

# Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 24, NUMBER 2, 2008



**American Red Cross**

# Immunohematology

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# Unexplained agglutination of stored red blood cells in Alsever's solution caused by the gram-negative bacterium *Serratia liquefaciens*

I. MARTINCIC, C. MASTRONARDI, A. CHUNG, AND S. RAMIREZ-ARCOS

Alsever's solution has been used for decades as a preservative solution for storage of RBCs. From October 2005 to January 2006, unexplained hemagglutination of approximately 10 to 20 percent of RBCs stored for several days in a modified version of Alsever's solution was noticed in quality control testing at the Canadian Blood Services Serology Laboratory. An investigation, including microbial testing, was initiated to determine the cause of the unexplained hemagglutination. The gram-negative bacterium *Serratia liquefaciens* was isolated from supernatant solutions of agglutinated RBCs. Further characterization of this strain revealed that it has the ability to form biofilms; presents high levels of resistance to chloramphenicol, neomycin, and gentamicin; and causes mannose-sensitive hemagglutination. The source of *S. liquefaciens* contamination in RBC supernatants was not found. However, this bacterium has not been isolated since January 2006 after enhanced cleaning practices were implemented in the serology laboratory where the RBCs are stored. This biofilm-forming, antibiotic-resistant *S. liquefaciens* strain could be directly linked to the unexplained hemagglutination observed in stored RBCs. *Immunohematology* 2008;24:39–44.

**Key Words:** hemagglutination, red blood cells, Alsever's solution, *Serratia liquefaciens*

Alsever's solution was first described by J.B. Alsever in 1941.<sup>1</sup> It is an isotonic solution (2.05% glucose, 0.42% sodium chloride, 0.8% trisodium citrate, 0.055% citric acid in double-distilled water [ddH<sub>2</sub>O]) routinely used as an anticoagulant and preservative, which permits the storage of whole blood and RBCs at refrigeration temperatures. The National Serology Laboratory at Canadian Blood Services (CBS) used to prepare a modified version of Alsever's solution (supplemented with 0.03% adenine, 0.2% inosine, 0.03% chloramphenicol, and 0.07% neomycin), which was sent to the hospitals for detecting antibodies of relevance in

transfusion medicine. Each new lot of modified Alsever's solution underwent quality control testing for stability every 8 months for a period of 24 months, which is the shelf life of this product. The quality control testing consisted of measuring pH, presence of hemolysis, and serologic reactivity for D, Fy<sup>a</sup>, and M. From October 2005 to January 2006, unexplained hemagglutination of approximately 10 to 20 percent of different types of RBCs stored for several days in modified Alsever's solution was noted during quality control testing. The CBS Serology Laboratory initiated an investigation, including microbial testing, to determine the cause of the unexplained hemagglutination. The gram-negative bacterium *Serratia liquefaciens* was isolated from supernatant solutions of agglutinated RBCs.

*Serratia* spp are opportunistic gram-negative bacteria that belong to the Enterobacteriaceae family and include several species, such as *Serratia marcescens* and *S. liquefaciens*. Once considered harmless, *S. liquefaciens* has emerged as an opportunistic pathogen in recent years and has been implicated in nosocomial infections and also in severe and in some cases fatal reactions associated with transfusion of contaminated RBCs or platelets.<sup>2–5</sup> The virulence of *S. liquefaciens* is related not only to the production of endotoxin but also to the organism's high antibiotic resistance and ability to form surface-attached cell aggregates embedded in a matrix, known as biofilms.<sup>6</sup> Adhesion, which is the first step of biofilm formation, is directly related to the presence of short, thin, hairlike projections called fimbriae, also known as "filamentous

hemagglutinins.<sup>7</sup> Mannose-sensitive hemagglutinin is associated with type 1 fimbriae whereas mannose-resistant hemagglutinins are linked with different types of fimbriae including type 3 fimbriae.<sup>7</sup>

This report describes a case of initially unexplained agglutination of stored RBCs that was subsequently determined to be caused by a hemagglutinin-positive, biofilm-forming, antibiotic-resistant *S. liquefaciens* strain.

## Materials and Methods

Microbial studies were performed on the following samples from CBS Serology Laboratory: supernatants of different lots of stored RBCs, samples of PBS used to wash RBCs, and water from the water bath used to thaw frozen RBCs. Samples were plated on 5 percent sheep blood agar, Luria Bertani (LB) agar, and trypticase soy agar. After identification of *S. liquefaciens* in the stored RBCs, the frozen RBCs, glassware, environmental samples (air and surfaces), and personnel were also tested. Different lots of modified Alsever's solution prepared by the Serology Laboratory, including those used to store the agglutinated RBCs, underwent sterility testing at an external microbiology laboratory (Nucro-Technics, Scarborough, ON, Canada).

The bacteriostatic or bactericidal effect of modified Alsever's solution on *S. liquefaciens* was investigated. Ten-fold serial dilutions ( $10^8$ – $10^2$  colony forming units [CFU]/mL) of *S. liquefaciens* isolated from RBC supernatants were prepared in modified Alsever's solution. One milliliter of the  $10^2$  CFU/mL dilution was inoculated into 500 mL of thioglycollate broth immediately after the dilutions were made (day 0) followed by 24-hour incubation at 30°C; this was used as a positive control. The  $10^2$  CFU/mL dilution was then kept at 2 to 8°C, and after vortexing, 1 mL was taken to inoculate 500 mL of thioglycollate at 8, 15, and 30 days after storage. The 1 to 500 dilution in thioglycollate allows bacteria to recover because it dilutes the antibiotics from the storage solution. The 2 to 8°C temperature was chosen for storage as this is the temperature at which RBCs are maintained in modified Alsever's solution.

Pulsed-field gel electrophoresis (PFGE) genotyping was performed for *S. liquefaciens* isolated from six supernatants of agglutinated RBCs at the Division of Microbiology of the Hospital for Sick Children (Toronto, ON, Canada).

Minimal inhibitory concentration (MIC) for chloramphenicol (Cl), neomycin (Neo), and gentamicin (Gm) was determined for *S. liquefaciens* isolated from RBC supernatants and for control strain *S. liquefaciens* ATCC

27592 according to the Clinical and Laboratory Standards Institute (previously known as National Committee for Clinical Laboratory Standards [NCCLS]).<sup>8</sup>

Ten-fold serial dilutions ( $10^9$ – $10^0$  CFU/mL) of *S. liquefaciens* cultures in PBS were tested in hemagglutination assays using 3 percent suspensions of group O and A RBCs previously stored frozen in liquid nitrogen. The RBCs were suspended in PBS with or without 50 mM D-mannose (Man), in modified Alsever's solution, and in "RBC storage solution" (Immucor Inc., Norcross, GA) following protocols previously established.<sup>7,9</sup> *S. liquefaciens* ATCC 27592 and *Escherichia coli* ATCC 25922 were used as negative and positive controls for hemagglutination, respectively.

*S. liquefaciens* isolated from RBC supernatants was assayed for its ability to form biofilms. Cultures were grown in LB broth supplemented with 0.2 percent glucose and 0.5 percent casamino acids (CAA) and incubated at 30°C for 24 hours. Biofilm formation was measured by crystal violet staining as previously described.<sup>6</sup> The presence of type 1 fimbriae was tested by PCR using primer pair SliqFW 5'-CTGGTATTGTCTGCAACTG-3' and SliqREV 5'-ACCTGCAGCACGTTATCTTG-3', which anneal at positions 433 and 1359, respectively, of the *S. marcescens* *fimA* gene (accession number AY730610). Primers were synthesized by Integrated DNA Technologies Inc. (Coralville, IA). PCR reactions were carried out in a Mastercycler ep gradient S (Eppendorf Corp., Mississauga, ON) as follows: 5 minutes at 95°C; 30 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 54°C, and extension for 1 minute at 72°C; 5 minutes at 72°C; and hold at 4°C. PCR was carried out in a final volume of 25 µL containing the following reagents: 22.5 µL of sterile ddH<sub>2</sub>O, 10 × PCR buffer supplemented with 1.5 mM MgCl<sub>2</sub> (Roche Diagnostics Corp., Indianapolis, IN), dNTPs (Boehringer Mannheim Corp., Indianapolis, IN), each primer (0.2 µg/mL), Hot Star *Taq* DNA polymerase (Roche), and 2.5 µL of template DNA. *S. liquefaciens* cell suspensions were prepared by diluting cells from overnight cultures in sterile ddH<sub>2</sub>O. Cell concentrations were adjusted using a 0.5 McFarland Equivalence Turbidity Standard (Remel, Lenexa, KS) to provide chromosomal DNA templates for PCR.

## Results

*S. liquefaciens* was only isolated from supernatants of RBCs that had been stored for several days in modified Alsever's solution and had presented

**Table 1.** Minimal inhibitory concentration and hemagglutination of *S. liquefaciens* isolated from stored RBCs

Isolate	MIC ( $\mu\text{g/mL}$ )*			HA			
	Cl	Neo	Gm	PBS		MAS <sup>†</sup>	Commercial solution <sup>†</sup>
				+ Man	- Man		
<i>S. liquefaciens</i> (RBC supernatant)	1024	1400	16	-	+	+	+
<i>S. liquefaciens</i> ATCC 27592	8	2	<1	-	-	-	-
<i>E. coli</i> ATCC 25922		NT		-	+	+	+

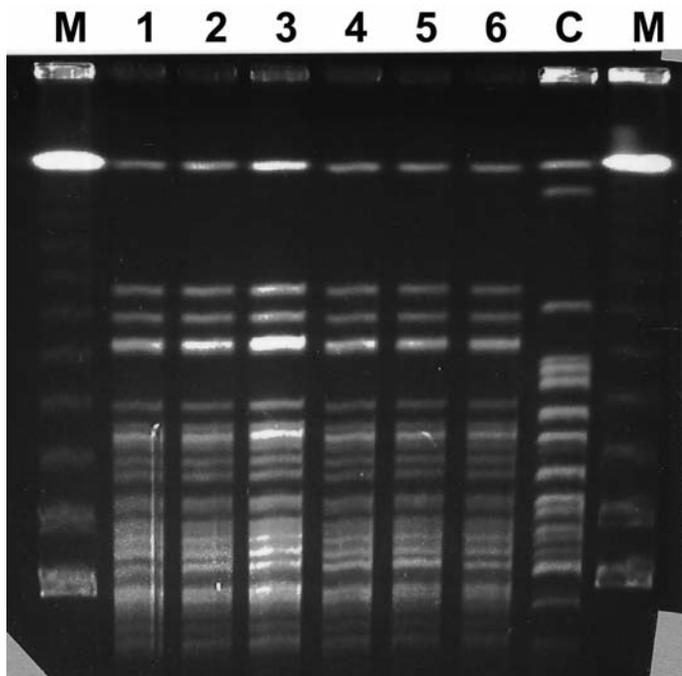
HA = hemagglutination

Man = mannose

NT = not tested

\*A bacterium is resistant to chloramphenicol (Cl), neomycin (Neo), and gentamicin (Gm) if minimal inhibitory concentrations (MICs) are > 32, > 8, and > 8  $\mu\text{g/mL}$ , respectively.†Modified Alsever's solution (MAS) contains 330  $\mu\text{g/mL}$  of Cl and 700  $\mu\text{g/mL}$  of Neo, and the commercial RBC storage solution is supplemented with 250  $\mu\text{g/mL}$  of Cl, 100  $\mu\text{g/mL}$  of Neo, and 50  $\mu\text{g/mL}$  of Gm.

unexplained agglutination. Unusual hemolysis was not noticed in the contaminated RBCs. Samples of the same RBCs which had not been stored in modified Alsever's solution as well as all of the other samples tested, including PBS, water from water baths, glassware, staff, and environmental samples, were negative for the presence of this bacterium. In addition, sterility testing of

**Fig. 1.** Genotyping of *S. liquefaciens* isolates. Genomic DNA was extracted from six strains of *S. liquefaciens* isolated from different RBC supernatants (lanes 1-6) and control strain ATCC 27592 (lane C) followed by digestion with *SpeI* and pulsed-field gel electrophoresis (PFGE) analysis. M, lambda ladder.

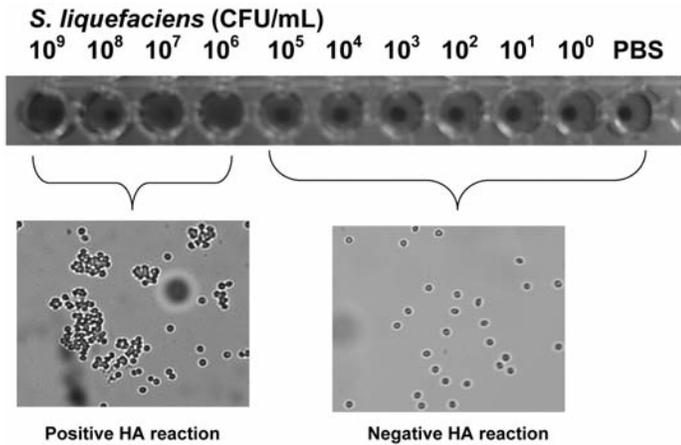
different lots of modified Alsever's solution repeatedly showed negative results. Interestingly, it was observed that this strain of *S. liquefaciens* grew better when incubated at refrigeration (2-8°C) and ambient (20-25°C) temperatures than when grown at higher temperatures (37°C).

The cell concentration of *S. liquefaciens* was substantially decreased with time when it was stored in modified Alsever's solution at 2 to 8°C. Weekly sampling of the 10<sup>2</sup> CFU/mL cell dilution was performed by taking 1 mL of the dilution for inoculation into 500 mL of thioglycollate followed by incubation at 30°C for 24 hours. Visual examination showed heavy growth of

the sample taken at day 0. Recovery of bacteria decreased proportionally with storage time of the bacterial suspension, being less heavy when the assay was performed after 8 days of storage and very light after 15 days, until no growth was observed after 30 days of storage.

PFGE analysis revealed that the six genotyped *S. liquefaciens* strains isolated from the RBC supernatants had the same band pattern (Fig. 1). This *S. liquefaciens* strain was resistant to Cl and Neo at concentrations used to prepare modified Alsever's and RBC storage solutions with MICs of 1024 and 1400, respectively (Table 1). This strain was also considered to be resistant to Gm based on the Clinical and Laboratory Standards Institute (formerly NCCLS) (MIC > 8  $\mu\text{g/mL}$ ).<sup>8</sup> In contrast, *S. liquefaciens* ATCC 27592, used as a control, was sensitive to all three antibiotics (Table 1).

*S. liquefaciens* isolated from the RBC supernatants caused mannose-sensitive hemagglutination of RBCs suspended in PBS supplemented with 50 mM of mannose. Hemagglutination was also present when this strain of *S. liquefaciens* was suspended in modified Alsever's solution or the commercial RBC storage solution at bacterial concentrations of at least 10<sup>6</sup> CFU/mL (Table 1, Fig. 2). Similar results were obtained for the positive control *E. coli* ATCC 25922, whereas the negative control *S. liquefaciens* ATCC 27592 did not cause hemagglutination in any of the conditions tested. The fimbrial hemagglutinin(s) of the *S. liquefaciens* isolated from the RBC supernatants do not share sequence homology at the DNA level with type 1 fimbriae of *S. marcescens* as PCR to amplify *fimA* yielded negative



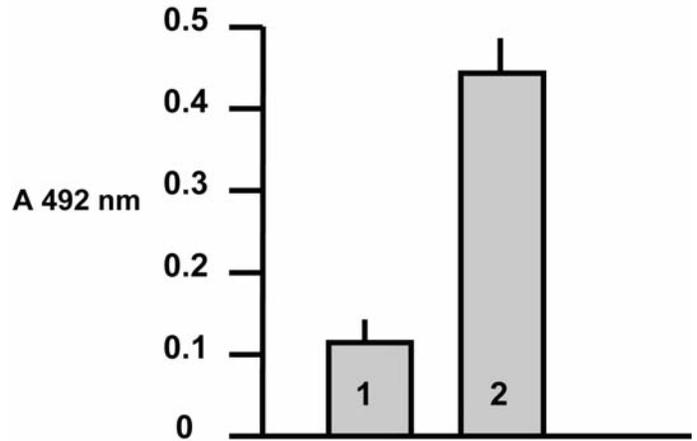
**Fig. 2.** Hemagglutination (HA) of *S. liquefaciens*. *S. liquefaciens* at concentrations  $\geq 10^6$  CFU/mL caused agglutination of RBCs suspended in modified Alsever's solution, which is observed as a diffuse mat of cells in a microplate assay (above) and as cell aggregates when observed under the microscope (below).

results (data not shown). As expected, positive hemagglutination was associated with biofilm formation of this *S. liquefaciens* isolate (Fig. 3) and with a microscopic filamentous phenotype (Fig. 4).

### Discussion

This report describes a case of agglutination of test RBCs stored in modified Alsever's solution caused by contamination with *S. liquefaciens*. With the exception of the supernatants of agglutinated RBCs, none of the other samples taken at the CBS Serology Laboratory during the investigation of this case were positive for the presence of *S. liquefaciens*. There is a recall report for modified Alsever's solution prepared by Gamma Biologicals, Inc., owing to microbial contamination.<sup>10</sup> However, the sterility testing of the modified Alsever's solution prepared at the CBS Serology Laboratory was negative, indicating that this was not the source of contamination. Because of the virulent characteristics of the *S. liquefaciens* strain isolated from the supernatants of agglutinated RBCs (i.e., highly resistant to antibiotics and ability for biofilm formation), it was speculated that the source of contamination was a clinical sample received at the CBS Serology Laboratory, although this could not be confirmed.

It is assumed that the level of *S. liquefaciens* contamination of the original source was high, probably with bacteria concentrations greater than  $10^6$  CFU/mL, because our results demonstrated that lower concentrations of *S. liquefaciens* did not cause hemagglutination.

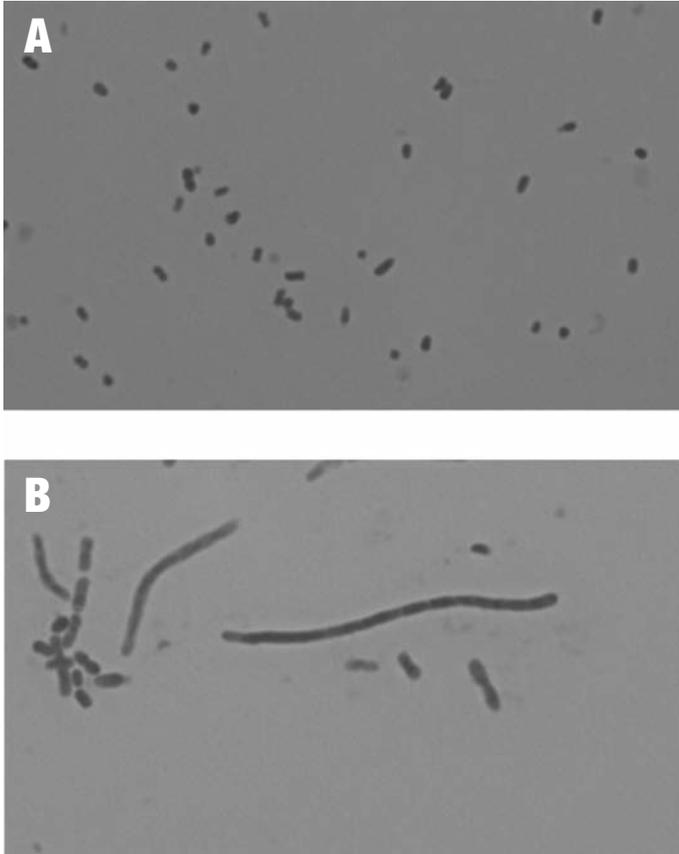


**Fig. 3.** Biofilm formation by *S. liquefaciens*. Measurement of biofilm formation by crystal violet staining.<sup>7,9</sup> (1) Negative control *S. liquefaciens* ATCC 27592, and (2) *S. liquefaciens* isolated from RBC supernatants, which showed substantially increased biofilm formation ability. The experiment was performed in duplicate, and bars reflect the means  $\pm$  SD of the two measurements.

In addition, we showed that modified Alsever's solution has a bactericidal effect at lower concentrations of this strain ( $10^2$  CFU/mL). This may be attributable to the synergistic effect of the two antibiotics present in the solution, Cl and Neo, or lack of nutrients. However, this should be further investigated as MICs to Cl or Neo alone revealed that this strain is highly resistant to each of these antimicrobials.

*S. liquefaciens* is an emergent nosocomial pathogen and a cause of severe reactions associated with transfusion of RBCs.<sup>3-5</sup> This bacterium is also a common contaminant of reagents used in clinical environments. There is a study reporting an *S. liquefaciens* outbreak in a hemodialysis center where the bacterium was isolated from empty vials of epoetin alfa as well as from antibacterial soap and hand lotion.<sup>2</sup> Isolation of *S. liquefaciens* from these sources, and in this case from agglutinated RBCs, indicates that this bacterium has the ability to survive under stressful conditions, likely by forming biofilms. This report confirmed the biofilm formation ability of the *S. liquefaciens* strain isolated at the CBS Serology Laboratory and demonstrated that this ability is linked to a high level of antibiotic resistance as occurs in other bacteria that form biofilms.

The agglutination observed in the stored RBCs was reproduced in in vitro hemagglutination assays. Our results showed that the fimbriae of *S. liquefaciens* responsible for this hemagglutination are functionally homologous to the type 1 fimbriae of *S. marcescens* or *E. coli* inasmuch as the agglutination was sensitive to the presence of mannose.<sup>6,7,9</sup> However, our PCR results



**Fig. 4.** Microscopic phenotype of *S. liquefaciens* isolated from supernatants of agglutinated RBCs. Contaminant *S. liquefaciens* presents normal morphology (i.e., coccobacilli) when grown on Luria Bertani plates (A) and filamentous phenotype when grown in Luria Bertani broth supplemented with glucose and casamino acids to induce biofilm formation (B).

demonstrated that the sequence of the gene encoding *S. liquefaciens* fimbriae is different from that of the *S. marcescens fimA* gene.<sup>11</sup>

PFGE genotyping showed that all of the RBC supernatants tested contained the same strain of *S. liquefaciens*, demonstrating that there was probably an initial introduction of the strain and subsequent cross-contamination in the laboratory during manipulation of these samples.

After this investigation was carried out, the CBS Serology Laboratory implemented enhanced cleaning practices including constant disinfection of work areas and flushing of Pasteur pipettes used to wash RBCs with 1% hypochlorite solution. The Pasteur pipettes are washed with a vacuum bottle system, and a sterile tip is attached so the pipette tips do not touch the RBCs. In addition, the pipette washing is followed by several rinses with PBS. After implementation of these new measurements, *S. liquefaciens* has not been isolated again, and no

other cases of unexplained hemagglutination have been reported by the CBS Serology Laboratory.

### Acknowledgments

We would like to thank Ms. S. Baldwin and Ms. J. Barnes (Serology Laboratory, Canadian Blood Services) for their technical assistance. We are also grateful to Ms. S. Hamilton (Quality Assurance, Canadian Blood Services) for her assistance with quality control testing and Dr. S. Richardson (The Hospital for Sick Children, Toronto, Ontario, Canada) for bacteria genotyping using pulse-field gel electrophoresis.

### References

1. Alsever JB, Ainslie RB. A new method for the preparation of dilute blood plasma and the operation of a complete transfusion service. *New York State J Med* 1941;41:126.
2. Grohskopf LA, Roth VR, Feikin DR, et al. *Serratia liquefaciens* bloodstream infections from contamination of epoetin alfa at a hemodialysis center. *N Engl J Med* 2001;344:1491-7.
3. Ramirez-Arcos S, Goldman M, Blajchman MA. Bacterial contamination. In: M. Popovsky, ed. *AABB transfusion reactions*. 3rd ed. Arlington, VA: American Association of Blood Banks, 2007:163-206.
4. Brecher ME, Hay SN. Bacterial contamination of blood components. *Clin Microbiol Rev* 2005;18:195-204.
5. Roth VR, Arduino MJ, Nobiletti J, et al. Transfusion-related sepsis due to *Serratia liquefaciens* in the United States. *Transfusion* 2000;40:931-5.
6. Labbate M, Queck SY, Koh KS, Rice SA, Givskov M, Kjelleberg S. Quorum sensing-controlled biofilm development in *Serratia liquefaciens* MG1. *J Bacteriol* 2004;186:692-8.
7. Adegbola RA, Old DC. New fimbrial hemagglutinin in *Serratia* species. *Infect Immun* 1982;38:306-15.
8. National Committee for Clinical Laboratory Standards M100-S12, 2002;22(1).
9. Bahrani-Mougeot FK, Buckles EL, Lockett CV, et al. Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic *Escherichia coli* virulence determinants in the murine urinary tract. *Mol Microbiol* 2002;45:1079-93.
10. <http://www.fda.gov/cber/recalls/gamals012505.htm>. Accessed on July 31, 2007.
11. Nichols WA, Clegg S, Brown MR. Characterization of the type 1 fimbrial subunit gene (*fimA*) of

*Serratia marcescens*. Mol Microbiol 1990;4:  
2119-26.

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# Hyperhemolytic transfusion reaction attributable to anti-Fy3 in a patient with sickle cell disease

M.A. REYES AND O.C. ILLOH

A case of hyperhemolytic transfusion reaction attributable to anti-Fy3 in a 30-year-old African American woman with a history of sickle cell disease is reported. The patient was admitted for vaso-occlusive sickle cell crisis and received 4 units of packed RBCs secondary to worsening symptomatic anemia (Hb 5.0 g/dL). On admission, the patient's antibody screen and identification showed anti-V and anti-E, and her antibody history included anti-E, -C, -Jk<sup>b</sup>, -N, -V, -S, -Sl<sup>a</sup>, and a cold agglutinin with possible anti-I specificity. A DAT performed on her RBCs was negative. RBC units that lacked E, C, Jk<sup>b</sup>, N, V, and S were transfused. Posttransfusion Hb was 8.9 g/dL. On day 10 she developed a fever of 103°F, and on day 11 her Hb decreased to 6.4 g/dL. She complained of severe back pain and dark urine. In addition, she became hypertensive, tachycardic, and jaundiced. The DAT indicated the presence of IgG on the patient's RBCs. Anti-Fy3 was identified in the serum and eluate. During the next 24 hours, her Hb decreased to 2.4 g/dL. The LDH level was 1687 U/L, and her reticulocyte count was 2.6%. A delayed hemolytic transfusion reaction with hyperhemolysis secondary to anti-Fy3 was suspected and was successfully treated with IVIG and high-dose prednisone. To the best of our knowledge, this is the first published case of hyperhemolysis in sickle cell disease attributable to anti-Fy3. *Immunohematology* 2008;24:45–51.

**Key Words:** hyperhemolysis, anti-Fy3, sickle cell disease

Delayed hemolytic transfusion reaction with hyperhemolysis (DHTR-H) is a serious complication of transfusion and alloimmunization that has been reported especially in patients with sickle cell disease. This condition is characterized by destruction of both donor and recipient RBCs and is associated with reticulocytopenia. This syndrome commonly occurs after an anamnestic response to a donor RBC antigen or secondary to the formation of new alloantibodies. Transfusion of more blood products can exacerbate hemolysis, and it is usually recommended that transfusions be withheld. Although the exact mechanisms behind hyperhemolysis are not clear, bystander hemolysis has been suggested as the possible pathogenesis of this condition.<sup>1-5</sup> Treatment with IVIG and steroids in severe cases of DHTR-H may be

lifesaving.<sup>4,6-8</sup> Anti-Fy3 is an uncommon antibody that has been described in Fy(a-b-) Caucasians and rarely in African Americans; it is associated with at least one report of an acute hemolytic transfusion reaction.<sup>9</sup> Although cases of hyperhemolysis syndrome have been described in the literature, none have been associated with anti-Fy3. This report describes a case of DHTR-H attributable to anti-Fy3 in an African American patient with sickle cell disease (SCD).

## Case Report

A 30-year-old African American woman with a history of sickle cell anemia (HbS 83.8%, HbF 11.7%, HbA2 4.5%), hepatitis C secondary to blood transfusion, deep venous thrombosis treated with Coumadin therapy, and previous episodes of acute sickle cell crisis was admitted to our hospital with complaints of fever; shortness of breath; and chest, neck, and back pain which were unresolved with her usual home medications. At the time of admission, the patient's Hb was 6.6 g/dL, Hct 20.1%, reticulocyte count 12.9% (corrected, 5.8%), and total bilirubin 2.5 mg/dL (indirect bilirubin, 1.7 mg/dL). Blood cultures were positive for *Enterobacter*, *Klebsiella*, and yeast (not *Candida albicans*). Despite aggressive hydration and initiation of antibiotic therapy, the patient's clinical status continued to deteriorate. Secondary to worsening anemia with a Hb of 5.0 g/dL, the patient was transfused with 4 units of leukocyte-reduced packed RBCs on hospital days 1 and 2. The patient's RBCs typed as group O, D+, C-, E-, c+, e+; M+, N-, S-, s+; P1+; K-; Fy(a-b-); and Jk(a+b-). The patient had a previous history of multiple alloantibodies including anti-E, -C, -Jk<sup>b</sup>, -N, -V, -S, and -Sl<sup>a</sup> and a cold agglutinin with possible anti-I specificity. Of the 4 units issued, 3 units were C-, E-, K-, Fy(a-b-),

Jk(b-), S-, and N-. Because of the difficulty of finding phenotypically matched RBCs for the patient, and the urgency with which it was needed, 1 unit that was C-, E-, K-, Fy(a-b+), Jk(b-), S-, and N- was also issued. The patient's Hb increased to 8.9 g/dL, and her clinical symptoms improved.

On hospital day 10, she developed a fever of 103°F without accompanying symptoms, and blood cultures were negative. By hospital day 11, her Hb decreased to 6.4 g/dL, and she complained of severe back pain and dark urine. In addition she became hypertensive, tachycardic, and jaundiced. The DAT performed on her RBCs was now positive with anti-IgG and negative with anti-C3. As there was clinical suspicion of a delayed hemolytic transfusion reaction, a blood bank investigation was initiated, and further transfusion was withheld. The initial work-up was suggestive of anti-Fy3. This was confirmed by the reference laboratory at Gulf Coast Regional Blood Center in Houston, Texas. Over the course of 24 hours, the patient's Hb continued to decrease, reaching a nadir of 2.4 g/dL. Her LDH level was 1687 U/L, total bilirubin was 2.7 mg/dL (indirect bilirubin, 1.4 mg/dL), and reticulocyte count was 2.6% (corrected, 0.4%). Haptoglobin measurement was not done. At this point DHTR-H was suspected. Her clinical condition continued to deteriorate, and she was started on high-dose prednisone (60 mg/day) and IVIG (400 mg/kg per day) for a treatment course of 5 days. On hospital day 13 the patient developed clinical symptoms of hemodynamic compromise including shortness of breath, tachypnea, systolic ejection murmur, and severe pallor. Her Hb was 2.6 g/dL. The decision was made to transfuse 1 unit of phenotypically matched (C-, E-, K-, Fy(a-b-), Jk(b-), S-, and N-), leukocyte-reduced packed RBCs and monitor closely for additional signs of hemolysis. Her Hb improved to 3.6 g/dL, and prednisone and IVIG treatment were continued. Her LDH level decreased during the ensuing days, and reticulocyte count increased to 6.3% (corrected, 1.5%). Her Hb continued to improve (up to 5.6 g/dL) and remained stable until hospital day 30 when the patient had an episode of epistaxis because of trauma from her nasal canula and baseline anticoagulation therapy for her history of deep vein thrombosis. Her Hb dropped to 2.6 g/dL. She was transfused with 1 unit of phenotypically matched (C-, E-, K-, Fy[a-b-], Jk[b-], S-, and N-), leukocyte-reduced packed RBCs. After transfusion her Hb increased to 3.3 g/dL and remained stable for the next day. Because her LDH level was 733 U/L and total bilirubin was 6.4 mg/dL with an indirect

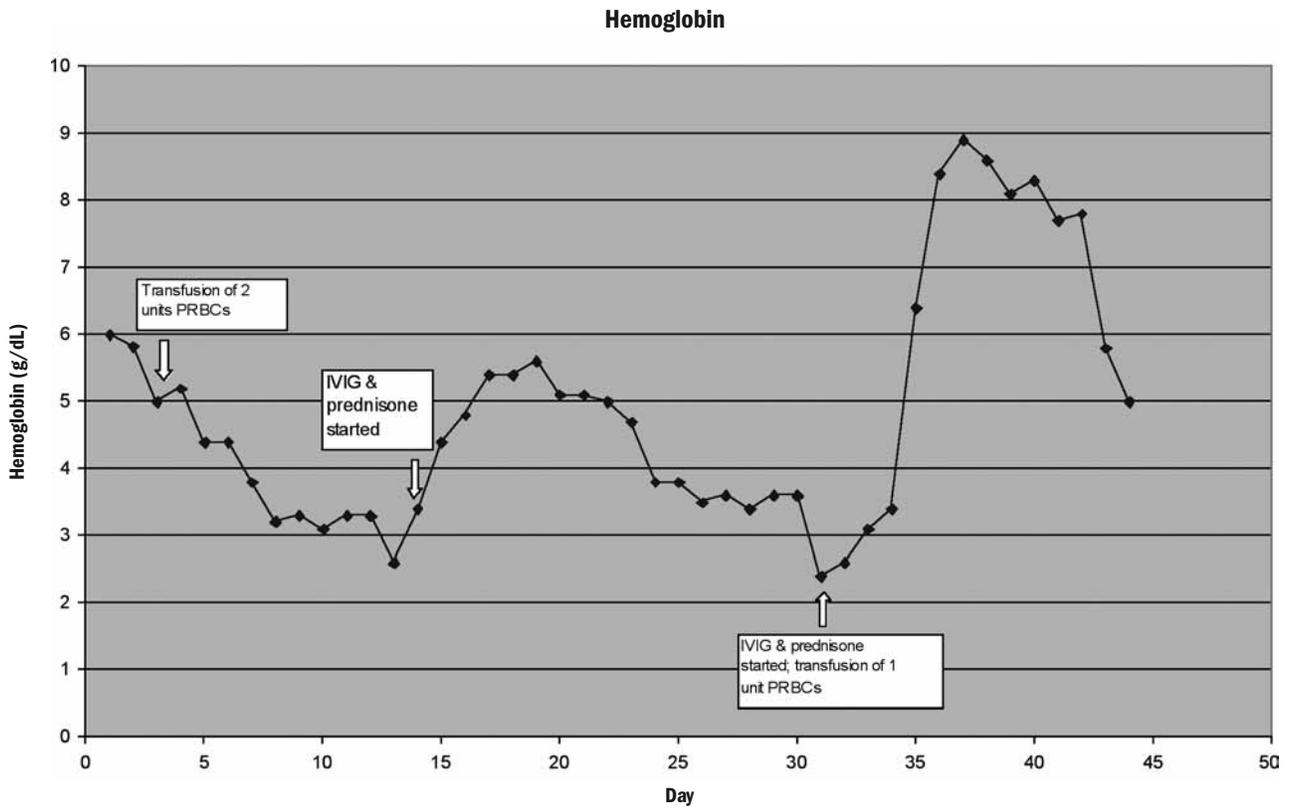
component of 3.4 mg/dL, hemolysis was again suspected. Her reticulocyte count was 15.1% (corrected, 2.6%) and haptoglobin was less than 6 mg/dL. The patient was restarted on high-dose prednisone (500 mg/day) and IVIG (400 mg/kg/day) immediately for a duration of 5 days. Her hemoglobin and clinical symptoms steadily improved, with no further evidence of hemolytic episodes. She was discharged with a Hb of 6.0 g/dL on hospital day 43. The hemoglobin and reticulocyte counts through her hospital course are shown in Figures 1 and 2.

## Materials and Methods

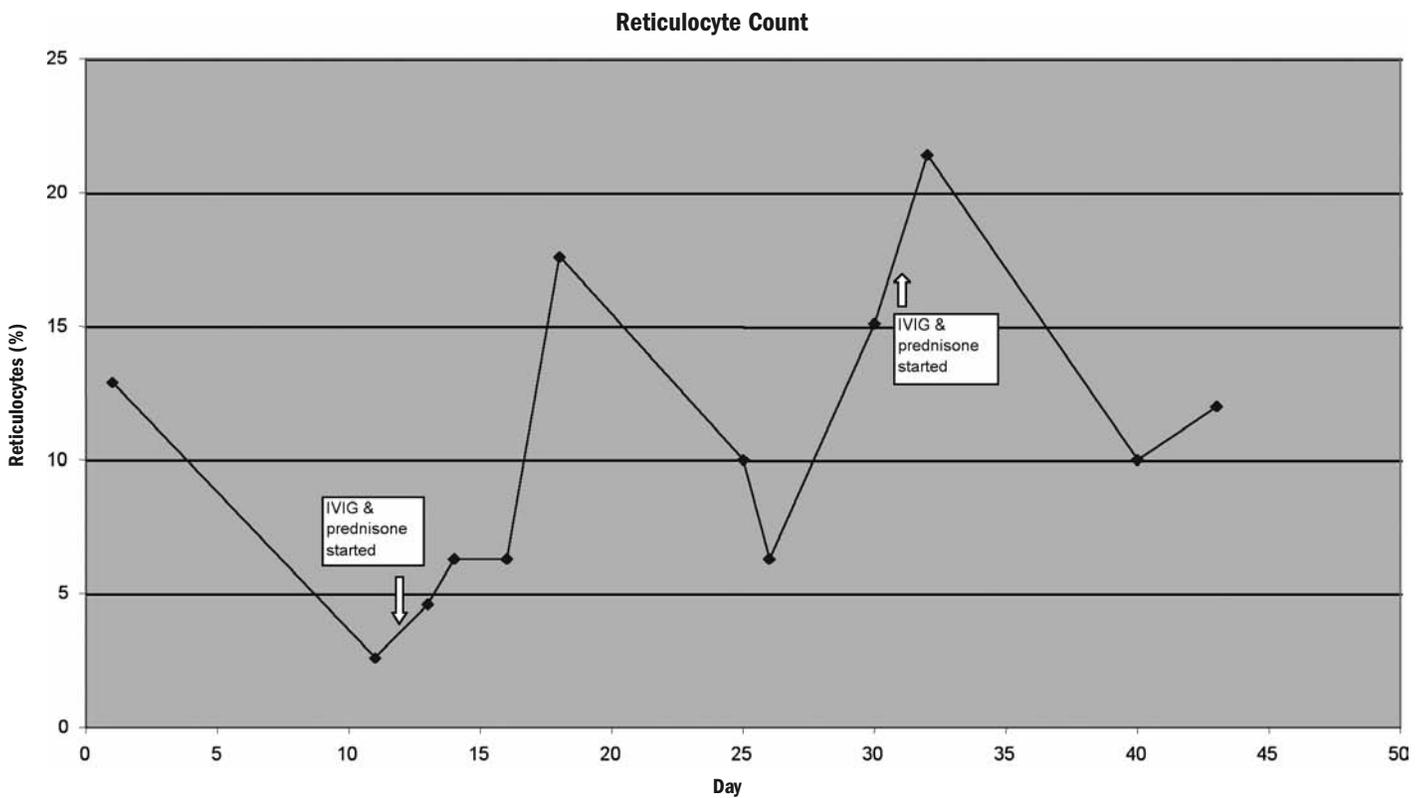
ABO and D testing were performed by standard tube testing using commercial reagents according to the manufacturer's protocol (Immucor, Inc., Norcross, GA). Antibody detection and identification tests were performed using gel methodology (ID-MTS Gel Test, Ortho-Clinical Diagnostics, Raritan, NJ). The patient's serum was tested against panels of commercial reagent RBCs (Ortho-Clinical Diagnostics) to determine antibody specificities. The DAT was performed with polyspecific antihuman globulin and monospecific anti-IgG and anti-C3d reagents (Gamma Biologicals, Inc., Houston, TX). The eluate, prepared by an acid elution method (ELU-KIT II, Gamma Biologicals, Inc.) was tested against panels of commercially available reagent RBCs (Ortho-Clinical Diagnostics). The patient's serum was also tested against ficin-treated RBCs (Ortho-Clinical Diagnostics). The patient's RBCs were typed for Rh, Kell, MNS, Lewis, Duffy, and Kidd major antigens using specific antisera according to the manufacturer's protocol (Immucor, Inc., Norcross, GA).

## Results

Pretransfusion, the patient typed as group O, D+ with a positive antibody screen. Anti-E and anti-V were identified in the sample. Historic records showed that she had multiple alloantibodies (anti-E, -C, -Jk<sup>b</sup>, -N, -V, -S, -I, and -Sl<sup>a</sup>). The phenotype on record was D+, C-, E-, c+, e+, M+, N-, S-, s+; P1+; K-; Fy(a-b-); and Jk(a+b-). The posttransfusion sample showed a positive antibody screen. The patient's serum was reactive with all Fy(a+) and Fy(b+) RBCs and only nonreactive with a donor RBC that matched her phenotype and was also Fy(a-b-). The serum sample showed reactivity after ficin treatment. The presence of anti-Fy3 was confirmed by the reference laboratory at Gulf Coast Regional Blood Center in Houston, Texas. The DAT was 3+ with polyspecific reagent, 3+ with anti-IgG, and negative with



**Fig. 1.** Patient hemoglobin levels throughout hospital admission. PRBCs = packed RBCs.



**Fig. 2.** Patient reticulocyte counts throughout hospital admission.

anti-C3d. The eluate showed reactivity with most donor RBCs except those that were Fy(a-b-). The phenotypes of the transfused units are as described earlier.

## Discussion

This report describes a case of DHTR-H. In this case, a new antibody (anti-Fy3) was detected in the patient's serum on repeat testing. To our knowledge, no case of DHTR-H attributable to the formation of anti-Fy3 alloantibody has been previously published.

Patients with SCD require frequent RBC transfusions or RBC exchange. Alloimmunization to RBC antigens occurs in anywhere from 18 to 47 percent of chronically transfused patients with SCD.<sup>10-12</sup> The formation of multiple antibodies presents a challenge in finding compatible blood units for these patients, and it is often not possible to provide completely phenotype-matched units and therefore prevent further antibody formation. Delayed hemolytic transfusion reaction (DHTR) is a common complication of alloimmunization. This reaction occurs 3 to 14 days after transfusion and presents with anemia, fever, and a positive DAT. On occasion, hyperhemolysis syndrome may occur as a life-threatening manifestation of DHTR. Hyperhemolysis syndrome is characterized by a drop in posttransfusion hemoglobin below pretransfusion levels, elevated LDH level and bilirubin, fever, pain, and hemoglobinuria. One unique finding in patients with this condition is reticulocytopenia. On many occasions the presenting symptoms of DHTR-H resemble manifestations of sickle cell crises.<sup>4,13-16</sup> Therefore, a DHTR must be kept in the differential diagnoses of all recently transfused patients with SCD with vaso-occlusive crises.<sup>4</sup> Delay in diagnosis may lead to inappropriate management and increased morbidity or mortality for these patients. In our case, sickle cell crisis was ruled out, and the presence of a newly detected antibody, combined with a severe drop in hemoglobin levels, supported the diagnosis of DHTR-H.

The mechanism of hyperhemolysis is not completely understood. Multiple studies have supported the fact that autologous RBCs are destroyed, in addition to destruction of transfused RBCs. A bystander hemolysis theory has been proposed.<sup>1-5</sup> Mechanisms by which this proposed bystander hemolysis occurs include epitope spreading and the defective regulation of the formation of the complement membrane attack complex in sickle RBCs.<sup>17</sup> Sickle RBCs more often expose cryptic antigens and therefore have a high level of surface IgG. This makes the RBCs more susceptible to

destruction by active macrophages.<sup>18,19</sup> Of interest, cases of hyperhemolysis have been reported in thalassemia patients after transfusion of RBC units.<sup>20</sup> This suggests that the presence of sickle RBCs alone is not enough to fully explain the hyperhemolysis phenomenon. In close to 50 percent of cases of hyperhemolysis, no new antibody is identified. Garratty<sup>21</sup> has proposed that in the absence of alloantibodies, other antibodies (including HLA antibodies) reacting with foreign proteins may cause complement activation and RBC hemolysis.

Typically in anemia caused by hemolysis, compensatory reticulocytosis is observed. However, many cases of DHTR-H show inappropriately low reticulocyte counts. The suggested mechanisms of reticulocytopenia include transfusion suppression of erythropoiesis, accelerated destruction of reticulocytes, and decreased levels of erythropoietin secondary to renal damage.<sup>2,10</sup> Previous case reports have shown an increase in reticulocyte production after the administration of IVIG or steroid treatment.<sup>6,8</sup> This supports the theory of antibody-mediated accelerated destruction of reticulocytes and hyperactivity of macrophages. In our patient, a drop in the reticulocyte count was observed despite worsening anemia. An increase in the reticulocyte count was observed only after treatment with IVIG and steroids commenced.

In contrast to the DAT in DHTR without hyperhemolysis, the DAT with hyperhemolytic syndrome is most often negative, even in the presence of a new antibody. In a study by Talano et al.,<sup>4</sup> patient RBCs in only two of nine episodes of DHTR-H were positive in the DAT. These were both associated with new antibodies (warm autoantibody and anti-E). The negative DAT can be explained by the hemolysis of transfused RBCs. Our patient's RBCs were negative in the DAT before transfusion, but at the time of the DHTR-H, the DAT was positive and anti-Fy3 was eluted from RBCs. However, the DAT was not repeated when her hemoglobin dropped further. The DAT remained positive even after the second episode of hyperhemolysis that occurred 3 to 4 weeks later with a negative eluate. Our findings and the results of others show that the DAT cannot not be used alone to diagnose DHTR-H.

Anti-Fy3 has been reported to cause both acute and delayed hemolytic transfusion reactions.<sup>9</sup> Several RBC antibodies have been implicated as a cause of DHTR-H, but there are no published reports of anti-Fy3 as a cause of such reactions. This antibody has been described in individuals with the Fy(a-b-) phenotype, but it very

rarely occurs in African Americans. Anti-Fy3 was first described in a Caucasian Australian woman whose RBCs were typed as Fy(a-b-). This antibody reacted with all RBCs that were Fy(a+b-), Fy(a-b+), and Fy(a+b+). Unlike anti-Fy<sup>a</sup> and anti-Fy<sup>b</sup>, anti-Fy3 reacted with enzyme-treated RBCs. Considering that the Fy(a-b-) phenotype is prevalent in the West African and African American populations, one would expect that anti-Fy3 would be a commonly encountered antibody. However, anti-Fy3 is rarely encountered, and the majority of cases have been reported in Caucasians. The molecular mechanisms behind the Fy(a-b-) phenotype in West African and Caucasian individuals differ. It is believed that most Africans are homozygous for an *FYB* allele with a point mutation in the promoter region of the gene that prevents expression of the Duffy antigens on RBCs.<sup>22</sup> However, it has been shown that Fy<sup>b</sup> is expressed on tissue cells of these same individuals, particularly the endothelial cells of postcapillary venules and Purkinje cells of the cerebellum.<sup>23</sup> Therefore, they are prevented from recognizing Fy<sup>b</sup> as foreign and from forming anti-Fy<sup>b</sup> or anti-Fy3. In Caucasians, it has been found that the Fy(a-b-) phenotype is the result of a deletion in the *FY* genes, and therefore there is a lack of expression of Duffy antigens on their RBCs and tissue cells.<sup>24</sup> These individuals are capable of forming antibodies to all of the Duffy antigens, including Fy3. Although this genetic mechanism is known to be found mostly in Caucasians, it has been proposed that both genetic mechanisms exist in the African population. Our patient is African American and therefore would not have been expected to make this antibody. Genotyping studies could not be performed, but it is proposed that her Fy(a-b-) phenotype is the result of a deletion in *FY* genes rather than a defect in the *FY* promoter. When one is looking for phenotypically matched RBCs for African American patients with the Fy(a-b-) phenotype, often the Fy(b-) requirement will be omitted to find units more quickly and because of the improbability of anti-Fy<sup>b</sup> formation in these patients. However, in our patient, giving a Fy(b+) RBC unit likely contributed to the initiation of DHTR-H.

Interestingly, this patient also had a history of anti-Sl<sup>a</sup>, which had been identified by another hospital approximately 10 years earlier. Anti-Sl<sup>a</sup> and anti-Fy3 can be difficult to distinguish serologically, and RBCs that are Fy(a-b-) also tend to be Sl(a-). However, whereas anti-Sl<sup>a</sup> reactivity is depressed with ficin treatment of RBCs, anti-Fy3 activity is unaffected by enzyme treatment. This can be an important distinguishing feature when

differentiating between these antibodies. In our patient, our reference laboratory (Gulf Coast Regional Blood Center, Houston, TX) did not identify anti-Sl<sup>a</sup> after multiple and extensive evaluations.

In patients with DHTR-H, transfusion of more blood may exacerbate hemolysis. Therefore the transfusion of RBCs is discouraged unless severe, life-threatening anemia occurs. In our patient, transfusions were withheld until the patient developed severe, symptomatic anemia. She appeared to have responded to IVIG and steroid treatment with an increasing hemoglobin and reticulocyte count. However, after a second transfusion, hyperhemolysis again occurred. Many studies have reported success with high-dose IVIG and corticosteroids, either alone to prevent hemolysis or in conjunction with RBC transfusions.<sup>6,8</sup> How these drugs help reduce hemolysis is not clear, but some of the proposed mechanisms include (1) IVIG prevention of contact-mediated lysis of the RBCs by blocking the adhesion of sickle cells, reticulocytes, and macrophages, and (2) IVIG and steroid suppression of hyperactive macrophages.<sup>8</sup> Additional trials and studies are needed to further define the role of these drugs in this condition. Although transfusion precipitates most cases of hyperhemolysis in patients with SCD, there have been reports of this condition occurring in patients with uncomplicated acute painful episodes.<sup>25</sup> It is possible the patient's underlying SCD and acute painful crisis, even without transfusion, could have contributed to the hyperhemolytic crisis.

Whether this reaction will occur again in patients who have had one episode of DHTR-H is not clear. Our patient seems to be one in whom DHTR-H will very likely recur. This is despite attempts to provide blood that matched her complete phenotype. Other reports have also shown that giving phenotypically matched blood did not prevent the occurrence of DHTR-H in some patients.<sup>3,4,10</sup> Since this episode, this patient has presented to the hospital multiple times but has not been transfused and has maintained baseline Hb of 5 to 6 g/dL.

## Conclusion

In conclusion, this is a report of DHTR-H attributable to anti-Fy3 in an African American woman with SCD. Anti-Fy3, although rare in African Americans and West Africans, can be associated with hyper-hemolysis. This report shows that hyperhemolysis is a serious complication that should be sought for early and that providing phenotypically matched units will not always prevent

DHTR-H from occurring. Withholding transfusions and initiating treatment with IVIG and steroids may be lifesaving. The complete pathogenesis of this condition and other treatment options available continue to be explored.

## References

1. King KE, Shirey RS, Lankiewicz MW, Young-Ramsaran J, Ness PM. Delayed hemolytic transfusion reactions in sickle cell disease: simultaneous destruction of recipients' red cells. *Transfusion* 1997;37:376-81.
2. Petz LD. Bystander immune cytolysis. *Transfus Med Rev* 2006;20:110-40.
3. Petz LD, Calhoun L, Shulman IA, Johnson C, Herron RM. The sickle cell hemolytic transfusion reaction syndrome. *Transfusion* 1997;37:382-92.
4. Talano JA, Hillery CA, Gottschall JL, Baylerian DM, Scott JP. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. *Pediatrics* 2003;111:e661-5.
5. Win N, Doughty H, Telfer P, Wild BJ, Pearson TC. Hyperhemolytic transfusion reaction in sickle cell disease. *Transfusion* 2001;41:323-8.
6. Cullis JO, Win N, Dudley JM, Kaye T. Post-transfusion hyperhaemolysis in a patient with sickle cell disease: use of steroids and intravenous immunoglobulin to prevent further red cell destruction. *Vox Sang* 1995;69:355-7.
7. Telen MJ. Principles and problems of transfusion in sickle cell disease. *Semin Hematol* 2001;38:315-23.
8. Win N, Yeghen T, Needs M, Chen FE, Okpala I. Use of intravenous immunoglobulin and intravenous methylprednisolone in hyperhaemolysis syndrome in sickle cell disease. *Hematology* 2004;9:433-6.
9. Olteanu H, Gerber D, Partridge K, et al. Acute hemolytic transfusion reaction secondary to anti-Fy3. *Immunohematology* 2005;21:48-52.
10. Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. *Transfusion* 2002;42:37-43.
11. Cox JV, Steane E, Cunningham G, Frenkel EP. Risk of alloimmunization and delayed hemolytic transfusion reactions in patients with sickle cell disease. *Arch Intern Med* 1988;148:2485-9.
12. Rosse WF, Gallagher D, Kinney TR, et al. Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease. *Blood* 1990;76:1431-7.
13. Chaplin H, Cassell M. The occasional fallibility of in vitro compatibility tests. *Transfusion* 1962;2:375-84.
14. Garratty G. Severe reactions associated with transfusion of patients with sickle cell disease. *Transfusion* 1997;37:357-61.
15. Diamond WJ, Brown FL Jr, Bitterman P, Klein HG, Davey RJ, Winslow RM. Delayed hemolytic transfusion reaction presenting as sickle-cell crisis. *Ann Intern Med* 1980;93:231-4.
16. Milner PF, Squires JE, Larison PJ, Charles WT, Krauss JS. Posttransfusion crises in sickle cell anemia: role of delayed hemolytic reactions to transfusion. *South Med J* 1985;78:1462-9.
17. Test ST, Woolworth VS. Defective regulation of complement by the sickle erythrocyte: evidence for a defect in control of membrane attack complex formation. *Blood* 1994;83:842-52.
18. Lubin B, Chiu D, Bastacky J, Roelofsen B, Van Deenen LL. Abnormalities in membrane phospholipid organization in sickled erythrocytes. *J Clin Invest* 1981;67:1643-9.
19. Schwartz RS, Tanaka Y, Fidler IJ, Chiu DT, Lubin B, Schroit AJ. Increased adherence of sickled and phosphatidylserine-enriched human erythrocytes to cultured human peripheral blood monocytes. *J Clin Invest* 1985;75:1965-72.
20. Grainger JD, Makar Y, McManus A, Wynn R. Refractory hyperhaemolysis in a patient with beta-thalassaemia major. *Transfus Med* 2001;11:55-7.
21. Garratty G. Immune hemolytic anemia associated with negative routine serology. *Semin Hematol* 2005;42:156-64.
22. Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nat Genet* 1995;10:224-8.
23. Peiper SC, Wang ZX, Neote K, et al. The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy negative individuals who lack the erythrocyte receptor. *J Exp Med* 1995;181:1311-7.
24. Mallinson G, Soo KS, Schall TJ, Pisacka M, Anstee DJ. Mutations in the erythrocyte chemokine receptor (Duffy) gene: the molecular basis of the Fya/Fyb antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a-b-) phenotype. *Br J Haematol* 1995;90:823-9.

25. Ballas SK, Marcolina MJ. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. *Transfusion* 2006;46:105-10.

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# Managing a pregnancy with antibodies: a clinician's perspective

M. TARSA AND T.F. KELLY

HDN, also known as erythroblastosis fetalis, is an immune-mediated anemia, caused by maternal antibodies against a specific fetal RBC antigen. The advent of Rh immune globulin (RhIG) almost 40 years ago has decreased the incidence of D-associated HDN. Maternal isoimmunization to other RBC antigens, for which there is no prophylaxis, continues to occur. The frequency of the latter has increased compared with that of Rh disease. Historically, HDN had a high rate of perinatal mortality. Advances, including improved neonatal care, amniocentesis, fetal RBC typing, and in utero transfusion, have decreased overall fetal loss rates to 2 to 3 percent.<sup>1</sup> Middle cerebral artery Doppler ultrasound has reduced the need of repetitive amniocenteses for assessment of fetal anemia. Determination of free fetal DNA in the maternal circulation is a new technique that may confirm the D status of a fetus, and thus is useful in identifying the fetus, parented by a heterozygous father, who is not at risk.

## Historical Review

HDN was first described in 400 BC by Hippocrates. In 1609, a French midwife reported a hydropic newborn girl who expired at birth with a twin who developed jaundice and neurologic complications and subsequently died.<sup>2</sup> Throughout the 19th century, the proposed cause of icterus gravis was the absence or obstruction of the bile duct. The term *erythroblastosis fetalis* connoted a hydropic stillborn by Rautmann in 1912.<sup>3</sup> It was not until 1931 that Buhrman and Sanford made the connection between erythroblastosis fetalis and newborn jaundice.<sup>4</sup> Landsteiner and Wiener described the Rh factor, and a year later Levine determined that antibodies against the D antigen were

instrumental in the cause of HDN.<sup>5,6</sup> The subsequent 60 years advanced the care of affected pregnancies including timing of delivery, amniocentesis for fetal assessment, in utero fetal transfusion, and noninvasive modalities for determining fetal risk. Improvements made in the care for this disorder cannot be overstated. Before 1945, 50 percent of all fetuses with hemolytic disease died, accounting for 10 percent of overall perinatal mortality.<sup>7</sup>

## RBC Alloimmunization

The antigens of the Rh system are the most common cause of immunization during pregnancy. Although more than 40 antigens belong to this system, D, C, c, E, and e are the most common. D- blood is found in about 15 percent of Caucasians and 5 percent of Africans. Other RBC antigens have been identified as the cause of HDN, including those in the Kell, Duffy, and Kidd blood group systems. HDN caused by antigens other than D is increasing in prevalence. The pathogenesis of HDN is similar, whether the disease is caused by D or another antigen, and for the purposes of this discussion D will be used.

A D- pregnant woman exposed to D antigen, usually as a result of transplacental passage, may develop alloimmunization. Events that are associated with fetal to maternal hemorrhage include delivery, abortion, molar or ectopic pregnancies, and procedures including amniocentesis, cordocentesis, chorionic villus sampling, and external cephalic version, as well as trauma and abruption. The maternal immune response is variable and is affected by the volume of fetomaternal hemorrhage, fetal zygosity for D, and ABO compatibility of mother and fetus.<sup>8</sup> Once a woman has developed D-specific IgG antibodies, a subsequent fetus is at risk of

hemolysis if its RBCs exhibit the offending antigen. The presence and severity of the anemia depend on both the antibody concentration and prior pregnancy history. Antibody subtype, frequency of expressed antigen, efficiency of placental immunoglobulin transport, and fetal spleen maturity all play a role in fetal RBC destruction.<sup>9</sup>

Fetal anemia and resultant extramedullary hemato-poiesis lead to a reduction of colloid osmotic pressure and liver congestion, respectively, producing portal hypertension. The fetus may then develop abnormal fluid collections, which is termed hydrops. Other RBC antigens including C, c, E, and e can provoke similar immune responses.

### Initial Workup of an Alloimmunized Pregnancy

For the purpose of consistency, Rh disease is described here. Data supporting the management of alloimmunization to antigens other than D is limited, but most authorities recommend management similar to that of D isoimmunization.<sup>10</sup>

Blood typing and an IAT should be performed on entry into prenatal care. The presence of a positive antibody screen suggests alloimmunization and requires characterization. Determining the antibody specificity, whether it is to D or another antigen, is necessary for two reasons: (1) some antibodies are not clinically significant and are not associated with HDN, and (2) paternal testing is available for most RBC antigens to assess for risk of transmission. For example, if the father is homozygous for D, all his offspring will be D+ and thus be at risk of HDN. Fifty-five percent of individuals are heterozygous for the antigen, which decreases the incidence of the fetus' RBCs being D to 50 percent. The difficulty with determining paternal zygoty for D is that no defined allele for the recessive condition has been identified. Therefore, serology testing for C, c, D, E, and e combined with ethnicity can provide an estimated risk of heterozygosity. Paternal testing is more helpful with other RBC antigens. In these cases, maternal sensitization may occur owing to a prior transfusion; thus the potential exists that the partner may not exhibit the corresponding RBC antigen. After phenotyping of the parental RBCs is completed, further management is dependent on the potential for the fetus' RBCs to exhibit the antigen (Fig. 1).<sup>11</sup>

If the father's RBCs are D+ and the couple does not have a history of an affected child, the patient may be given one of two options: (1) serial monitoring of

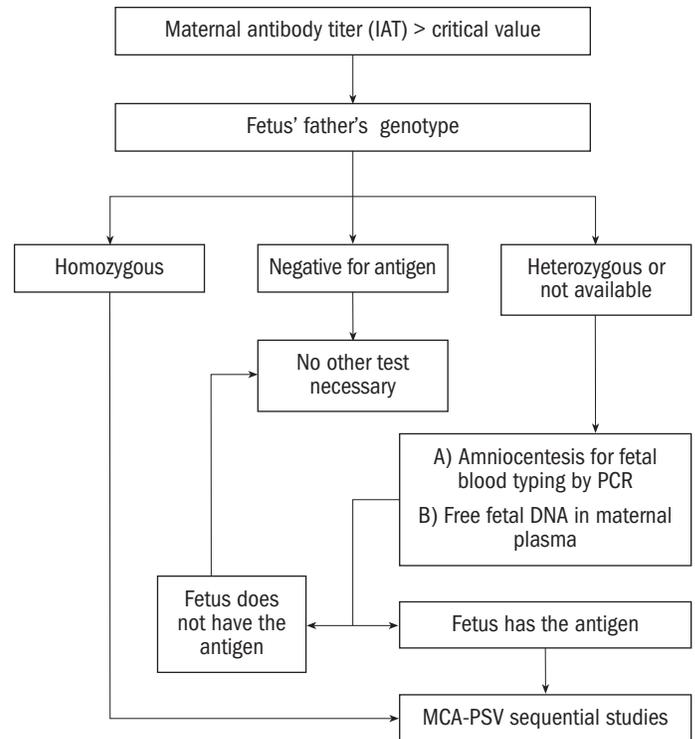


Fig. 1. Algorithm for workup in the alloimmunized pregnancy.<sup>11</sup>

maternal antibody titer on a biweekly or monthly basis until it reaches a critical value; or (2) offering an amniocentesis to investigate fetal D (or any other antigen) status using PCR.

Fetal DNA from nucleated RBCs has been isolated in maternal blood and used for fetal RhD genotyping.<sup>12</sup> A recent meta-analysis reported 94.8 percent accuracy of noninvasive fetal Rh determination using maternal blood. In alloimmunized pregnancies, 91.8 percent of the cases were correctly diagnosed. Free fetal DNA in maternal serum or plasma had higher diagnostic accuracy when compared with RNA or DNA extracted from maternal blood.<sup>13</sup> These techniques are currently not believed to be definitive. The potential error of not correctly identifying a D+ fetus in an alloimmunized mother is not completely preventable at present. Hopefully in the near future such a test will achieve 100 percent accuracy and become the preferred method for determining fetal D status and eliminating unnecessary workups for fetuses not at risk for HDN.

### Estimating Risk of HDN

HDN is classified as mild, moderate, and severe.<sup>14</sup> The majority of cases present as mild, requiring newborn phototherapy. Only 30 percent of affected newborns have moderate and 20 percent have severe

disease. Moderate cases have anemia and may require preterm delivery and exchange transfusion. Fetuses with severe disease are hydropic. Fortunately, less than 10 percent of patients have severe disease at less than 34 week' gestational age, the time when in utero therapy would be necessary. Estimating the risk of HDN relies on prior history and maternal antibody titer. A patient without a prior history of sensitization would be expected to have a good outcome. Conversely, one with a prior affected pregnancy or certainly a prior hydropic fetus or adverse postnatal course would be at risk for an adverse outcome. Antibody titers alone are unreliable for prediction when a patient has had a prior affected fetus or infant.

### Maternal Antibody Titer

Methods for determining the titer vary by institution, and the critical value for the potential of fetal anemia is usually established in each laboratory. A titer between 8 and 32 is considered critical. This applies to Rh as well as other antigens. The exception is K, in which there are affected cases when maternal titers are less than 16. In a pregnancy without a prior affected fetus, the titer is repeated every 2 to 4 weeks as long as it remains below the critical threshold. Once the value is met, serial assessments for the possibility of fetal anemia must be undertaken, by either amniocentesis or middle cerebral artery Doppler ultrasound. In cases in which there is a history of a prior severely affected fetus, serial surveillance is warranted regardless of the antibody titer.

### Amniocentesis

Yellow amniotic fluid in anemic fetuses with severe hemolytic disease correlates with its high bilirubin content and is likely derived from fetal pulmonary and tracheal secretions. Liley<sup>15</sup> first described, in 1961, a qualitative analysis of amniotic fluid bilirubin for HDN, which is measured by spectrophotometry, absorbing light at a wavelength of 450 nm. The resulting deviation from baseline ( $\Delta OD_{450}$ ) is plotted against gestational age (Fig. 2). The graph is divided into three zones with zone I indicating a low likelihood of severe anemia, zone II intermediate, and zone III a high probability of severe fetal anemia. The prediction of fetal anemia requires repeated samplings every 1 to 3 weeks depending on the initial value. Decreasing values are reassuring, but a rising curve indicates active hemolysis. Values in upper zone II or zone III require further assessment with cordocentesis to determine fetal hemoglobin or consideration of delivery if beyond 34 weeks' gestation.

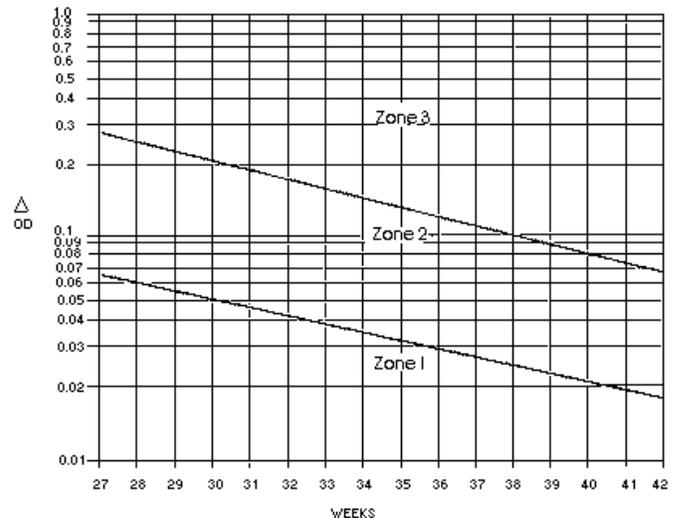


Fig. 2. Liley curve. Optical density at 450 nm is measured ( $\Delta OD_{450}$ ) and plotted against gestational age.<sup>15</sup>

The Liley curve was modified by Queenan using data from the early second trimester, allowing earlier diagnosis and treatment of fetal anemia in pregnancy.<sup>16</sup>

Amniotic fluid PCR can be used to document the fetal antigen status for D and other common antigens.<sup>17</sup> A number of blood banks provide this testing, usually requiring amniotic fluid or cultured amniocytes and parental blood. A fetus subsequently found lacking the gene that encodes the antigen requires no further workup or monitoring. Amniocentesis is not without risks. The common complications include infection and preterm membrane rupture. Furthermore, it may potentially cause an anamnestic maternal immune response, placing the fetus at further risk of anemia.

### Ultrasound

During the past 20 years investigators have attempted to use ultrasound to indirectly screen for fetal anemia as a result of the concerns about amniocentesis complications. Parameters that can be monitored by ultrasound include umbilical vein diameter, fetal spleen or liver length, biventricular outer diameter, and placental thickness. Unfortunately, most of these poorly predict fetal anemia, and their use in current clinical practice is limited.<sup>18</sup> The only validated tool for fetal anemia prediction is middle cerebral artery Doppler ultrasound. Anemic fetuses have increased cardiac output, decreased blood viscosity, and thus increased flow velocity. Mari et al.<sup>19</sup> in 2000 showed that measurement of peak systolic velocity in the middle cerebral artery accurately predicts severe fetal anemia (Fig.3). Once the

critical antibody screening titer has been achieved, cerebral Doppler ultrasounds are performed on a weekly or biweekly basis. When peak systolic values reach 1.5 times the median of those expected for gestational age, cordocentesis is indicated for determining fetal hemoglobin level, blood type, and potential intrauterine transfusion. After 35 weeks' gestation this modality becomes less reliable, and there is an increased false-positive rate.<sup>20</sup> Depending on the clinical history, consideration should be given to documenting fetal lung maturity and determining the  $\Delta$ OD 450 via amniocentesis at around 36 weeks.

Recently in a multicenter trial of detection of severe fetal anemia, Doppler ultrasound and amniotic fluid  $\Delta$ OD 450 were compared for sensitivity and accuracy. Results revealed that Doppler was more sensitive and accurate when compared with  $\Delta$ OD 450 using the Liley method and was as sensitive and accurate when compared with Queenan's method.<sup>21</sup> The conclusion of the trial was that Doppler can safely replace the more invasive amniocentesis for detection of fetal anemia.

### Intrauterine Fetal Transfusion

Intrauterine peritoneal blood transfusion using x-ray guidance was first described by Liley in 1963.<sup>22</sup> Since this groundbreaking step toward intrauterine treatment of fetal anemia, many modifications of the procedure have been made with the hope of improving the safety of the procedure. The purpose of fetal transfusion is to reestablish normal fetal hematocrit with donor RBCs that lack the offending antigen(s) and suppress subsequent fetal bone marrow production. Donor blood is group O, D-. The blood is packed to a Hct of 75% to 80%, is screened for infections including CMV, and is irradiated to prevent graft-versus-host reactions. The amount of transfused blood depends on pretransfusion and desired posttransfusion hematocrits (usually 45–50%), gestational age, and hematocrit of the donor.<sup>23</sup>

Transcutaneous approaches can be either fetal intraperitoneal or intravascular, and both performed under ultrasound guidance. Intraperitoneal transfusion places the donor blood into the fetal peritoneal cavity and relies on the absorption of the RBCs via the subdiaphragmatic and thoracic ducts. The utility of this method is limited in fetuses with hydrops because the absorption into the lymphatics is impaired and is associated with a higher fetal death rate. Intravascular access has the advantage of direct and immediate delivery of donor RBCs into the fetal vasculature. Both the umbilical vein and the intrahepatic vein have been

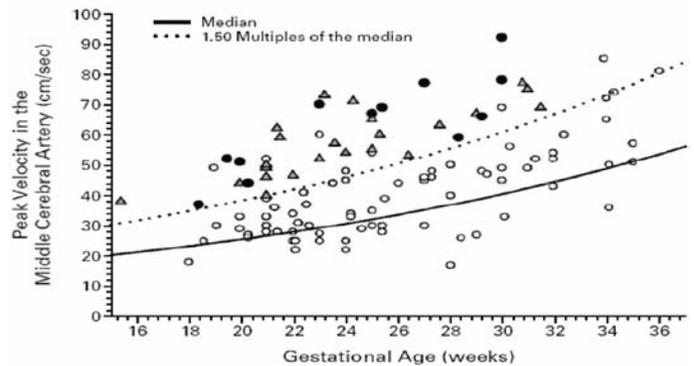


Fig. 3. Peak systolic velocity in the middle cerebral artery of pregnancies at risk for fetal anemia. Open circles indicate no fetal anemia or mild anemia. Triangles indicate moderate to severe anemia. Solid circles indicate fetuses with hydrops.<sup>19</sup>

used for access. Exact placement to some extent depends on fetal and placental position, ease of access to the cord root, and operator training. Free loops of cord are avoided because of the potential of tearing with fetal movement.

The timing of fetal blood sampling and potential transfusion can be challenging. The goal is to initiate the process once the fetus has moderate to severe anemia but before development of hydrops. Intravascular fetal transfusion is now considered to be a safe procedure with an overall survival rate of 89 percent and procedure-related rate of loss of 1.6 percent per procedure. Complications such as rupture of membranes, intrauterine infection, emergency cesarean section, fetal death, and neonatal death have been reported.<sup>24</sup>

### Timing of Delivery and Intrapartum Issues

The fetal risks of HDN increase as gestation advances. Therefore an alloimmunized pregnancy is preferably delivered before the estimated due date. Actual timing of the delivery should be dictated by the estimated severity of disease, balancing the risks of ongoing hemolysis and need for in utero transfusion versus the risks of prematurity. Patients with mild or moderate disease usually are delivered at around 36 to 37 weeks, after confirmation of fetal lung maturity. Severely affected fetuses are usually delivered when the risks of transfusion outweigh the risks of long-term disability from a preterm birth, or if one can estimate the need for transfusion based on the last known fetal hematocrit after 34 weeks. If an atypical antibody is detected, the woman's blood sample should be typed and crossmatched once labor occurs. Delivery should

be preferably at an institution adept at caring for neonates with the possibility of severe anemia.

### Conclusion

RhIG, Doppler ultrasonography, and intrauterine fetal transfusion have improved the care of pregnancies affected by D or isoimmunization by other antigens including K. Once thought to be a fatal disease, in utero severe fetal anemia can now be reversed with about 90 percent survival rate. The care of a mother affected by RBC alloimmunization can be challenging and time-consuming, especially in cases in which fetal RBC antigen type is unknown. The hope is that within the next decade advances in determination of free fetal DNA in the maternal blood will significantly improve care and avoid many unnecessary procedures.

### References

1. Queenan JT. Erythroblastosis fetalis: closing the circle. *N Engl J Med* 1986;314:1448-9.
2. Bourgeois L. Observations Diverse sur la Sterilite Perte de Fruiot, Foecondite, Accouchments, et Maladies de Femmes, et Enfants Nouveaux-naiz, Paris, 1609.
3. Rautmann H. Ueber blutbildung bei fotaler allgemeiner wassersuch. *Beit Z Path Anat u z allg Path* 1912;54:332.
4. Buhrman WL, Sanford HN. Is familial jaundice of newborn infants erythroblastosis fetalis? *Am J Dis Child* 1931;41:225-35.
5. Landsteiner K, Wiener AJ. An agglutinable factor in human blood recognized by immune sera for rhesus blood. *Proc Soc Exp Biol Med* 1940;43:223-4.
6. Levine P, Burnham L, Katzin EM, et al. The role of isoimmunization in the pathogenesis of erythroblastosis fetalis. *Am J Obstet Gynecol* 1941;42:825-7.
7. Bowman J. Thirty-five years of Rh prophylaxis. *Transfusion* 2003;43:1661-6.
8. Kumpel BM. *Transfusion* 2006;46:1652-6.
9. Hadley AG. Laboratory assays for predicting the severity of haemolytic disease of the fetus and newborn. *Transpl Immunol* 2002;10:191-8.
10. Said S, McParland P. Update on the management of non anti-D antibodies. *Ir Med J* 2006;99:55-6.
11. Detti L, Akiyama M, Mari G. Doppler blood flow in obstetrics. *Curr Opin Obstet Gynecol* 2002;14:587-93.
12. Geifman-Holtzman O, Bernstein IM, Berry SM, et al. Fetal RhD genotyping in fetal cells flow sorted from maternal blood. *Am J Obstet Gynecol* 1996;174:818-22.
13. Geifman-Holtzman O, Grotegus CA, Gaughan JP. Diagnostic accuracy of noninvasive fetal Rh genotyping from maternal blood: a meta-analysis. *Am J Obstet Gynecol* 2006;195:1163-73.
14. Bowman JM. Hemolytic disease (erythroblastosis fetalis). In: Creasy R, Resnik R, eds. *Maternal-fetal medicine*, 4th ed. Philadelphia:WB Saunders, 1999:736-67.
15. Liley AW. Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. *Am J Obstet Gynecol* 1961;82:1359-70.
16. Queenan JT, Tomai TP, Ural SH, et al. Deviation in amniotic fluid optical density at a wavelength of 450 nm in Rh-immunized pregnancies from 14-40 weeks' gestation: a proposal for clinical management. *Am J Obstet Gynecol* 1993;168:1370-6.
17. Faas BH, Maaskant-Van Wijk PA, von dem Borne AE, et al. The applicability of different PCR-based methods for fetal RHD and K1 genotyping: a prospective study. *Prenat Diagn* 2000;20:453-8.
18. Dukler D, Oepkes D, Seaward G, Windrim R. Noninvasive tests to predict fetal anemia: a study comparing Doppler and ultrasound parameters. *Am J Obstet Gynecol* 2003;188:1310-4.
19. Mari G, Deter RL, Carpenter RL, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. *N Engl J Med* 2000;342:9-14.
20. Zimmerman R, Carpenter RJ Jr, Durig P, et al. Longitudinal measurement of peak systolic velocity in the fetal middle cerebral artery for monitoring pregnancies complicated by red cell alloimmunisation: a prospective multicentre trial with intention-to-treat. *Br J Obstet Gynaecol* 2002;109:746-52.
21. Oepkes D, Seaward PG, Vandenbussche FP, et al. The Diamond Study Group. Doppler ultrasonography versus amniocentesis to predict fetal anemia. *N Engl J Med* 2006;355:156-64.
22. Liley AW. Intrauterine transfusion of foetus in haemolytic disease. *Br Med J* 1963;5365:1107-9.
23. Nicolaidis KH, Soothill PW, Rodech CH, et al. Rh disease: intravascular fetal blood transfusion by cordocentesis. *Fetal Ther* 1986;1:185-92.

24. Van Kamp IL, Klumper FJ, Bakkum RS, et al. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red cell alloimmunization. *Am J Obstet Gynecol* 2005;192: 171-7.

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# Antibodies to Le<sup>a</sup> and Le<sup>b</sup>: a challenge to identify regardless of how you detect them

L. ERNSTER

## Clinical Case Presentation

A 25-year-old African American woman who was 17 weeks pregnant presented in the emergency room with vaginal bleeding. This was her first pregnancy and she denied ever receiving a transfusion. She had no history of blood bank testing at this hospital.

## Immuno-hematologic Evaluation and Results

Forward and reverse ABO and D typing was performed using a standard test tube method, and the patient was found to be group A, D- (Table 1). No discrepancy was observed.

The antibody detection test using the gel method (ID-MTS anti-IgG cards and 0.8% Selectogen reagent RBCs; Ortho-Clinical Diagnostics, Raritan, NJ) produced agglutination (2+) with cell I, and no agglutination (0) with cell II (Table 2).

An RBC panel used for antibody identification (0.8% Resolve Panel A, Ortho-Clinical Diagnostics) was tested using gel. Eight of 11 reagent RBC samples were reactive, with seven of those samples demonstrating mixed cell agglutination and one sample demonstrating 2+ agglutination (Table 3). The patient's auto-control run in parallel with the RBC panel was negative.

A second RBC panel (0.8% Resolve Panel B, Ortho-Clinical Diagnostics) was tested using gel and demonstrated agglutination with only one RBC sample producing mixed cell agglutination (Table 4).

Additional selected reagent RBCs were tested using gel to exclude all other commonly encountered antibodies. Sufficient rule-outs were obtained (Table 5).

The inability to definitively confirm antibody specificity and the observance of numerous mixed cell reactions prompted testing with alternative antibody detection methods. An antibody detection test using

**Table 1.** Standard test tube method for forward and reverse ABO and D typing

Additive	Forward			Reverse	
	Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
Immediate Spin	4+	0	0	0	4+

PEG (PeG and Panocreen I and II; Immucor/Gamma, Norcross, GA) was performed and resulted in agglutination (2+) at the IAT phase only with both screening cells (Table 6).

A cold panel consisting of antibody detection cells (Panocreen I and II, A<sub>1</sub> and A<sub>2</sub> RBCs, Referencells-4, Immucor/Gamma), group O cord RBCs, and the patient's RBCs was performed. If the mixed cell reactions were caused by a cold reacting antibody, the cold panel would aid in establishing whether the antibody is autoimmune or alloimmune in nature, and would confirm its specificity. The immediate spin phase was nonreactive with all RBC samples, but the 15-minute room temperature incubation produced agglutination (2+) with the antibody detection RBCs, A<sub>1</sub> RBCs, and A<sub>2</sub> RBCs (Table 7). Agglutination was observed with all RBC samples tested at 18°C and 4°C.

A selected RBC panel (Panocell, Immucor/Gamma) was tested using PEG, which demonstrated agglutination (1+ to 2+) at the IAT phase with Le(a+) or Le(b+) RBC samples. At least one expression of all other commonly encountered antigens was represented on Le(a-b-) RBC samples. All were nonreactive at all phases (Table 8).

## Interpretation

This case describes classic identification of anti-Le<sup>a</sup> and anti-Le<sup>b</sup>, demonstrating seemingly nonspecific reactivity at first glance yet highly suggestive reactivity after further review of gel testing. The reactivity of most

**Table 2.** Antibody detection test

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		Xg <sup>a</sup>	GEL, 15' IAT	
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>			
1) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	0	0	+	+	+	2+
2) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	+	+	0

**Table 3.** Antibody identification panel

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		Xg <sup>a</sup>	GEL, 15' IAT
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>		
1) R <sub>1</sub> R <sub>1</sub>	+	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	0	0	+	+	+	+	0
2) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	0	+	0	0	+	+	+	+	+	0
3) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	0	+	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0	0
4) R <sub>0</sub> r	+	0	0	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	0	0	+	+	MC
5) r'r	0	+	0	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	+	0	+	0	+	MC
6) r''r	0	0	0	+	+	+	+	+	+	+	+	0	+	+	0	+	0	+	0	0	0	+	+	+	+	2+
7) rr	0	0	0	0	+	+	+	+	+	0	+	0	+	+	+	0	+	+	0	0	0	+	0	+	+	MC
8) rr	0	0	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	0	+	0	+	0	+	MC
9) rr	0	0	0	0	+	+	+	+	0	+	0	0	+	+	+	0	0	+	0	0	0	+	+	+	+	MC
10) rr	0	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	0	+	+	MC
11) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	MC
12) Patient																										0

MC = mixed cell agglutination

**Table 4.** Alternative antibody identification panel

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		Xg <sup>a</sup>	GEL, 15' IAT
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>		
12) rr	0	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0	+	0
13) rr	0	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	0	0	0	+	+	0	+	0
14) rr	0	0	0	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	0
15) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	+	+	+	0	+	0	0	+	0	+	0	0	+	0	+	0	+	0
16) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	0	+	0	0
17) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	0	+	0	0	+	+	0
18) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	0	+	+	+	0	+	S	+	0	0	+	0	0	0	+	+	0	+	MC
19) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	0	0	+	+	0
20) R <sub>2</sub> r'	+	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	0	+	+	0
21) r''r	0	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	+	+	0	0	+	+	+	+	+	0
22) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	+	+	+	0

MC = mixed cell agglutination

Le(a+) or Le(b+) RBC samples on the initial antibody identification panel in conjunction with the patient's diagnosis suggested the presence of both antibodies and warranted further testing to identify them by alternative methods. Both anti-Le<sup>a</sup> and anti-Le<sup>b</sup> were confirmed by tube testing using PEG. These serologic findings are supported by the reactivity of the cold panel at the 15-minute room temperature phase with RBC samples known or likely to express the Le<sup>a</sup> and Le<sup>b</sup> as opposed to nonreactivity with cord RBCs likely to lack or have weak expressions of both antigens.

### Discussion

Anti-Le<sup>a</sup> and -Le<sup>b</sup> are usually IgM in nature, with optimal reactivity at room temperature or lower. As previously stated, the ID-MTS gel system was used as the primary antibody detection method and uses anti-IgG specific cards that are not known to be sensitive for IgM antibodies. However, IgM antibodies can yield positive reactions in anti-IgG cards if antibodies and RBCs agglutinate in the card's reaction chamber before centrifugation.<sup>1</sup> Presumably, if not all RBCs in the reaction chamber agglutinate, a mixed cell reaction could occur.

**Table 5.** Additional cells

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		GEL, 15'	
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	IAT
1) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	0	0	+	0	+	S	+	0	0	+	0	0	+	0	+	0	0	0
2) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	+	+	0	0	+	+	+	0	0	+	0	0	+	+	0	+	+	MC
3) R <sub>1</sub> R <sub>1</sub>	+	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	+	+	0	0	+	+	+	0	+	W+
4) R <sub>1</sub> R <sub>1</sub>	+	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	0	0	+	0	+	+	0
5) R <sub>1</sub> R <sub>1</sub>	+	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	0	+	0	+	+	+	MC

MC = mixed cell agglutination

**Table 6.** PEG antibody detection test

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		PEG, 15'				
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	IS	37	IAT	CC
1) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	+	0	NH	2+	NT
2) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	0	+	+	0	+	+	0	NH	2+	NT

NH = no hemolysis NT = not tested

Of the 29 total reagent panel cells tested using gel, two cells were Le(a-b-). Both these RBC samples were nonreactive. There were 13 reactive RBC samples, all of which expressed either Le<sup>a</sup> or Le<sup>b</sup>. All but two of the reactive RBC samples demonstrated mixed cell reactivity, which is consistent with IgM antibodies.

Although the cold panel demonstrated agglutination with all RBC samples tested at the latter phases of 18°C and 4°C, consistent with a cold autoantibody, the room temperature agglutination observed on the antibody detection RBCs, A<sub>1</sub> RBCs, and A<sub>2</sub> RBCs suggests the presence of antibody or antibodies that are alloimmune in nature. The manufacturer listed the antibody detection RBCs as having expressed either Le<sup>a</sup> or Le<sup>b</sup>. The A<sub>1</sub> and A<sub>2</sub> RBCs were manufactured from a pool of donors, known only to be negative for D, C, and E.<sup>2</sup> All other non-ABO blood group antigens, including Le<sup>a</sup> and Le<sup>b</sup>, could be expressed on these reagent RBCs. Lewis system antigens are poorly developed at birth, explaining the nonreactivity of the cord blood RBCs.<sup>3</sup>

Both antibody detection RBC samples agglutinated (2+) at the IAT phase of a PEG-IAT. Some IgM antibodies are known to react at PEG-IAT, including those in the Lewis system.<sup>1</sup> The decision was made to test a panel of

**Table 7.** Cold panel antibody detection test

Phase:	Short Cold Panel			
	IS	RT	18°C	4°C
SC I	0	2+	3+	3+
SC II	0	2+	3+	3+
Auto	0	0	W+	3+
0 Cord Cells	0	0	2+	3+
A <sub>1</sub> Cells	0	2+	3+	3+
A <sub>2</sub> Cells	0	2+	2+	3+

RBC samples using a PEG additive under the belief that the 2+ agglutination observed with the antibody detection RBC samples indicated that consistent reactivity on Le(a+) and Le(b+) RBCs would be attainable. The RBCs selected for the PEG panel consisted of three Le(a+), three Le(b+), and one with double-dose expression of all other commonly encountered antigens. A double-dose RBC for K (K+k) was unavailable so a single-dose (K+k+) RBC sample was substituted. Although all other antibodies had already been excluded using gel, the Le(a-b-) RBC samples were tested as a negative control to exclude any other antibodies that may only react by PEG-IAT. The negative reactions on these RBCs ensure the reactivity observed on all of the Le(a+) and Le(b+) RBCs was attributable to anti-Le<sup>a</sup> and -Le<sup>b</sup>.

Had the PEG testing been nonreactive at all phases, the next logical step would have been to test the same selected cell panel using a 15-minute room temperature incubation. The results of the cold panel suggest reactivity with only the Le(a+) and Le(b+) RBCs would have been observed.

In the absence of sufficient Le(a-b-) reagent RBCs, the presence of Lewis antibodies can be confirmed by incubating the patient's plasma with commercially available Lewis substance before testing against Le(a+) or Le(b+) panel RBCs. Lewis substance contains the Le<sup>a</sup> and Le<sup>b</sup> antigens, which neutralize the antibodies in the patient's plasma. After neutralization, reactivity with Le(a+) or Le(b+) panel RBCs would not be expected. However, validation of testing Lewis-neutralized plasma using the gel method has not been documented.

Antibodies to Lewis antigens are common in pregnant patients. Drastic increases in lipoprotein levels

**Table 8.** PEG test on selected cell panel

	Rh							MNS				Lu		P	Lewis		Kell				Duffy		Kidd		PEG, 15'				
	D	C	C <sup>w</sup>	E	c	e	f	M	N	S	s	Lu <sup>a</sup>	Lu <sup>b</sup>	P <sub>1</sub>	Le <sup>a</sup>	Le <sup>b</sup>	K	k	Kp <sup>a</sup>	Js <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	IS	37	IAT	CC
1) rr	0	0	0	0	+	+	+	+	0	+	0	0	+	0	0	0	0	+	0	0	0	+	+	+	+	0	NH	0	✓
2) rr	+	+	0	0	+	+	+	0	+	0	+	0	+	+	0	0	0	+	0	0	0	0	+	0	0	0	NH	0	✓
3) R <sub>1</sub> R <sub>1</sub>	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	0	+	+	+	+	+	0	NH	0	✓
4) R <sub>2</sub> R <sub>2</sub>	+	0	0	+	+	0	0	+	0	+	+	0	+	0	0	0	0	+	0	0	+	0	+	+	+	0	NH	0	✓
5) r'r	0	+	0	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	NH	2+	NT
6) r''r	0	0	0	+	+	+	+	+	0	+	0	0	+	+	0	0	0	+	0	0	0	+	0	+	0	0	NH	0	✓
7) rr	0	0	0	0	+	+	+	+	+	0	+	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	NH	2+	NT
8) rr	0	0	0	0	+	+	+	+	0	+	+	0	+	+	0	+	0	+	0	0	+	0	0	+	0	0	NH	1+	NT
9) rr	0	0	0	0	+	+	+	+	0	+	+	0	+	+	0	+	0	+	0	0	0	0	+	+	0	0	NH	1+	NT
10) rr	0	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	0	NH	2+	NT
11) R <sub>0</sub> r	+	0	0	0	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	0	0	0	+	0	+	0	NH	1+	NT
12) Patient																										0	NH	0	✓

NH = no hemolysis NT = not tested

can cause a patient that normally expresses Lewis on her RBCs to lose her antigen expression, allowing for the formation of naturally occurring antibodies to the Le<sup>a</sup> and Le<sup>b</sup> determinants.<sup>4</sup> In addition, approximately 22 percent of African Americans are genetically Le(a-b-).<sup>5</sup> The patient's admitting diagnosis and ethnicity support antibodies to the Lewis system.

Because anti-Le<sup>a</sup> and -Le<sup>b</sup> are usually clinically insignificant antibodies,<sup>5</sup> RBCs that are crossmatch-compatible in a 37°C test (i.e., gel or PEG-IAT) are suitable for transfusion. Typing units of RBCs for either Le<sup>a</sup> or Le<sup>b</sup> is not necessary.<sup>3</sup>

**Closing Comments**

Many different factors can aid in the identification of unexpected antibodies. This patient's diagnosis, ethnicity, and unusual reactivity with the initial antibody identification panel were highly suggestive of anti-Le<sup>a</sup> and -Le<sup>b</sup>. Each antibody identification method has its own nuances. Knowing what unexpected results can indicate and correlating them to the patient's history and clinical situation can reduce unnecessary testing and lead to more efficient problem resolution.

**References**

1. Harmening DM. Modern blood banking and transfusion practices. 5th ed. Philadelphia, PA: F.A. Davis, 2005:153-5,300.
2. Immucor, Inc. Referencells package insert. Revised 02/2006.
3. Brecher ME, ed. Technical manual. 15th ed. Bethesda, MD: American Association of Blood Banks, 2005: 306.
4. Issitt PD and Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:261.
5. Reid ME, Lomas-Francis C. The blood group antigen factsbook. 2nd ed. San Diego, CA: Academic Press, 2004:212-4.

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# 2008 Immunohematology Reference Laboratory Conference

## Summary of presentations

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

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

Following are summaries of the presentations given at the 2008 IRL conference that was hosted by the ARC from April 11 to 13 in Scottsdale, Arizona.

### Leadership

Many individuals believe that to lead you must be at the very top of an organization, but nothing could be further from the truth. In successful organizations, much of the leadership comes from below the top; in fact, most comes from the middle of the organization. Each of us can be a good leader, but we must work to obtain the skills that good leaders possess. Leadership

is all about influence, getting people to follow you not because of your position but because of who you are and what you do. John Maxwell, a renowned author on leadership principles, states "The true measure of leadership is influence, nothing more, nothing less." Influence is defined as a power affecting a person, thing, or course of events, especially one that operates without any direct or apparent effort. Influence is not something that is tangible, but the ability to influence is something you have to earn and you can learn how to do it.

One can have influence by position, but the leadership that results from this is very limited. People follow you because they have to follow you, not necessarily because they want to follow you. Leadership by position doesn't get one very far; it is limited by job description, and although you do have influence, that influence is limited. The ideal state is to be able to lead individuals because they want you to lead them; they allow you to influence them. They do this because they respect you or admire you and know you care about them as people. They see you as a person who gets things done; they like what you are doing. They want to be on your team!

To lead, one must prepare. A favorite quote comes from John Wooden. "When opportunity comes, it is too late to prepare." How true this is! We must be ready to lead whenever and wherever the opportunity presents itself. So how do we do that? First of all, as John Maxwell discusses in his book *The 360° Leader*; we must learn to manage ourselves. Sometimes this is one of the most challenging aspects of being a leader. A good leader knows when to show emotions and when to hold them back. At times holding back one's feelings can be best for others; at other times a little show of emotion from you is just what is needed by your team. Knowing when and where to let your emotions out is important. For example, you may be angry during a

meeting because you are not getting the cooperation you need from some of those at the table. Letting out that anger inappropriately (i.e., pounding your fist on the table or throwing something) will only make things worse and lessen you in the eyes of those you lead. Handling the situation in a calm, rational, and professional manner will elevate you with others. They want to be like you; you have more influence over them.

Leaders must also prioritize. All of us have many things on our plate, and if we try to do everything, we become ineffective, especially as leaders. Things slip through the cracks and we constantly play catch up. Learning to say “no” is difficult to do, but the good leader is able to do this to benefit the organization as a whole. A good leader delegates and allows others to do the work, providing guidance as needed without micro-managing.

Another area of leadership that requires self-management is thinking. It is true that if you think bad thoughts, bad things can happen. If you think someone is less than a “10,” you may not give them a chance to show you they are a “10.” If you think you can’t succeed, you won’t. As a leader, you must also manage what you say. Thinking before you speak is always a good practice, and making sure that what you say has value is another. Have you ever been in a meeting with someone who always has to say something even when they have nothing valuable to say? It is frustrating for everyone. If people are going to spend their time listening to you, what you say has to have value; otherwise, you are wasting their time. Remember sometimes saying nothing at all is best, especially if you have nothing valuable to say.

Lastly, and I believe most importantly, a good leader manages his or her personal life. Making time for family and friends is critical, and doing things to get away from the everyday drudgery of work is as well. Good leaders have hobbies and outside interests; for example, they watch or participate in sports, they read, and they travel (not work travel, of course). Although it isn’t always possible, good leaders also take care of physical needs such as eating well, getting plenty of rest, and exercising. Failing to take care of those physical needs can make one an ineffective leader very quickly.

So what are the characteristics of an effective leader? Leaders are secure and do not feel threatened by others. They have vision and see the big picture; their scope is outside of what is good for them and them alone. They unselfishly look at what is good for those whom they lead: the department, the organization, or

beyond. Real leaders are not chameleons; they do not flip-flop on decisions, saying one thing to one person or group because that is what they want to hear and something different to another person or group because that is what they want to hear. They make decisions and use a good decision-making process. They know that failing to make a decision is a decision and often a bad one.

Leaders delight when those who follow them succeed. They are not jealous that someone else is getting some limelight; they are happy to share the kudos. Leaders see everyone as a “10” and allow them a chance to be that “10” by placing them in their strength zones. Leaders truly care about the individuals they lead. They see them as people with lives. Investing time in getting to know those you lead not only helps you to understand them better but it also builds trust. This trust allows you to gain influence with them, and they are more willing to follow you. A good leader invests power in others, setting reasonable goals and supporting individuals when needed but not totally controlling them. Leaders praise accomplishments publicly and constructively criticize privately behind closed doors. Good leaders are loyal to those who follow them. Additional good leadership practices include the following:

- Being prepared—Leaders do their homework. They understand what the desired results are, how they are trying to achieve those results, and what the issues in achieving those results are.
- Communicating well—Leaders communicate horizontally and vertically. They communicate clearly and directly. They listen well and they inform others appropriately as needed.
- Setting good goals—Leaders set goals that are realistic but they push the envelope, challenging within limits those who follow them. They don’t ask others to do what they would not do.
- Modeling the behavior you want to see in others (i.e., walking the talk)—Leaders are role models and exhibit those characteristics they want to see in those who follow them. They never just give “lip service.”
- Using data and facts to make decisions—Whereas sometimes good leaders go with intuition, whenever possible, they make decisions based on good data and facts.
- Managing conflict instead of avoiding it—Leaders are not afraid of conflict. Although they don’t seek to create it, when it occurs they facilitate its resolution.

As you can see, leadership is much more than management. Management is about projects; leadership is about people. Management is about procedures and rules; leadership is about vision and relationships. The leader looks to the future; the manager maintains and implements what is the current state.

Not everyone is born a leader and not everyone can be at the top of the organization, but everyone can lead. Working on those areas discussed above will prepare you to lead whenever and wherever the opportunity arises.

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### **Decision-Making When All RBCs Tested Are Incompatible**

When all, or most, RBCs react during compatibility testing, there are several causes to be considered, and questions to be answered, depending on whether the RBCs in question are from donor units, reagent RBCs, or both:

1. Autoantibody or alloantibody, or both?
2. Alloantibody to high-prevalence antigen?
3. Mixture of several alloantibodies?
4. Not an antibody to RBC antigens?
5. Is antibody clinically significant?

Items 1, 2, and 3 are the “bread and butter” of IRLs, so will not be discussed in great detail. Questions 4 and 5 are sometimes more difficult for even a sophisticated IRL to answer.

When all tested reagent RBCs react, sometimes it is attributable to antibodies that are reacting with antigens that are not blood group antigens. These antigens may be chemicals (e.g., antibiotics, sugars, EDTA, citrate, hydrocortisone, inosine) in commercial reagents such as RBC suspending media, antisera (e.g., dyes), or potentiators (Paraben, azide, thimerosal). This becomes obvious sometimes when reagents from other companies and donor RBCs do not react. Other targets for non-blood group antigens may be the senescent cell antigen present on older RBCs. An unusual phenomenon has been described in which serum reacts with all RBCs but plasma does not, and the DAT is positive on RBCs from a clot but not an EDTA sample. This condition is associated with ulcerative colitis. It has been suggested that it is caused by antibody to serine proteases (e.g., produced during clotting).

Several approaches have been used to predict the clinical significance of an antibody (e.g., thermal amplitude, specificity, functional cellular assays, and 51Cr RBC survival). A major problem is defining what clinical significance means. Do we want tests to predict whether transfused RBCs will survive normally, or are we satisfied if the antibody does not cause morbidity in the patient? It may be that the first is better for patients with hematologic disease, but the second is acceptable for most other patients.

### *References*

1. Garratty G. In vitro reactions with red blood cells that are not due to blood group antibodies: a review. *Immunohematol* 1998;14:1-11.
2. Arndt PA, Garratty A. retrospective analysis of the value of monocyte monolayer assay results for predicting clinical significance of blood group alloantibodies. *Transfusion* 2004;44:1273-81.
3. Daniels G, Poole J, deSilva M, Callaghan T, MacLennan S, Smith N. The clinical significance of blood group antibodies. *Transfus Med* 2002;12: 287-95.

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### **Compliance to Quality: A Journey**

We are a focused industry, focused on compliance, and maybe sometimes so focused on compliance that we let quality slide. Now, there is a controversial statement. In a world in which the Food and Drug Administration rules and we have the AABB Standards to abide by, how can such a statement be made? Many of us look at compliance and quality as totally interchangeable, but in reality they are not. To explore how we might want to change our focus from one bent on compliance to one bent on quality, let's talk about a journey—a journey from compliance to quality. By the way, for those of you who are gasping for air about now, I am not saying *away* from compliance but rather from compliance focused to quality focused. This doesn't mean you are going to stop being compliant—quite the opposite. In a true quality organization, compliance is a byproduct in all you do.

First of all, let's talk about the differences between compliance and quality. Compliance by definition means bending to the will of others; it is the act of submitting. A total focus on compliance stifles creativity, is reactive, and implies something is done to you. It can,

and often does, create adverse relationships with those who regulate us. Quality, on the other hand, leads to compliance, generates new ideas, is proactive, and promotes good relationships with regulators. Quality is more than just compliance. So what is quality?

Quality is a matter of perception—McDonald's versus Chez Jardin. Which restaurant represents quality depends on the requirements. Do you want a fast-food meal that is consistently the same or a gourmet meal in a quiet, candlelit dining room?

A good definition for quality is *doing the right things right the first time*. If we know what the requirements are, we do what we should do; we are compliant. If we do things right the first time, we are not only effective but we are also efficient. Quality is the result of effectiveness and efficiency. You do what you say you are going to do and you do it right with the least expenditure of resources. A quality organization is by definition also a compliant organization because you are doing the right things right. You also please your customers because you know what their requirements are and you work to meet those requirements each time you do your work.

So, how do we achieve a quality focus? First of all there are some important questions to ask. Does what you are doing make sense? Is it the right thing to do? Is it the right thing to do *now*? Is there an easier way to do it (simpler is better)? Does the fear of making a mistake paralyze you? Does change overwhelm you? What are your customers' requirements? Knowing the answers to these questions and dealing with the difficult ones before you begin the journey toward a quality focus is critical to your success.

There are also some cautions you should remember along the way:

- (1) Don't head down the wrong path by implementing complex systems that hinder you instead of helping you.
- (2) Don't yield to the temptation to tackle everything at once.
- (3) Know your capabilities.
- (4) Be accountable.

Accountability, according to *The OZ Principle*, a book by Roger Connors, Tom Smith, and Craig Hickman, is the process of seeing, owning, solving, and doing. It is a perspective that embraces both current and future efforts rather than reactive and historical expectations.

Considering accountability, you will find some dangerous detours in your journey to quality. Let's take a brief look at these.

#### *Number 1—Ignoring problems or denying that you have problems*

People pretend not to know that there is a problem, remain unaware that the problem affects them, or choose to altogether deny the problem. This just doesn't work. You can't fix problems unless you pay attention to them. This is sort of like sticking your head in the sand. You eventually are going to have to breathe, and the problems won't have gone away.

#### *Number 2—It is not MY job!*

In this case, there is an awareness that something needs to be done to get the results, but there is also a lack of responsibility or desire to involve oneself. Assuming someone else will always pick up the ball is a mistake; things fall through the cracks or, even worse, nothing is done.

#### *Number 3—Pointing fingers*

People deny their own responsibility for poor results and seek to shift the blame to others. This is a total lack of accountability; someone else is always responsible for the state of things. This is ignoring your role in the situation, and usually you do have some role in why things are as they are.

#### *Number 4—Excuses*

Making up excuses doesn't get things fixed and may even lead to solving the wrong problem.

#### *Number 5—Confusion—Tell me what to do*

People cite confusion as an excuse to avoid accountability. If they don't understand the problem or the situation, surely they can't be expected to do anything about it. Or if you tell me what to do, then I can't be held accountable for what happens.

#### *Number 6—Cover your tail*

This happens when people seek "protection" by developing "stories" as to why they couldn't possibly be blamed for something that might go wrong. Individuals can be quite creative in making up these stories; again, they hide the truth and avoid true accountability.

#### *Number 7—Wait and see*

In this dangerous detour, people choose to wait and see whether things will get better. Typically, things only get worse. Solutions get swallowed up in a swamp of inaction. This type of behavior may be motivated by fear of failure, risk aversion, or an unrealistic desire for a better solution.

The road to quality is not an easy road; avoiding those detours can be hard. However, the road to quality is a rewarding one. A key factor is to celebrate successes (especially small ones), catch people doing the right things right, create heroes, and recognize and reward performance. Achieving quality is going beyond compliance, and it allows you to meet your goals and results in a strong organization. In a true quality organization, the compliance is there. Lastly, remember, quality is a journey and one which is truly worth making.

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### **You Have Options . . . Immucor, Inc.**

As the need for accurate, timely, and cost-effective immunohematology diagnostic testing increases dramatically across the world, Immucor stands as the largest and best equipped corporation in North America to provide the solutions needed to meet the needs of the industry. Based in Norcross, Georgia, Immucor believes in exceeding the needs of the customer. This starts by understanding the requirements of the industry through “voice of the customer” research. In 2007, this extensive market research identified five key market needs in our industry. Based on these findings, we concluded that the blood bank customer most desires the following:

1. Full and reliable blood bank automation
2. A broad testing menu
3. A continuous access platform
4. A fast turnaround time
5. Automation and test methods that are easy to use

Immucor is meeting these needs through the delivery of superior products in our Scalable Solutions automated product line, which includes the Capture workstation, the Galileo, and the Galileo Echo.

Immucor’s manual Capture workstation features the P2 dual-plate incubator, the CSW100 plate washer, and the Immuspin centrifuge. Perfect for smaller laboratories or as a backup to large, fully automated laboratories, the Capture workstation provides standardized test results every time. For more than 20 years, thousands of laboratories across the globe have trusted Capture solid-phase technology, with more than 30 million Capture tests performed annually.

Galileo, Immucor’s flagship instrument, has revolutionized transfusion diagnostics with its speed and flexibility. Galileo was the first automated system

designed to meet the workflow of a transfusion diagnostics laboratory, not change it. With a broad test menu, high throughput coupled with a quick turnaround time, reflex testing, and an intuitive user interface, Galileo has become the gold standard in automated platforms.

With high demand for the features of the Galileo in a smaller package, Immucor developed the Galileo Echo—a revolutionary instrument that delivers the benefits of Galileo for smaller laboratories. Echo was designed to perform a record number of assays with the industry’s smallest footprint.

However, meeting the customer’s needs requires not only superior products, but also a superior approach. Anchored by our Blood Bank System Specialists, the Immucor team is dedicated to providing a solution for these key industry needs, or “drivers.” This includes coupling blood bank reagent options and instrument platforms with an implementation process called “Lean.”

Lean is a continuous improvement philosophy that is used to eliminate waste and variation. It is widely used today in the health-care diagnostics industry to improve quality and response times while maintaining or decreasing expenditures. Immucor has invested in our people to make them Lean-certified under the Lean HealthCare Institute banner, with the express purpose to pass on their skills to Immucor customers by installing and integrating all products into the customer’s specific environment appropriately.

This results in a customized solution for each laboratory location. This customized solution may include layout and process enhancements through the Lean methodology. It might also include technology enhancements through Immucor’s networked automation. Immucor, through a partnership with Data Innovations, uses a variety of networking methods, including remote access and wide area networked innovations, to meet the data needs of the customer, ensuring that they have the right information at the right time.

Through dedication and the drive to exceed expectations, Immucor is committed to meet and exceed the needs of the customer.

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### **The Journey to Licensure**

The Diagnostic Manufacturing Division (DMD) of the American Red Cross (ARC) fully manufactures licensed and 510(k) cleared immunohematology

reagents in accordance with current Good Manufacturing Practices and 21CFR, parts 600 and 800. These products are distributed to ARC National Testing Laboratories and Immunohematology Reference Laboratories and various non-ARC organizations. As a constituent of the ARC, the mission of the DMD is to support its customers with the highest quality, cost-effective reagents and ancillary products. To that end, DMD uses a quality system that includes change management, document control, training, supplier evaluation, incoming goods specifications and inspection, in-process controls, contamination controls, environmental monitoring, labeling and packaging controls, final product testing, design control, equipment and process validation, equipment maintenance and calibration, deviation management, corrective and preventive action plans, nonconforming product control, product complaint management, internal audits, and management review.

Established in 1976, DMD has manufactured from four locations. The 1998 move to the current location required DMD to once again obtain licensure to manufacture licensed blood grouping reagents through the Biologics License Application (BLA) process.

A 2001 BLA supplement was withdrawn primarily because of an inability to meet FDA sterility requirements. A different approach using a microbiologically controlled process was developed, and a manufacturing process was designed to ensure the microbial level will not adversely impact product performance. The philosophy is based on two distinct elements and the results of supportive studies:

#### Environmental Monitoring

- Air
- Surfaces
- Water
- Personnel (gowning)

#### Bioburden Reduction

- Product filtration
- Process equipment cleaning
- Heat treatment of product components (containers/closures)
- Product preservative (sodium azide, 0.1%)

#### Supportive Validation Studies

- Bioburden monitoring of in-process material
- Airborne baseline levels
- Sodium azide effectiveness
- Equipment cleaning
- Closure integrity of filtration vessel
- Closure integrity of final product containers

- Media fill—tryptic soy broth substituted for product

Final product contamination testing demonstrates the product meets the DMD microbiologically controlled standard.

A BLA supplement was submitted in 2005 that contained the following required elements:

- Cover letter
- FDA forms (2)
- Introduction—executive summary
- Proposed labeling (all labels and package inserts) and packaging
- Chemistry, manufacturing, and controls (CMC)
  - Extensive description of in vitro substance (active ingredient) and in vitro product
    - Composition—ingredients and formulas
    - Characterization—test method descriptions and applications
    - Method of manufacture—raw materials and acceptance criteria, flow charts
    - Process controls—in-process controls, process validation, bioburden assessment
    - Reference standards
    - Specifications and analytical methods
    - Container closure system—description and validation
    - Stability data
  - Environmental monitoring data
    - Air
    - Water
    - Surfaces
    - People
    - In-process bioburden
  - Batch records—6 conformance lots
  - Validation (process and equipment) summaries
- Establishment description
  - Physical description, floor plans
  - Cross-contamination controls
  - Environmental monitoring
  - HVAC system and validation
  - Water system and validation

The DMD submission totaled 1315 pages that thoroughly explained the microbiologic control “story,” supplied data to support the “story,” and provided details about the product composition, manufacturing process, test methods, and conformance lot production.

DMD was granted licensure in 2006 for anti-Fy<sup>a</sup> and anti-K and in 2007 for anti-S and anti-k. Another BLA supplement will occur in 2008 for anti-M, anti-s, anti-Kp<sup>a</sup> and anti-Kp<sup>b</sup>.

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## Serologic Investigation of Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia can be classified as those anemias associated with (1) antibodies reacting optimally at 37°C (“warm type” AIHA [WAIHA] = 80%), and (2) antibodies reacting optimally below 10°C (cold agglutinin syndrome [CAS] = 18%, and paroxysmal cold hemoglobinuria [PCH] = 2%).

- WAIHA: Usually IgG autoantibody, but can be IgM or IgA. DAT: IgG + C3 (67%), IgG without C3 (20%), C3 without IgG (13%). In vitro lysis of RBCs is uncommon (0.8% untreated, 13% enzyme-treated); 35 percent will have cold agglutinins active at room temperature. Usually extravascular lysis. Most common specificity is Rh-related, but almost all high-frequency antigens have been involved.
- CAS: IgM high titer/thermal amplitude ( $\geq 30^{\circ}\text{C}$ ); cold agglutinin. Cold monophasic lysis usually present. DAT: C3dg only. Extravascular in vivo lysis. Usually anti-I.
- PCH: Rarest type of AIHA (<2%). More common in children (infection associated) than adults. Usually intravascular lysis. DAT: C3dg only. Biphasic (sensitized in cold, hemolysis when moved to 37°C) cold lysis detected by Donath Landsteiner (DL) test. DL antibody has anti-P specificity.
- DAT-Negative AIHA: Approximately 10 percent of WAIHA. Can be caused by RBC-bound IgG below DAT sensitivity threshold, low-affinity autoantibody, IgA or IgM warm antibody. Helpful tests are direct Polybrene test, DAT using anti-IgA/IgM, using ice-cold saline or LISS washes for DAT, or flow cytometric DAT.
- WAIHA Associated With IgM Autoantibodies: Often, severe AIHA, sometimes intravascular lysis. Spontaneous agglutination of patients' RBCs often (78%) occurs; C3dg on RBCs (90%); IgM on RBCs (62%) but not often (30%) detected by DAT.

Sometimes (25%) IgG also present. Serum usually (80%) contains 37°C-reactive agglutinins.

### *Childhood AIHA:*

- Acute transient: Acute transient AIHA 10× more common in children than adults; 82 percent in first 4 years; 68 percent associated with infection; 45 percent have hemoglobinuria (71% of sudden onset); 59 percent have only C3dg on RBCs; 12 percent have positive DL test. Respond well to steroids. Low fatality rate.
- Chronic WAIHA: Often (58%) associated with systemic disorders; only 44 percent in first 4 years; 85 percent have IgG, with or without C3, on RBCs. Variable response to steroids; 12 percent mortality.

### *Reference*

1. Petz LD, Garratty G. Immune hemolytic anemias. 2nd ed. Philadelphia: Churchill Livingstone, 2004.

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*George Garratty, PhD, FRCPath, Scientific Director, American Red Cross Southern California Region, Pomona, CA.*

## Controversies in Testing

Although the practice of blood banking and transfusion medicine may, at times, seem to be driven by regulators, there still remain many opportunities for variation in applying the regulations to actual daily operations. Adaptation of new technologies also affords the possibility of different applications. Controversies over what acceptable or best practices may look like arise. Several of these were more closely examined.

### *Criteria for Exclusion of Alloantibodies*

A critical control point in identification of RBC antibodies is antibody exclusion. This ensures that there are no other unexpected antibodies in the sample that may have been masked by the reactivity of the known antibodies. The process of antibody exclusion requires decisions on what alloantibodies will routinely be investigated and excluded and what criteria will be used for exclusion. The following options exist for exclusion: (1) using RBCs that carry a single dose or presumed double dose of the antigen in question; (2) using only in-date reagent RBCs or allowing the use of expired reagent RBCs; or (3) excluding an antibody specificity on the basis of one nonreactive antigen-positive RBC sample or requiring two or more antigen-positive RBC samples to be nonreactive. Decisions on these options constitute the policy for antibody exclusion in a given facility.

The biggest controversy in antigen exclusion centers on the number of antigen-positive RBC samples that must be nonreactive to exclude the antibody. Use of one RBC sample for exclusion allows antibodies to be excluded more easily with minimal sample. It also can affect the selection of units for transfusion because when an antibody is excluded, donor units negative for that antigen are not required for transfusion. However, reliance on a single RBC sample for exclusion does not allow for any error in testing or unknown variation in antigen expression. A policy that requires nonreactivity with two or more antigen-positive RBC samples to exclude an antibody compensates for these unexpected events. However, this practice can require a greater inventory of test RBCs and will likely use more patient sample.

An e-mail survey of AABB-accredited Immunohematology Reference Laboratories (IRL) and American Red Cross (ARC) IRLs was conducted in mid-2007 to assess policies related to the number of exclusion RBCs required. Of 63 laboratories responding, 42 facilities (67%) used only one nonreactive RBC sample to exclude an alloantibody. Comments from the responding laboratories were "require one example of double-dose RBCs but more than one example if the RBCs express a single dose of the antigen," or "one double dose required except for anti-K and anti-C or anti-E in presence of anti-D." Several laboratories required more than one antigen-negative RBC sample for clinically significant alloantibodies but only one example for antibodies not generally clinically significant.

Considering the pros and cons of various exclusion issues, one protocol that might be used would be as follows:

- For Rh, Fy, Jk, Ss antibodies
  - At least two antigen-positive RBC samples are nonreactive
  - At least one example of the RBCs has a double dose expression of the antigen except when excluding anti-C or anti-E in the presence of anti-D or excluding anti-K
  - If the exclusion RBC sample does not have a double dose of the antigen, the test method used must be PEG, gel, solid-phase, or enzyme (if appropriate)
  - If expired RBCs must be used, they must be double dose
- For MN, Lewis, P1 antibodies
  - One RBC sample for exclusion
  - Double dose, if appropriate

### *Reactivity in Various Test Methods*

Many test methods are available for use in blood bank testing today: tube techniques (LISS or PEG) and non-tube techniques (automated and manual gel or automated and manual solid phase). In general, comparative studies have shown that non-tube methods are more sensitive than tube methods in detecting clinically significant alloantibodies. However, non-tube methods can also be less specific than tube methods. That is, when reactivity is detected in a tube method, it is more likely to be an alloantibody.

Most participants in the ARC IRL Conference represented laboratories receiving the majority of their samples from other blood bank laboratories. These referral laboratories primarily used tube techniques for routine testing; some could also perform gel testing. A very few could perform solid-phase tests. The hospital laboratories represented reported using both tube and gel methods. Virtually all hospital laboratories used the same method for initial antibody identification as antibody detection, although a second method would be used when the primary method does not indicate clear specificity.

Various scenarios were posed giving options for testing when nonspecific reactivity or panagglutination was observed in initial non-tube identification tests. Conference participants often indicated that tube test methods (LISS or PEG) would be used when an apparent autoantibody was detected in non-tube tests. Sporadic reactivity in non-tube tests would most often be approached by performing antibody exclusions in the non-tube test followed by selection of donor units based on a compatible crossmatch. When initial non-tube tests that react with many cells on a panel are encountered, some of the laboratories will revert to non-tube methods to look for specificity.

When designing antibody identification protocols combining tube and non-tube tests, it must be remembered that no single protocol is correct. The selected protocol must balance appropriate investigation of reactivity with timely provision of test results and blood products. Change in the reactivity in a patient's sample after transfusion may be an indicator for more rigorous testing by non-tube methods.

### *Provision of Genotype-Matched Units for WAA Patients*

The availability of large scale, batch genotyping for patient and donor samples has opened new possibilities for selection of units for transfusion. Patients with warm autoantibody in the serum can require complex

adsorption studies to exclude alloantibodies. It has been suggested that genotyping for common RBC antigens could eliminate the need for evaluation of the sample using adsorptions if genotypically matched donor units were selected for transfusion.

Several concerns of both serologic and logistic nature have been raised for this type of protocol. Although readily available in the commercial setting, genotype testing must be performed in laboratories having personnel with the appropriate skill set for accurate performance, interpretation, and application of the test. A patient's genotype may not represent the RBC phenotype. Because of unrecognized mutations, a patient may appear to be antigen positive but still be capable of making the alloantibody. Logistically, the genotyping assays are not licensed for labeling of donor units with phenotype information. RBC phenotypes must be confirmed serologically. The impact on blood suppliers must also be considered. Large-scale donor phenotyping is not routinely available in donor centers. If a hospital transfusion service implements a protocol of genotype matching, the donor center must identify the matched units through labor intensive, manually performed serologic antigen typing. The blood centers must be prepared to allocate financial and personnel resources for this approach.

The protocol may first be used in patient populations such as patients with sickle cell anemia in whom genetic variation in RBC antigens is more commonly encountered and the benefits of phenotypically matched units have been demonstrated. Routine use in warm autoantibody patient populations for the purpose of streamlining pretransfusion testing may not be justified until genotyping in donor centers is readily available.

Controversies in testing patterns and protocols will routinely present themselves as new technologies and techniques are developed. Critical evaluation of the information and analysis of the impact on both patient care and provision of blood products for transfusion will result in wise choices that meet the needs of patients and transfusion medicine providers.

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## **Blood Doping in Athletes—Detection by Flow Cytometry**

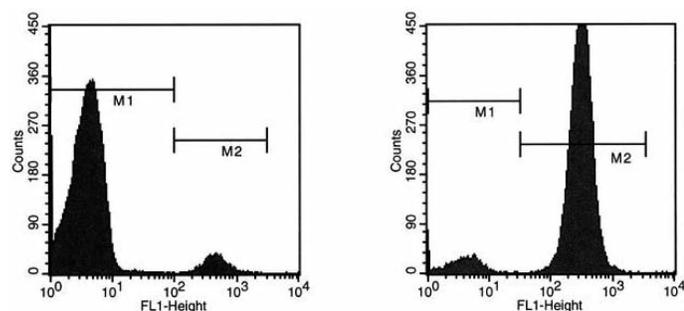
In sports, the term “doping” refers to the use of performance-enhancing substances or methods. Anti-

doping rules have been adopted not only for the health and safety of the athlete but also to “protect the ethics underlying sports.”<sup>1</sup> Blood doping refers to the use of methods to increase circulating hemoglobin levels. The resulting increase in the oxygen concentration of arterial blood can be an advantage for athletes both in competition and in training. Three methods athletes can use for this are (1) autologous or allogeneic blood transfusion, (2) pharmacologic products (e.g., recombinant human erythropoietin, rHuEPO) that stimulate overproduction of RBCs in the bone marrow, and (3) blood substitutes. Transfusion has been rumored to be used by athletes since the 1960s. There are anecdotal reports of its decline when rHuEPO was introduced in the late 1980s, but purportedly implementation of a test to detect rHuEPO in 2000 has driven athletes back to transfusion. Flow cytometry was first used for the detection of allogeneic transfusion in athletes at the 2004 Summer Olympics in Athens (there are currently no methods to detect autologous transfusion).

Flow cytometry is ideally suited for the detection and quantitation of mixed cell populations because each cell is analyzed individually. Subpopulations of RBCs can be detected based on their antigenic differences (via fluorochrome-labeled antibodies), and quantitative results can be obtained. Flow cytometry has been successfully used since the 1980s for the detection and quantitation of minor RBC populations in the following situations: fetal-maternal hemorrhage, survival studies in transfused patients, individuals with blood group mosaicism, and hematopoietic chimerism in twins and bone marrow/stem cell transplant patients. Recent publications have addressed the use of flow cytometry in the setting of blood doping.<sup>2-6</sup>

### *Technical Considerations*

Flow cytometry is widely used for WBC analyses and less commonly for RBC analyses. Thus, most flow cytometry operators are not familiar with issues associated with testing RBCs (e.g., problems associated with agglutination); some methods that pertain to WBCs (e.g., incubations at 4°C to prevent capping) do not apply to RBCs. Agglutination is the biggest problem facing those analyzing RBCs by flow cytometry. The best flow cytometric test would involve incubation of test RBCs with a nonagglutinating, strongly fluorescent IgG blood group antibody. Unfortunately, these types of antibodies are not readily available. The usual testing protocol involves incubating test RBCs with a commercial blood group antibody (e.g., anti-c, -K, -Fy<sup>a</sup>, etc.) and then a fluorochrome-labeled secondary



**Fig. 1.** Mixed RBC populations. Fluorescence histograms showing results of 5 percent s+ in s- RBCs (left histogram) and 5 percent s- in s+ RBCs (right histogram) after incubation with polyclonal anti-s and then a fluorochrome-labeled Fab anti-human IgG. Markers are set electronically around the two populations (M1 = antigen-negative events, M2 = antigen-positive events) and quantitative results are obtained.

antibody, e.g., FITC anti-IgG. Agglutination can occur due to the primary or the secondary antibody and can affect the results (the flow cytometer will count both a single RBC and an agglutinated group of RBCs as one event each). Agglutination can be avoided by using nonagglutinating primary antibodies (if available) and Fab fragments of fluorochrome-labeled secondary antibodies. In some cases, chemical fixation of RBCs may be needed to minimize agglutination. Primary and secondary antisera used for this testing will need to be standardized for use with RBCs by flow cytometry.

The RBC antigens that will be most helpful in detection of transfused RBCs in athletes are those of moderate frequency (A, B, D, C, c, E, M, N, S, s, K, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>). To be able to clearly distinguish antigen-positive RBCs from antigen-negative RBCs (Fig. 1), it is important to use strong antibodies. Other factors that affect the ability to discriminate antigen-positive from antigen-negative RBCs include background fluorescence, the number of antigen sites, and the method used to sensitize or label the RBCs. The sensitivity for detection of an antigen-positive minor RBC population is less than 1 percent, but detection of an antigen-negative minor RBC population is more difficult (because of the presence of nonfluorescent background events).

A false-positive result would involve the detection of a second population of RBCs in an untransfused athlete. This could be attributable to poor laboratory techniques (wrong sample tested or contaminated sample) or the presence of WBCs in the sample. To have confidence in the results, mixed populations for at least two blood group antigens should be detected and the percentages of the minor populations should be similar. Antidoping test laboratories require repeat testing on a

stored aliquot if the initial results are positive. Subsequent blood samples should be obtained from an athlete to demonstrate a decrease in the minor population over time. The athlete may claim that the second population is the result of chimerism rather than transfusion, but chimerism is unusual and could be detected by molecular testing.

A false-negative result would involve not detecting a second population of RBCs in a transfused athlete. It would be unlikely that there would be no antigen mismatches between the donor and recipient (unless they were identical twins), but a difference in antigens may not be detected if too few antibodies (or the wrong antibodies) are tested. Poor techniques (insufficient washing after incubation with antisera, presence of agglutination, or suboptimal instrument set-up), insufficient labeling of antigen-positive RBCs, or poor quality of the blood sample could all lead to false negative results.

In conclusion, flow cytometry is a good method to detect allogeneic transfusion. It is important that the testing laboratory be familiar with testing RBCs by routine serology as well as by flow cytometry.

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#### References

1. Pound RW. Inside dope: how drugs are the biggest threat to sports, why you should care, and what can be done about them. Ontario, Canada: Wiley, 2006.
2. Nelson M, Ashenden M, Langshaw M, Popp H. Detection of homologous blood transfusion by flow cytometry: a deterrent against blood doping. *Haematologica* 2002;87:881-2 (letter).
3. Nelson M, Popp H, Sharpe K, Ashenden M. Proof of homologous blood transfusion through quantification of blood group antigens. *Haematologica* 2003;88:1284-95.
4. Nelson M, Cooper S, Nakhla S, Smith S, King M, Ashenden M. Validation of a test designed to detect blood-doping of elite athletes by homologous transfusion. *Aust J Med Sci* 2004;25:27-33.
5. Voss SC, Thevis M, Schinkothe T, Schänzer W. Detection of homologous blood transfusion. *Int J Sports Med* 2007;28:633-7.
6. Arndt PA, Kumpel BM. Blood doping in athletes—detection of allogeneic blood transfusion by flow cytometry. *Am J Hematol* 2008 (in press).

***Blood Group Antigens & Antibodies: A Guide to Clinical Relevance & Technical Tips***

M.E. Reid and C. Lomas-Francis. Long Island City, NY: Star Bright Books, Inc. 2007. 211 pp.

Marion Reid and Christine Lomas-Francis published the second edition of *The Blood Group Antigen FactsBook* in 2004. Transfusion medicine specialists thought it was THE reference book for facts and information about blood group antigens. Although this is still true today, it is somewhat difficult carrying a 561-page book on rounds or between laboratories. Reid and Lomas-Francis recently published the pocketbook sized *Blood Group Antigens & Antibodies: A Guide to Clinical Relevance & Technical Tips* with helpful variations in formats.

Individual antigens are listed in alphabetical order, but are also color-coded (blue, green, and red representing polymorphic and low- and high-prevalence antigens). Information is easier to locate as antigens are arranged in color-coded tabs at the edge of each page. A consistent format is used to provide the following information on each antigen: clinical significance, number of antigen-negative donors per 100, in vitro

characteristics of the alloantibody, technical tips, and comments. Several informative tables, such as low-prevalence antigens present in ethnic populations and the effects of enzymes and chemicals on antigens, are provided.

I do miss the molecular diagrams and references from the earlier edition. However, including them would add pages, and the pocket-size book would no longer be easy to carry. I am thus looking forward to Reid and Lomas-Francis's electronic version of *Blood Group Antigens & Antibodies* in the not-too-distant future.

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***Methods in Molecular Medicine, Volume 134, Bone Marrow and Stem Cell Transplantation***

M. Beksac, ed. Totowa, NJ: Humana Press, 2007. 234 pp.

The basic biology of stem cell activation and perpetuation is by no means a mature field, still more mysterious than resolved. However, there is a tendency toward rapid transfer of method from the bench to the clinic. Thus, given the rich advances made in both stem cell transplantation (SCT) and molecular medicine during the last decade, a textbook devoted to relevant laboratory methods is a welcome necessity.

This volume, one of an extensive series devoted to such applications in various medical specialties, begins with a brief overview of the topics covered and their clinical relevance. The first chapter devotes itself to evaluation of stem cells from the standpoint of quiescence versus activation, and self-renewal versus differentiation. A means of characterizing the distinct gene expression signatures of murine stem cells in different states is outlined, with detailed discussion of quality control and bioinformatics strategies for analysis. Chapter 2 describes in vivo imaging methods, which

reveal the fate of labeled, transplanted stem cells in mice and provide insight into stem cell trafficking. The eventual development of translational approaches seems certain, and the clinical utility of such methods is obvious.

Chapters 3 through 6 focus on HLA. Chapter 3 gives a nice overview of available molecular typing methods and utilization strategies in the context of SCT. Chapter 4 focuses on sequence-specific primed PCR in the typing of HLA Class I and II, providing a rationale for its preferential use in unrelated transplantation (compared with sequence-specific oligonucleotide-primed PCR [SSO-PCR]) and a comprehensive description of the steps involved. Chapter 5 complements this with a similar description of SSO-PCR and its rendering on the Luminex, one of two available commercial systems. Chapter 6 discusses yet a third typing method and its role in the repertoire: sequencing-based typing, with its unique ability to define HLA type unambiguously at the allelic level.

Chapters 7 through 10 examine molecular typing for non-MHC loci. Chapter 7 discusses the major impact minor histocompatibility antigens can have on outcome in HLA-matched SCT, lists available information about these antigens and primary references, and summarizes the typing methods. Chapter 8 delves into non-HLA gene polymorphisms involving immune regulatory molecules, such as cytokines, steroid receptors, and response mediators, and their effect on SCT outcome. The author contrasts the simpler genomic variations found in such polymorphisms with those involving HLA and addresses study design and data interpretation issues as well as relevant methods. The authors of Chapter 9 report an association between single nucleotide polymorphisms in the intracytoplasmic epithelial receptor, NOD2/CARD15, and severe GVHD and mortality in HLA-identical SCT. They include discussion of their findings and a detailed methods section. Chapter 10 reviews the role of natural killer cells and killer immunoglobulin-like receptors (KIR) in posttransplant immunologic dynamics, the structure of the relevant genes, and a fully elaborated PCR strategy for typing KIR polymorphisms.

Chapter 11 returns to the theme of stem cell trafficking, now in the context of histologic evaluation of human tissue, and presents methods for identifying marrow-derived nonhematopoietic cells by double labeling with immunohistochemistry and in situ hybridization. The applications, utility, and limitations of the approach are also discussed.

Chapters 12 and 13 tackle minimal residual disease (MRD), a key area of interest for clinicians. In Chapter 12 the author covers relevant chromosomal aberrations and those methods applicable to autologous and

allogeneic SCT for chronic and acute myeloid disorders. These methods include real-time PCR (RT-PCR), and multiplex RT-PCR. Chapter 13 switches to lymphoid disorders, and the use of qualitative and quantitative PCR and quantitative RT-PCR in documenting MRD and monitoring tumor burden.

Chapter 14 shifts to the related area of molecular surveillance of hematopoietic chimerism. The author provides a thorough technical and theoretical description of the use of lineage-specific chimerism analysis in the detection of impending graft rejection and relapse. He compares this method favorably from the standpoint of both sensitivity and specificity with the common approach of microsatellite analysis by PCR.

In the book's final chapter, the authors leave genetic analysis altogether, reporting on the utility of proteomic screening as a means of assessing patients for complications after allogeneic SCT. The specific method described is capillary electrophoresis coupled on-line to an electrospray-ionization-time-of-flight-mass spectrometer for the analysis of biomarkers in human urine.

In summary, *Methods in Molecular Medicine, Bone Marrow and Stem Cell Transplantation* offers detailed instructions for a range of sophisticated methods and couches them in brief reviews of both the reported experience and suggestions for application. It is recommended as a resource for those involved in SCT as either clinicians or investigators.

David Moolten, MD  
Medical Director

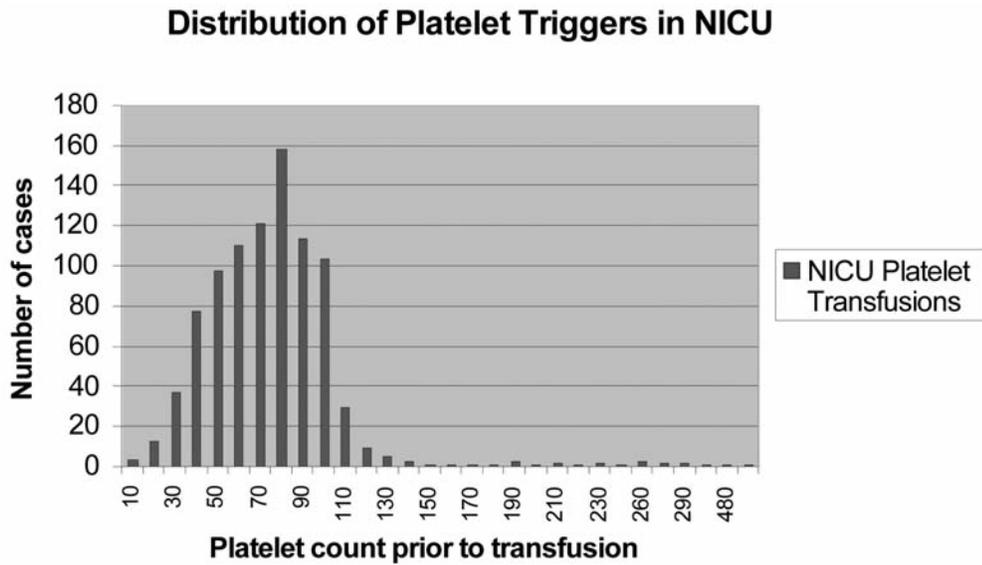
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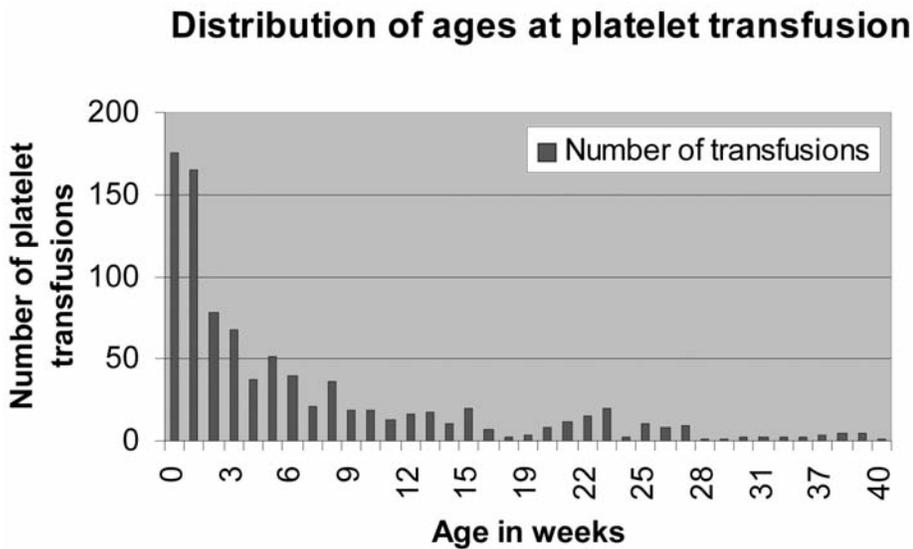
Vol. 24, No. 1, 2008; page 18

**Neonatal and infant platelet transfusions**

The author has informed the editors of *Immunohematology* that there are errors on page 18, Fig. 1 and Fig. 2. The x-axis labels for each table should read thus:



**Fig. 1.** Frequency distribution of 909 pretransfusion platelet counts in infants who received platelet transfusions while admitted to The Children’s Hospital of Philadelphia NICU between July 1, 2006, and June 30, 2007.



**Fig. 2.** Frequency distribution of patient ages at the time of platelet transfusion (N = 909) in infants who received platelet transfusions while admitted to The Children’s Hospital of Philadelphia NICU between July 1, 2006, and June 30, 2007.

**Meeting!**

**September 18 National Institutes of Health, Department of Transfusion Medicine, Symposium**

The National Institutes of Health, Department of Transfusion Medicine, 27th Annual Symposium: Immunohematology and Blood Transfusion, co-hosted by the Greater Chesapeake and Potomac Region of the American Red Cross, will be held on September 18, 2008. The symposium is free of charge; advanced registration is encouraged. **Contact** Karen Byrne at NIH/CC/DTM, Bldg.10/Rm 1C711, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184 or: KByrne@mail.cc.nih.gov or visit our Web site: [www.cc.nih.gov/dtm/education](http://www.cc.nih.gov/dtm/education)

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For additional information, contact: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021 / e-mail: ghalverson@nybloodcenter.org (phone 212-570-3026, FAX: 212-737-4935) or visit the Web site at <http://www.nybloodcenter.org> >research >immunochemistry >current list of monoclonal antibodies available.

**Specialist in Blood Bank (SBB) Program**

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## **Blood Group Antigens & Antibodies**

### **A guide to clinical relevance & technical tips**

BY

MARION E. REID AND CHRISTINE LOMAS-FRANCIS

The authors are using royalties generated from the sale of this pocketbook for educational purposes to mentor people in the joys of immunohematology as a career. They will accomplish this in the following ways:

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#### ***About the book***

This compact “pocketbook” from the authors of the *Blood Group Antigen FactsBook* is a **must** for anyone who is involved in the laboratory or bedside care of patients with blood group alloantibodies.

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Test methods:

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

For information, e-mail: [immuno@usa.redcross.org](mailto:immuno@usa.redcross.org)  
or call:

Maryann Keashen-Schnell  
(215) 451-4041 office  
(215) 451-4205 laboratory

Sandra Nance  
(215) 451-4362

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

Indications for granulocyte serology testing include:

- Alloimmune neonatal neutropenia (ANN)
- Autoimmune neutropenia (AIN)
- Transfusion related acute lung injury (TRALI)

Methodologies employed:

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

TRALI investigations also include:

- HLA (PRA) Class I and Class II antibody detection

For further information contact:

Neutrophil Serology Laboratory  
(651) 291-6797

Randy Schuller  
(651) 291-6758  
schullerr@usa.redcross.org

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# Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

## INSTRUCTIONS FOR AUTHORS

### I. GENERAL INSTRUCTIONS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Double-space throughout the manuscript. Number the pages consecutively in the upper right-hand corner, beginning with the title page.

### II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW

**A. Each component of the manuscript must start on a new page in the following order:**

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

### B. Preparation of manuscript

1. Title page
  - a. Full title of manuscript with only first letter of first word capitalized (bold title)
  - b. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
  - c. Running title of ≤40 characters, including spaces
  - d. Three to ten key words
2. Abstract
  - a. One paragraph, no longer than 300 words
  - b. Purpose, methods, findings, and conclusion of study
3. Key words
  - a. List under abstract
4. Text (serial pages): *Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections*
  - a. Introduction  
Purpose and rationale for study, including pertinent background references
  - b. Case Report (if indicated by study)  
Clinical and/or hematologic data and background serology/molecular
  - c. Materials and Methods  
Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient's names or hospital numbers.
  - d. Results  
Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
  - e. Discussion  
Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
5. Acknowledgments: *Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.*
6. References
  - a. In text, use superscript, Arabic numbers.
  - b. Number references consecutively in the order they occur in the text.
7. Tables
  - a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of . . .) use no punctuation at the end of the title.

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

### 8. Figures

- a. Figures can be submitted either by e-mail or as photographs (5" × 7" glossy).
- b. Place caption for a figure on a separate page (e.g. Fig. 1 Results of. . .), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
- c. When plotting points on a figure, use the following symbols if possible:  
○ ● △ ▲ □ ■.

### 9. Author information

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

### III. EDUCATIONAL FORUM

**A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:**

1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
2. Annotated conference proceedings

### B. Preparation of manuscript

1. Title page
  - a. Capitalize first word of title.
  - b. Initials and last name of each author (no degrees; all CAPS)
2. Text
  - a. Case should be written as progressive disclosure and may include the following headings, as appropriate
    - i. Clinical Case Presentation: *Clinical information and differential diagnosis*
    - ii. Immunohematologic Evaluation and Results: *Serology and molecular testing*
    - iii. Interpretation: *Include interpretation of laboratory results, correlating with clinical findings*
    - iv. Recommended Therapy: *Include both transfusion and nontransfusion-based therapies*
    - v. Discussion: *Brief review of literature with unique features of this case*
    - vi. Reference: *Limited to those directly pertinent*
    - vii. Author information (see II.B.9.)
    - viii. Tables (see II.B.7.)

### IV. LETTER TO THE EDITOR

#### A. Preparation

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

Send all manuscripts by e-mail to [immuno@usa.redcross.org](mailto:immuno@usa.redcross.org)

## Becoming a Specialist in Blood Banking (SBB)

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

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

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

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

### 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	Phone Contact	Email Contact	Website	On site or On line Program
Walter Reed Army Medical Center	William Turcan	202-782-6210	William.Turcan@NA.AMEDD.ARMY.MIL	www.militaryblood.dod.mil	On site
American Red Cross, Southern CA Region	Michael Coover	909-859-7496	CooverM@usa.redcross.org	none	On site
ARC-Central OH Region	Joanne Kosanke	614-253-2740 x2270	kosankej@usa.redcross.org	none	On site
Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403	Lynne.Lemense@bcw.edu	www.bcw.edu	On site
Community Blood Center/CTS Dayton, OH	Nancy Lang	937-461-3293	nlang@cbccts.org	http://www.bcbcts.org/education/sbb.htm	On line
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201	cwong@giveblood.org	www.giveblood.org/education/distance/htm	On line
Hoxworth Blood Center, Univ. of Cincinnati	Susan Wilkinson	513-558-1275	susan.wilkinson@uc.edu	www.hoxworth.org	On site
Indiana Blood Center	Jayanna Slayten	317-916-5186	jslayten@indianablood.org	www.indianablood.org	On line
Johns Hopkins Hospital	Jan Light	410-955-6580	jlight5@jhmi.edu	http://pathology2.jhu/departments/divisions/transfusion/sbb.cfm	On site
Medical Center of Louisiana	Karen Kirkley	504-903-3954	kkirkk@lsuhsc.edu	none	On site
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335	kbyrne@mail.cc.nih.gov	www.cc.nih.gov/dtm	On site
Rush University	Veronica Lewis	312-942-2402	Veronica_Lewis@rush.edu	www.rushu.rush.edu/health/dept.html	On line
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 x 1514	mdoty@fbsblood.org	www.fbsblood.org	On line
University of Texas Health Science Center at San Antonio	Linda Myers	210-731-5526	lmyers@bloodntissue.org	www.uthscsa.edu	On site
University of Texas Medical Branch at Galveston	Janet Vincent	409-772-3055	jvincent@utmb.edu	www.utmb.edu/sbb	On line
University of Texas SW Medical Center	Barbara Laird-Fryer	214-648-1785	barbara.fryer@UTSouthwestern.edu	http://telecampus.utsystem.edu	On line

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

Revised August 2007

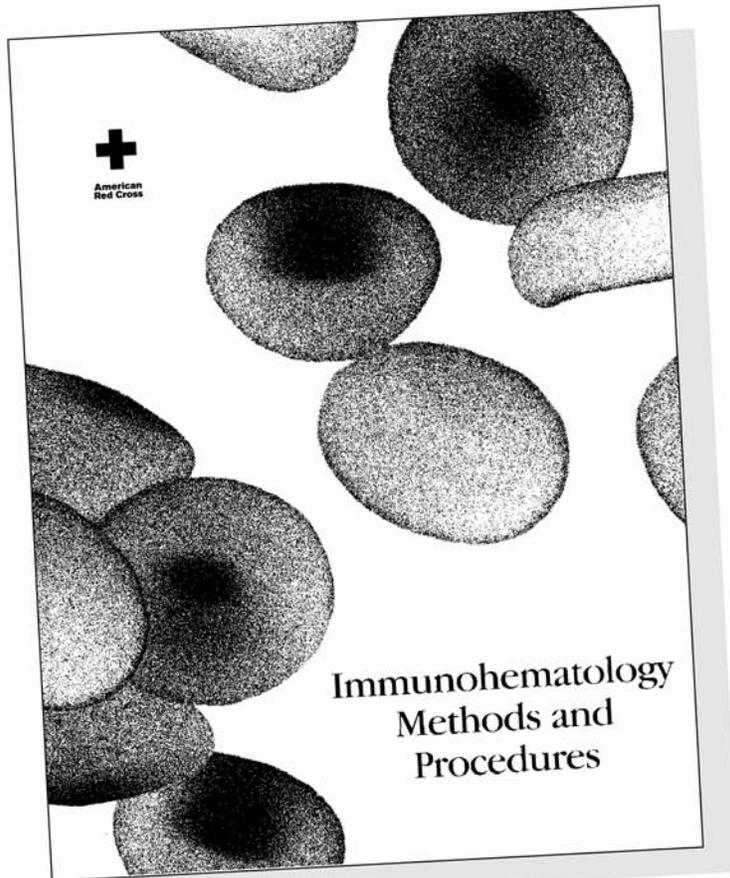
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