in Caucasians. In a study of the first five HPA systems, the gene frequencies of *HPA-2b* and *HPA-5b* in 100 African American women were found to be 18 percent and 21 percent, respectively, compared with 9 percent and 11 percent seen in Caucasians.<sup>10</sup> This indicates a higher potential risk for alloimmunization for HPA-2 and HPA-5 antigens in African Americans than in Caucasians. The case reported here occurred in an African American patient.

Anti-HLA antibody was also identified in the maternal serum in this case. Alloimmunization to HLA antigens is not uncommon in pregnancy. Anti-HLA antibodies are usually IgG, but usually do not harm the fetus because antibodies against HLA antigens expressed by the fetus are thought to be adsorbed by HLA antigens on the placenta. In this case, the development of anti-HLA antibodies seemed to have no effect on the response to platelet transfusion in the fetus; a posttransfusion platelet count of 166,000/ $\mu$ L was obtained. Anti-HLA antibodies together with anti-HPA-1a antibody formation have been reported<sup>11</sup>; there was demonstration of poor increments to HPA-1a negative platelet transfusions that were administered in utero. The mother had not been transfused herself, but had three prior pregnancies affected by NAIT, with in utero transfusions given. The likely source of alloimmunization to nonpaternal HLA antigens was thought to be these previous fetal platelet transfusions. NAIT due to HLA antibody has been described, most recently in a series of three cases from Japan, where platelet-specific antibodies were not identified.<sup>12</sup>

The purpose of antenatal management of NAIT is to prevent intracerebral or other hemorrhage. The mainstay of treatment appears to be maternal intravenous injection of IgG and weekly intrauterine platelet transfusion with antigen-negative platelets, derived from either the mother or an antigen-negative donor.<sup>13</sup> The first PUBS is recommended at around the 20th week of gestation to assess fetal status and determine the platelet phenotype. If there is thrombocytopenia, in addition to platelet transfusion, the mother is started on intravenous IgG, and a second PUBS with transfusion is performed 8 weeks later. If there has been no response, corticosteroids may be added to the mother's regimen. If the fetal platelet count is above 50,000/ $\mu$ L just prior to delivery, a vaginal delivery may be attempted, if below 50,000/ $\mu$ L, an additional transfusion prior to delivery should be given.

However, current management of these cases is not without controversy. In a recent study to determine the effectiveness of IVIG and the safety of cordocentesis in NAIT,<sup>14</sup> 18 mother-infant pairs were examined. Seven fetuses had adequate platelet counts, 11 fetuses were thrombocytopenic. Eight of the thrombocytopenic fetuses were treated with maternally administered IVIG, and two fetuses were given IVIG delivered to the umbilical vein. The platelet counts of six of the eight fetuses given maternally-administered IgG increased, but the counts of the two given direct injection did not improve. In addition, two of 38 (5.3%) PUBS procedures had hemorrhagic complications despite platelet transfusion. Even with platelet transfusion, as in the case we report, PUBS has a significant complication rate due to hemorrhage.

In conclusion, this is a case of NAIT due to anti-HPA-5b antibodies that was identified only after referral to a high-risk obstetrics clinic. Alloimmunization to the HPA-5b antigen accounts for only 4.3 percent of all NAIT cases. The clinical presentation of such cases is described as milder than that of NAIT due to alloimmunization to the HPA-1a antigen. The severe effects in this patient are an exception.

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# Patellar dislocation: a case report and a review of other uncommon adverse events associated with blood donation

G.M. MENY AND S. MURPHY

Patellar dislocation is a fairly common occurrence usually associated with various activities such as sports or dancing. A case report of patellar dislocation associated with blood donation in a 17-year-old female is described. A review of uncommon adverse events associated with blood donation is also presented. *Immunohematology* 2003;19:132–134.

**Key Words:** patellar dislocation, adverse blood donation events

The knee is one of the most commonly injured joints in the human body.<sup>1</sup> Patellar dislocation may be seen in various activities such as dancing or turning quickly in sports,<sup>2</sup> but has never been reported as a complication of blood donation. We describe a case of patellar dislocation associated with blood donation.

## Case Report

The donor was a 17-year-old female who presented for her first whole-blood donation at a high school blood drive. The predonation history was significant for a 2-week trip to Italy and a 1-week trip to France within 1 year prior to the attempted donation. No medical conditions or medications were noted. The donor described herself as weighing 135 lbs. Temperature was 97.2°F, blood pressure 104/60 mm Hg, pulse 64 bpm, and Hct 39%.

While attempting to lie down on the 34-inch-high mobile donor bed, the donor noted severe pain in her left patellar region. She was transported to a local emergency room, where she was diagnosed with a dislocated patella. The patella was reduced and the left leg immobilized with a cast. Follow-up after 3 weeks revealed complete recovery.

## Discussion

Patellar dislocation (PD) occurs after contraction of the quadriceps muscle in combination with sudden external rotation and flexion of the tibia on the femur. PD is fairly common among adolescents, females, and the obese.<sup>1,2</sup> See Table 1 for other predisposing factors for PD.

**Table 1.** Predisposing factors for patellar dislocations

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Genu valgum (“knock knee”)
Weak vastus medialis
Excessive tibial torsion
Patella alta (“high-riding patella”)

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The patella usually dislocates laterally because the medial femoral condyle is larger than the lateral femoral condyle. Many dislocations reduce spontaneously, while others require treatment (reduction) at a hospital. After reduction a knee immobilizer is placed for 3–7 weeks, with progressive weight bearing.<sup>1</sup>

Several published series of adverse effects in donors after whole-blood or apheresis donation do not describe patellar dislocation among any donors.<sup>3–7</sup> Thirty-six percent of 1000 donors interviewed 3 weeks after donation described adverse effects in a study by Newman et al.<sup>3</sup> Bruising (22.7%), arm soreness (10%), fatigue (7.8%), and vasovagal symptoms (5.3%) were the more common adverse events. Popovsky et al.<sup>6</sup> characterized complications requiring hospitalization in approximately 4.1 million autologous and allogeneic whole-blood donation records. Allogeneic blood donation complications result in a hospitalization rate of 1 per 198,119 donations. Complications from vasovagal attacks were the most frequent cause of

hospitalization (67%), while the least frequent were arteriovenous fistula (3.0%), phlebitis (3.0%), and pseudoaneurysm (3.0%). While the complications of vasovagal attacks rarely, if ever, cause death, the most significant adverse event is the fracture or laceration that occurs as a result of the syncopal episode.<sup>5</sup> These injuries should be referred for immediate medical attention.

Uncommon adverse events during whole-blood donation include 12 reported cases of arterial venipuncture identified in 410,000 blood donations (1/34,000).<sup>4</sup> Eleven cases had a blood flow rate of < 4 minutes; nine units were bright red; and the needle was pulsating in four cases. One case of arterial venipuncture was diagnosed when a brachial artery pseudoaneurysm developed in a donor 3 days after donation.

A pseudoaneurysm (or "false aneurysm") is an extravascular hematoma that communicates with the internal vascular space. It resembles a true aneurysm, which is an abnormal dilated vessel. A true aneurysm, in contrast to a pseudoaneurysm, is surrounded by the artery wall. Pseudoaneurysm of the brachial artery was first described by Popovsky et al. in 1994.<sup>8</sup> After whole-blood donation, a 49-year-old female noted ecchymosis extending to the axilla and numbness in all fingers. An egg-size lump developed beneath the skin at the phlebotomy site. Ultrasound confirmed the diagnosis. A pinpoint-size hole was found in the artery when the pseudoaneurysm was excised.

In a review of other uncommon donor reactions and injuries, Newman notes that arteriovenous fistula and compartment syndrome can occur, but are extremely rare.<sup>5</sup>

An acquired arteriovenous (AV) fistula can present as a pulsating mass with a bruit and palpable thrill because a channel has formed between an artery and a vein. AV fistula was first described by Lung et al. in 1971.<sup>9</sup> A 25-year-old male was noted to have a bruit over the antecubital fossa. A unit of blood was donated 6 months before the examination. It was noted that the blood container filled unusually rapidly, perhaps indicating an arterial venipuncture. An arteriogram revealed the presence of an AV fistula. Repair was accomplished surgically without difficulties.

Compartment syndrome occurs when a significant amount of bleeding into a closed space occurs. Pressure builds, which causes venules and capillaries to close, leading to tissue necrosis. Pain, partial paralysis, parathesia, and a swollen fascial compartment may be

noted in this condition. Surgical treatment is required to prevent permanent damage. One case was reported from 23 million donations during a 4-year period.<sup>5</sup> A 71-year-old female noted swelling at the venipuncture site 1.5 minutes into a blood donation. The venipuncture was discontinued. Ten minutes of pressure and a pressure bandage were applied. The donor lost feeling in the arm within 4 hours and was hospitalized. A 10 × 5 × 3 cm hematoma was surgically removed from beneath the biceps muscle and a fasciotomy of the upper arm was performed. Evidence of an arterial venipuncture was not found.

Infections, including location infections at the venipuncture site (e.g., abscess formation or acute cellulitis), and thrombophlebitis have been described. Newman<sup>5</sup> estimated the incidence of local infections to be less than 1 in 200,000 donations. Treatment includes the application of warm soaks. The use of antibiotics and drainage of pus are recommended, if applicable. The incidence of thrombophlebitis was estimated to be 1 in 50,000 to 1 in 100,000 donations. Linear red lines are rarely observed. Treatment includes the application of heat and aspirin.

Fatalities due to blood donation are uncommon. The FDA required the reporting of transfusion-associated fatalities by all registered facilities beginning in November, 1975. Three-hundred fifty-five reports were received from 1976 to 1985. Of these, twelve were reports of donors dying during or as a result of blood or plasma donation (see Table 2).<sup>10</sup> Three donors died from acute myocardial infarction (MI) and two died from possible MI. In its 2002 Annual Report (10-01-2001 through 09-30-2002),<sup>11</sup> the FDA received 95 reports of transfusion-associated fatalities. Ten of these reports were related to blood collection. Although

**Table 2.** Blood or plasma donor fatalities in the United States, 1976 to 1985

Age	Sex	Cause of Death
31	F	Unknown
33	M	Convulsion; MI?
?	M	Ruptured cerebral vascular aneurysm
?	M	Digoxin toxicity
?	?	Convulsion; MI?
48	F	Pheochromocytoma
?	?	Group O donor received group A RBCs
47	M	MI
44	M	MI (18 hours after phlebotomy)
37	F	MI concurrent
?	M	Staphylococcal sepsis, endocarditis
47	M	Stroke (3 hours after donation)

some investigations remain open, occult heart disease is the most frequently determined cause of death at this time. Kasper et al.<sup>12</sup> performed a literature search of the English- and German-language literature between 1977 and 1997 and found nine reported deaths among at least 17,227 autologous blood donations before elective cardiac surgery, for an estimated mortality rate of 0.12 percent. This compared with a 2.2 percent mortality rate found in a series of patients on a waiting list for elective coronary artery bypass grafting.

In conclusion, this is the first report of a patellar dislocation in a donor during an attempted blood donation. Not surprisingly, the majority of uncommon adverse events related to blood donation noted above are related to the vascular system. Orthopedic injuries, if associated with blood donation, are usually related to other adverse events, such as falling off the bed during a syncopal episode. This donor was attempting to lie down on the donor bed and dislocated her patella, which is a not uncommon injury in young females. With appropriate care, complete recovery can be anticipated.

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## BOOK REVIEW

*Technical Manual, 14th edition.* Mark Brecher, MD, ed. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2002. 792 pp. List price: \$179; member price: \$114; student price \$99. ISBN: 1-56395-155-X. Stock number: 023013. To order: fax: +1-301-951-7150; phone: 866-222-2498 (within the US); and +1-301-215-6499 (outside the US); online: [www.aabb.org/Marketplace/Bookstore](http://www.aabb.org/Marketplace/Bookstore)

As participants in an SBB program, this is our year to discover the many resources available that will provide us with information we need to take us to the next level. The classic requisite text with which we are becoming intimately familiar is the AABB *Technical Manual*. The current edition is a special one: the 50th anniversary edition. We, as SBB students, shared the task of reviewing this book to learn the technical aspects of blood banking and as an exercise in journalism, each selecting sections to review, as well as the methods and appendices. We hope that others interested in blood banking can appreciate our review of this book, although it is from our somewhat unique student/instructor perspective. As we reviewed each chapter and section, we thought of additional readings that may be used as study guides for students in SBB programs. These suggestions can be found in the addendum. References and additional readings noted after each chapter in the *Technical Manual* are excellent resources and it should be noted that those in our addendum are not inclusive, but what we have found most useful.

### Section: Quality Issues

#### *Chapters 1-3*

The first section of the *Technical Manual* discusses Quality Issues. This section comprises three chapters: Quality Systems, Facilities and Safety, and Blood Utilization Management. Chapter 1 compares the AABB Quality System Essentials (QSEs) and the International Organization for Standardization (ISO) 9001:2000. Also, this chapter serves as a good introduction to quality in blood banks for beginners in the field. The second chapter deals with safety issues in blood banks, e.g., blood-borne pathogens and chemical and radiation safety. Chapter 3, Blood Utilization Management, provides practical information

about the function of blood banks. It discusses the considerations that blood bankers should keep in mind when determining inventory levels, e.g., minimum inventory levels, daily usage, and factors affecting outdates. It briefly presents issues related to managing special products such as CMV-reduced-risk units, HLA-matched platelets, and irradiated components. This section is easy to read and packed with useful information for managing a blood bank.

### Section: Blood Donation and Collection

#### *Chapters 4-8*

The initial chapter in this section, Donor Selection and Blood Collection, gives an overview of the donor screening process and the procedure for collecting whole blood as well as postdonation care and guidelines for handling adverse reactions. The appendix includes a list of "Some Drugs Commonly Accepted in Blood Donors" and the most recent version (at the time of publication) of "Uniform Donor History Questions," which no longer includes the brief explanation of the rationale for each question present in the previous edition. The chapter Autologous Blood Donation and Transfusion is an extremely detailed presentation of several methods for providing autologous blood and a discussion of many controversial issues that apply. This chapter is very comprehensive and, short of the opportunity to observe a perioperative collection first hand, provides a more than adequate explanation of the topic. The chapter Apheresis describes the principles of the technique and its application for donor collections as well as therapeutic uses. The goal of covering an enormous subject in just one chapter is well accomplished. Blood Component Testing and Labeling is a brief chapter, outlining the testing and labeling requirements set forth by the FDA and AABB Standards. Chapter 8, Preparation, Storage, and Distribution of Components from Whole Blood Donations, is a practical collection of information. It provides a substantial gathering of facts about anticoagulants, then describes the unique properties and handling of each blood component. The reader is frequently referred to procedures in the Methods section of the text, which are very specific and helpful.

**Section: Immunologic and Genetic Principles**

*Chapters 9-12*

The Molecular Biology in Transfusion Medicine chapter basically has stayed the same as in the last edition except that two paragraphs, DNA microarrays and gene therapy, have been added to the application of molecular biology. The progression from DNA to mRNA to protein is described with figures to support the text. The molecular techniques section covers isolation of nucleic acids, polymerase chain reaction, restriction endonucleases and DNA profiling, cloning, and sequences. Some of the figures in chapter 10, Blood Group Genetics, have been updated and the basic principles of genetics and heredity are reviewed. The four pedigrees showing different patterns of inheritance are represented in figure 10-7. There is a section that discusses nomenclature, which has a table that gives examples of correct and incorrect terminology. An appendix of blood group genetics terms has been added. The chapter Immunology starts with a description of the immune response and describes cells, cytokines, and their interactions. Three figures have been added, illustrating different mechanisms of alloantibody production (red cell and HLA). A fourth figure summarizes intravascular and extravascular red cell destruction. The last chapter, Red Cell Antigen-Antibody Reactions and Their Detection, reviews red cell antigen-antibody reactions and test systems used to detect them. The basics, sensitization and agglutination, are reviewed in detail and good figures accompany the text. Ways to enhance antibody detection, as well as other methods such as solid phase red cell adherence tests, are reviewed. Methods sections two and three should be read in conjunction with this chapter, as many procedures are described.

**Section: Blood Groups**

*Chapters 13-17*

For many blood bankers, this is the heart of the text. The beginning chapter, ABO, H, and Lewis Blood Groups, and Structurally Related Antigens, approaches a fairly complicated subject in a methodical manner. This is also the chapter where ABO discrepancies are addressed, a section commonly bookmarked in many a working technical manual. The simplistic chapter title, The Rh System, belies the chapter's highly complex subject matter, from the existence of multiple terminologies to the fact that there are 53 recognized

antigens (there are probably more by now). This chapter briefly describes many of the essential concepts at the molecular level, the serologic level, and devotes much attention to reagents and testing for Rh antigens. A valuable chart listing the blood groups, their membrane components, and their chromosome locations is contained in the Other Blood Groups chapter. The major blood groups are discussed at moderate length, and the blood groups for which antibodies are less commonly encountered are presented more briefly. The reader is invited to refer to other texts and reviews for more detail. Chapter 16, Platelet and Granulocyte Antigens and Antibodies, contains a useful chart with a compilation of the HPA system antigens and their alleles, frequencies, and GP class location. The determination of platelet refractoriness is discussed along with diseases associated with platelet antibodies and methods of detecting them. Neutrophil antigens and antibodies and their effects are also presented in this chapter. The concluding chapter of this section, The HLA System, describes the genetics, inheritance, biochemical structure, and function of the HLA antigens. Methods of detection, as well as methods of testing for compatibility, are reviewed.

**Section: Serologic Principles and Transfusion Medicine**

*Chapters 18-20*

Chapter 18, Pretransfusion Testing, provides information regarding pretransfusion testing, from the transfusion request to issue of the requested product. Table 18-1 lists causes of positive pretransfusion tests. Chapter 19, Initial Detection and Identification of Alloantibodies to Red Cell Antigens, contains information that can be used as a refresher for any blood banker working with patients' samples. The layout of the chapter remains the same as in the previous edition and is easy to read because of the progression from simple to complex antibody identification. A flowchart (figure 19-1) provides logical steps to follow when presented with a positive antibody screen. This would help one work efficiently. The last chapter, The Positive Direct Antiglobulin Test and Immune-Mediated Cell Destruction, could be a book on its own. It contains so much information that it should be read carefully and repeatedly so as not to miss anything. Classifications of immune hemolytic anemias are given with practical approaches to investigate and resolve them. A section on drug-induced immune hemolytic

## BOOK REVIEW CONT'D

anemia is included with a new flowchart for investigating a positive DAT. A comprehensive list of drugs associated with immune hemolysis and/or a positive DAT is included as an appendix.

### Section: Clinical Considerations in Transfusion Practice

#### *Chapters 21-28*

This section concerns the administration of blood products to adult, pediatric, and neonatal patients. It also deals with maintaining the safety and purity of the blood supply. Chapters 21 and 22, Blood Transfusion Practice and Administration of Blood and Blood Components, contain indications and contraindications to be followed for administration of blood products to patients. Chapter 21 presents pharmacologic alternatives to transfusion. Chapters 23 and 24 discuss the importance of perinatal, neonatal, and pediatric transfusions. HDN and neonatal alloimmune thrombocytopenia are discussed in detail. It also covers the significance of testing maternal serum for antibodies and various causes of HDN. The Methods section at the end of the book contains easy-to-follow procedures for the evaluation of HDN. Chapter 25, which is about the evolving field of hematopoietic transplantations, presents the materials in a well-organized fashion that is simple to follow. It is a useful guide for selecting donors based on FDA regulations and AABB standards. Information about blood collection, processing, and transplantation of peripheral blood, umbilical cord, and bone marrow cells is presented as it relates to a cell processing laboratory. The chapters Tissue and Organ Transplantation, Noninfectious Complications of Blood Transfusion, and Transfusion-Transmitted Diseases have tables that give the reader an overview of the essential materials that are covered in the main text. This is a helpful summary and serves as a quick reference. Noninfectious Complications of Blood Transfusion presents transfusion reactions in a table form as well as a text description. This section includes many formulas for the determinations of a CRYO dose for fibrinogen content, factor VIII, and quantification of fetal-maternal hemorrhage.

### Conclusion

The *Technical Manual* continues to be a basic necessity for SBB students as well as anyone in the field of blood banking. It is a perfect starting point and provides an outline for tackling the voluminous

amount of information available on the subject. The references provide a guide to additional readings if a more in-depth understanding is desired. The tried and true Methods section, plus the listings of normal laboratory result values, QC performance intervals, and the directories, found in the appendix, are invaluable resources. This 50th anniversary edition deserves much celebration, as it represents what we in the field consider to be the definition of blood banking.

### Addendum (alphabetical)

1. American Association of Blood Banks, American Blood Centers, American Red Cross. Circular of Information on the use of human and blood products. Bethesda, MD: American Association of Blood Banks, July 2002.
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## COMMUNICATIONS

*Immunohematology*—20th Year—2004

!!! HAPPY ANNIVERSARY !!!

Letter From the Editors:

Many things are planned for the four 20th anniversary issues to be published in 2004. All four issues will be devoted to reviews. In addition, any original articles that are submitted for that issue, and accepted, will be included. The March issue will have four blood group reviews. Christine Lomas-Francis, *Immunohematology's* own technical editor, is spearheading this issue and has a stellar lineup of authors who are preparing the four exciting reviews to begin this anniversary year.

Fumiichiro Yamamoto, PhD, will bring us ABO; Franz Wagner, PhD, will present Rh; Marion Reid, PhD, FIBMS, and Susan Westhoff, MT(ASCP)SBB, will review Kell, Kid, and Duffy; and Karen Byrne, MS, MT(ASCP)SBB, and Peter Byrne will cover other important blood groups—no small feat!

In that issue we will also hear from Sandy Ellisor, MS, MT(ASCP)SBB; Marion Reid, PhD; and Helen Glidden, MT(ASCP)SBB, who 28 years ago started the Red Cell Free Press (the precursor of *Immunohematology*). It was the American Red Cross

Newsletter published by National Headquarters for the reference laboratories. We will publish some of the interesting items from the Red Cell Free Press during 2004 that may spark memories, smiles, and even a bit of nostalgia for the older American Red Cross reference laboratory staff.

We will also hear from the first author published in Volume 1, #1—Denise Harmining, PhD, who also has graciously agreed to comment after 20 years. Her article was a very comprehensive review of warm autoimmune hemolytic anemia.

The reviews in the remaining three issues are equally exciting and subsequently will be previewed!

Don't forget to send your original articles to Mary McGinniss who is, as usual, ready and waiting for them.

Dolores Mallory  
Editor-in-Chief

Mary McGinniss  
Managing Editor

## COMMUNICATIONS CONT'D

Letter From the Editors:

### To Contributors to the 2003 Issues

The journal depends on readers, authors, editorial board, peer reviewers, and our Penn-Jersey staff. We wish we could thank all of you personally, but doing so is not practical. Instead, we thank each of you as members of an honored group.

First and foremost, we thank the authors for their reviews, scientific articles, case reports, book reviews, and letters to the editors that come, not only from the United States but from many countries of the world. This has given the journal an international flavor.

Our editorial board is a prestigious one and we depend on them, not only for peer reviews, but for guidance in policy and suggestions for improvements. Special thanks go to S. Gerald Sandler, MD, our Medical Editor, who reviews every article for medical content, and to Christine Lomas-Francis, MSc, our Technical Editor, who reads every article for technical content. In this issue, we would like to welcome W. John Judd, FIBMS, MiBiol, to the board. He has served as an excellent peer reviewer for many years. The current board is listed by name in the front of each issue of the journal.

Our peer reviewers do a wonderful job. In each December issue we list them by name with thanks to each.

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We also want to thank the staff at Penn-Jersey, Linda Berenato and Marge Manigly, who do everything else to get the journal ready for press. They word-process all articles, keep up with subscriptions, and handle all e-mail, to name a few tasks. We also thank Lucy Oppenheim, our copy editor; George Aydinian, our proofreader; and Paul Duquette, our electronic publisher.

Finally, thanks go to our readers, whose enthusiasm and interest in the journal make it all worthwhile.

Dolores Mallory  
Editor-in-Chief

Mary McGinniss  
Managing Editor

## IN MEMORIAM

### **David E. Hatcher** 1923–2003



David Ellison Hatcher was born in Pinckard, Alabama, in 1923. He joined the Navy on December 8, 1941, and served in the Medical Corps on a cruiser in the South Pacific for 3 years despite an inner ear problem. After he was discharged, he became the first male to be accepted into the medical technology program at the University of Alabama Medical Center and he became certified in Medical Technology. He eventually accepted a sales position with Knickerbocker, a blood bank manufacturing company. In early 1960, he cofounded his first blood bank reagent manufacturing company, Spectra Biologicals, and in 1970, he cofounded Gamma Biologicals.

David Hatcher made many contributions to blood banking in addition to being cofounder, president, and CEO of two successful biotechnology companies. In 1957, while with Knickerbocker, he started the first Antibody Club in the United States, in Houston, Texas. Unlike Antibody Clubs of today that meet periodically to hear guest lecturers, the original club was designed to also share rare serum, cells, and donors.

In 1981, David Hatcher, BS, MT(ASCP), and his then wife, Betty Hatcher, jointly received the Ivor Dunsford Memorial Award from the American Association of Blood Banks. He received it for original work with Di<sup>b</sup>, Dc-/Dc-, V and VS, and S- s- U+ RBCs and for establishing the first Antibody Club.

David was well known for his generosity. He willingly shared reagents with scientists who needed them for their studies. He brought exceptional colleagues to the United States to study and to lecture. And always, he shared his vast knowledge of blood bank stories from the “good old days.” With his passing we look on those days with even more fondness.

## IN MEMORIAM CONT'D

### **Professor Walter Morgan 1900–2003**

Walter Morgan was born in Ilford, England, on October 5, 1900. He was a pioneer in what was to become molecular immunogenetics, elucidating the precise structure of the major blood group antigens of the ABO and Lewis systems. He attended Raynes Foundation School in Ilford, then joined the Royal Navy for a short time at the end of the First World War. He studied chemistry at London University and in 1925 started his lifelong career in research at the Lister Institute of Preventive Medicine, studying chemistry of carbohydrates. He transferred to the Serum and Vaccines Department of the Lister Institute in 1929, studying immunology.

When the Second World War started, resulting in a need for immunologically specific human blood grouping reagents for the newly developing transfusion services and donor centers, he was ready to adapt his work in carbohydrate chemistry to human blood groups. Better and more reliable reagents were needed and he learned from the experts at the South London Transfusion Depot in Sutton, Barbara Dodd and Kathleen Boorman. Professor Morgan began working with purified water-soluble forms of A, B, and H substance to chemically characterize blood group antigens and to correlate this information with their genetic background. This would be his work for 35 years and it made him one of the first molecular biologists. After the war, he continued his work with Dr. Winifred Watkins and other colleagues and determined the immunodominant sugars of the A, B, H, Le<sup>a</sup>, and Le<sup>b</sup> antigens. Morgan and Watkins also proposed the genetic pathway for the biosynthesis of these antigens. All was confirmed!

Professor Morgan continued to work at the Lister Institute until his first retirement, as Deputy Director in 1968. He was recalled to serve as Director, which he did from 1972 to 1975. He worked until he was 89 years old and during that time characterized the P<sub>1</sub> and Sd<sup>a</sup> antigens.

Professor Morgan received many awards, including his appointment as CBE in 1959 and as Vice President of the Royal Society; the Royal Medal of the Royal Society, 1968; the Landsteiner Award of the American Association of Blood Banks (jointly with Winifred Watkins); the Philip Levine Award of the American Society of Clinical Pathology; and Honorary Fellowship of the Royal College of Physicians.

Professor Morgan married Dorothy Price, who died in 1993. They had one son and two daughters. Well known for his great enthusiasm and kindness, he was one of the great cornerstones of the field.

Delores Mallory  
Editor-in-Chief

## ANNOUNCEMENTS

**Monoclonal antibodies available.** The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive DAT. Anti-Rh17 is a direct agglutinating monoclonal antibody. Anti-Fy<sup>a</sup>, anti-K, anti-Js<sup>b</sup>, and anti-Kp<sup>a</sup> are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. **Contact:** Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

**Workshop on Blood Group Genotyping.** The ISBT/ICSH Expert Panel in Molecular Biology has recommended that a workshop be held on blood group genotyping by molecular techniques. The results would culminate in a report at the ISBT Congress in 2004 in Edinburgh. It was decided that only laboratories that provide a reference service in blood group genotyping would be included in the workshop. One of the aims of the workshop would be to establish an external quality assurance plan. If you have any suggestions as to how the workshop should be organized, we would be grateful for your opinions. If you are interested in taking part in such a workshop, please **contact** Geoff Daniels (geoff.daniels@nbs.nhs.uk). Offer presented by Geoff Daniels, Martin L. Olsson, and Ellen van der Schoot.

**HEMATOLOGÍA HABANA' 2005—First Announcement.** The 5th National Congress and the 7th Latin American Meeting in Hematology, Immunology, and Transfusion Medicine will present a scientific program at the International Conference Center, Havana, Cuba, May 16–20, 2005. A preliminary program lists malignant hemopathies, disorders of RBC membranes, immunotherapy, histocompatibility, immunohematology, hemolytic disease of the newborn, and blood components as some of the topics. For more information **contact:** Prof. José M. Ballester, President, Organizing Committee, Hematology Habana' 2005, Apartado 8070, Ciudad de la Habana, CP 10800, Cuba.

## **Masters (MSc) in Transfusion and Transplantation Sciences**

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Further information can be obtained from the Web site:

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For further details and application forms please contact:

**Professor Ben Bradley**

**University of Bristol, Department of Transplantation Sciences**

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**FAX +44 1179 595 342, TELEPHONE +44 1779 595 455, E-MAIL: [ben.bradley@bristol.ac.uk](mailto:ben.bradley@bristol.ac.uk)**

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# Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

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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:

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5. References
6. Author information
7. Tables—see 6 under Preparation
8. Figures—see 7 under Preparation

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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

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  - 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

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  - 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

Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.

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- B. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
- C. Place explanations in footnotes (sequence: \*, †, ‡, §, ¶, \*\*, ††).

### 8. Figures

- A. Figures can be submitted either by e-mail or as photographs (5" × 7" glossy).
- B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
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### 9. Author information

- A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and **complete** address (including zip code) for **all** authors. List country when applicable.

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5. References—limited to ten.
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## Becoming a Specialist in Blood Banking (SBB)

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

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

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

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

### Who are SBBs?

Supervisors of Transfusion Services	Managers of Blood Centers	LIS Coordinators Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers
Quality Assurance Officers	Technical Representatives	Reference Lab Specialist

### Why be an SBB?

Professional growth	Job placement	Job satisfaction	Career advancement
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### How does one become an SBB?

- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience

**Fact #1:** In recent years, the average SBB exam pass rate is only 38%.

**Fact #2:** In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

### Conclusion:

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

### Contact the following programs for more information:

PROGRAM	CONTACT NAME	CONTACT INFORMATION
Walter Reed Army Medical Center	William Turcan	202-782-6210; William.Turcan@NA.AMEDD.ARMY.MIL
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Medical Center of Louisiana	Karen Kirkley	504-903-2466; kkirk1@lsuhsc.edu
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335; Kbyrne@mail.cc.nih.gov
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ARC-Central OH Region, OSU Medical Center	Joanne Kosanke	614-253-2740 x 2270; kosankej@usa.redcross.org
Hoxworth Blood Center/Univ. of Cincinnati Medical Center	Catherine Beiting	513-558-1275; Catherine.Beiting@uc.edu
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201; cwong@giveblood.org
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Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403; Irlemense@bcsew.edu

*Additional Information can be found by visiting the following Web sites: [www.ascp.org](http://www.ascp.org), [www.caahep.org](http://www.caahep.org), and [www.aabb.org](http://www.aabb.org)*



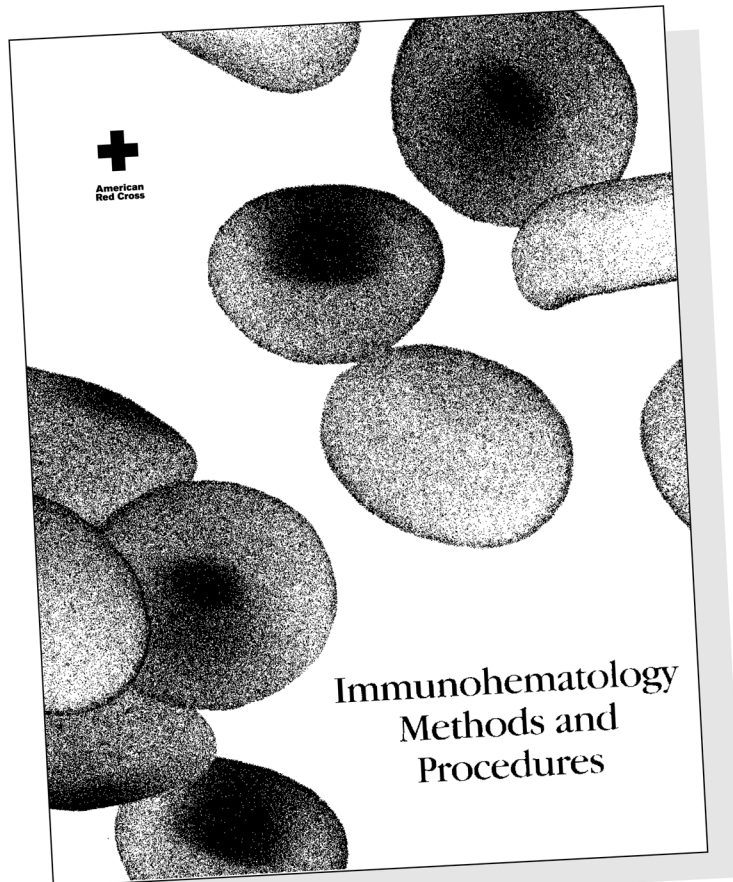
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