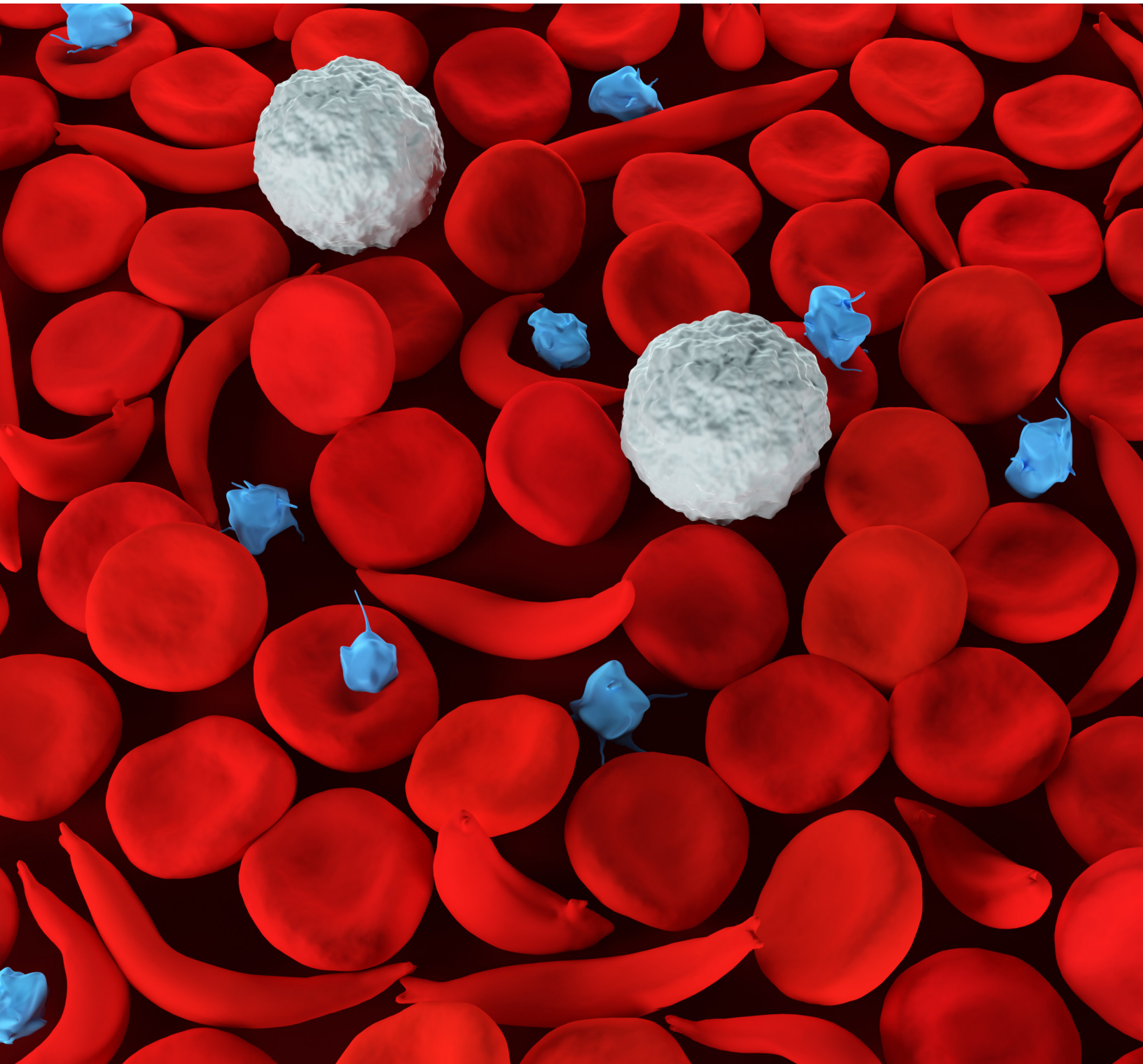




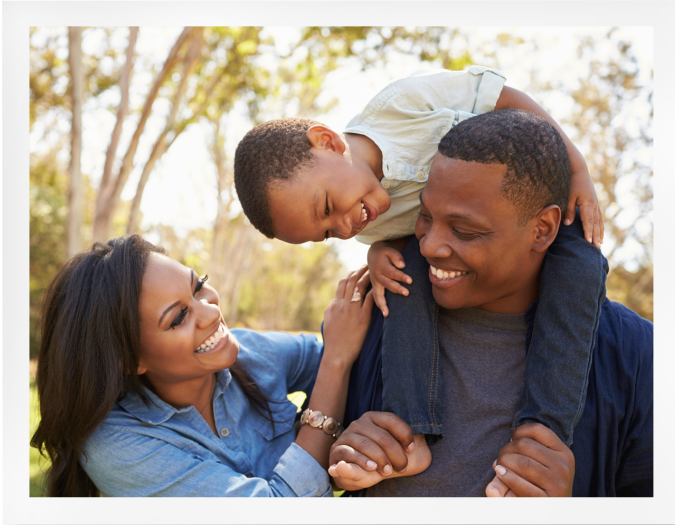
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Using molecularly and racially matched units to support adult sickle cell disease patients



Alloimmunization among the sickle cell patient population is an ongoing problem but there are possible strategies to minimize the risk such as expanded antigen matching, early characterization of Rh variants, and increased donor recruitment and retention among African American donors.

A three-year study (2010–2013), was undertaken to examine the RBC antigen types of both donors and patients (54 adult patients with sickle cell disease). The donor antigen frequencies (as determined by molecular typing) were evaluated and compared to the antigen frequencies observed in the patient population (as assessed by serologic typing) supported by these donations. Also observed was

the alloimmunization rate over the study timeframe and the frequency with which D negative units were provided for transfusion to D positive patients. Only three patients were genotyped, because they developed an antibody during the study period.

During the study timeframe, a total of 6066 self-identified African American donors were typed using molecular methods. Previous studies have shown that transfusing phenotypically matched blood (C, c, E, e, K) has been associated with a decrease in alloimmunization as the most common African American phenotype is D-, C-, E-, K-, a combination found in only 3% of Caucasians. This study demonstrates that antibody formation rates among the chronically-transfused sickle cell disease patient population remains disproportionately high, with 9.3% forming a new antibody during the study period, despite the availability of genotyped units from African American donors. Even with adherence to a prophylactic antigen-matching protocol, there were patients in the cohort who developed Rh antibodies due to undetected Rh variants and patients who developed alloantibodies against antigens that were not included in the prophylactic protocol (for example, Jsa, Cw, Cob, Lua.)

There was also a significant frequency of transfusion of D negative units to D positive patients. Only 10% of D positive patients received only D positive units and 40% of units transfused to D positive patients were from donors who were negative for D, C, E, and K antigens. This utilization pattern could be due to the limited availability of D positive antigen

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Cover photo: 3d rendering of sickle cell blood cells. Getty Images.

This issue of PLUS was written by Jimmy E. Lowery II, MT(ASCP)SBBCM.

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matched units or because it is simply faster to provide D negative units for those D+, C-, E-, K- patients (which are stocked by the institution) rather than request D+, C-, E-, K- units from the blood supplier. Increased recruitment and retention of African American donors, particularly those donors who are found to be D+, C-, E-,K- could be key to reducing the overutilization of D negative units among this patient population.

One of the most interesting, observations from this study was the frequency, or lack thereof, of repeat donors among the African American donor population. 57% of the donors

donated only once during the three-year study period and only 20% donated twice during the same period. Molecular typing of donors who do not become repeat donors is not going to mitigate the alloantibody formation problem in the sickle cell patient population. The challenge of developing effective recruitment techniques must be met to increase not only the overall recruitment of African American donors, but also to retain these donors and encourage repeat donations.

Karafin, MS, et al. Barriers to using molecularly typed minority red blood cell donors in support of chronically transfused adult patients with sickle cell disease. *Transfusion*, 2015; 55: 1399–1406.

Report: Conflicting trends in red blood cell distribution and the impact on patient care

Patient blood management initiatives, intraoperative cell salvage and improved surgical techniques have led to a significant decline in blood transfusions over the past several years in the United States, which has in turn led to a decline in the number of units of red blood cells (RBCs) distributed to hospitals by blood centers. Much has been written regarding this movement, which overall has been seen as a positive trend and an aligning of US blood utilization with what is current practice in other countries.

Although the total number of RBC units has declined, a troubling countertrend has been recognized; the simultaneous increase in the number of antigen-negative red blood cell (RBC) units distributed over the same time period. While there has been a 27% decline in the total number of RBCs distributed during the time period studied (2009-2016), an almost 40% increase in the number of antigen-typed units was observed over the same stretch of time. These are blood products that have been typed by the blood center for specific antigens as requested by the hospital or transfusing facility and are typically ordered for patients who have previously identified alloantibodies or as prophylaxis for patient populations determined to be at risk for developing alloantibodies (e.g., sickle cell disease.)

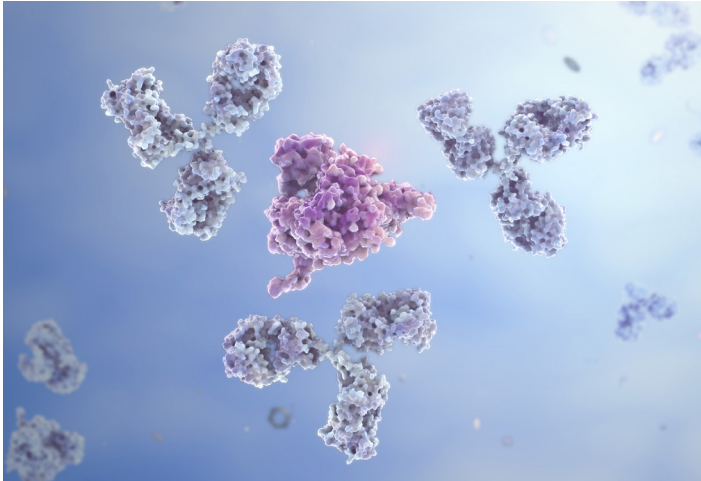
When the distributed units were stratified by race/ethnicity, as self-reported by the donors at the time of donation, it was observed that the majority of the total units distributed as



well as the antigen-negative units distributed were collected from donors who self-identified as white. It was also noted that antigen-negative RBCs from African-American donors were excessively high in proportion to all RBC distributions. This is presumed to be a result of the increased likelihood of finding a match for patients of a particular ethnicity among similar donors and underscores the importance of identifying and encouraging blood donation among African-Americans.

Yazer, MH, et al. Trends in antigen-negative red blood cell distributions by racial or ethnic groups in the United States. *Transfusion*, 2017. Epub ahead of print.

Are antigen matching strategies to mitigate alloimmunization in sickle cell disease cost-effective or clinically-effective?



Alloimmunization occurs in approximately 30% of transfusions to sickle cell disease (SCD) patients and is unpredictable; the pathologic process is poorly understood. In their review, Gehrie and colleagues outline possible risk factors for and adverse effects of alloimmunization in SCD patients and consider the economic impact and effectiveness of different approaches to these problems. Risk factors such as the total number of RBC transfusions received, the age of the patient, degree of genetic difference between donor and recipient, and the mechanism of exposure (simple or exchange transfusion) have been studied and shown to possibly influence the risk of alloimmunization but with no single factor or set of factors demonstrating a definitive link. For SCD patients, alloimmunization causes clinically-significant delays in care due to longer wait times for transfusions, potential delayed hemolytic transfusion reactions (DHTRs), and hemolytic disease of the fetus and newborn (HDFN).

Facilities have implemented various strategies to reduce the incidence of alloimmunization among SCD patients, the most common of which is to provide serologically matched

red cells for transfusion. Programs vary in degree of match required, some only C,E,K while others also match for Fya, Fyb, Jka, Jkb and sometimes MNS. Serologic matching protocols have shown success, resulting in as much as a 50% reduction in the rate of alloimmunization of SCD patients. However, there are limitations, such as alloimmunization associated with variant or partial RH alleles which cannot be identified using serologic methods or alloimmunization resulting from an emergency transfusion or a transfusion received at a facility that does not have such a matching program. Another approach to alloimmunization prevention is to provide genotype-matched RBCs for transfusion of SCD patients. Genotyping has several advantages over serologic testing, such as the ability to identify weak or partial alleles as well as the ability to type SCD patients who may have been recently transfused. However, there is currently only one FDA- approved testing method that does not require confirmation by serologic methods as well as extended time—as much as 36 hours—to complete the testing and complex systems like RHD are not included in that platform.

A strength of this report is the thoughtful discussion of the economic implications of matching programs, since cost-saving projects are highly dependent on laboratory platforms and it is difficult to confidently model the financial impact of alloimmunization on SCD patients. These authors make a clear call for researchers to develop not only methods for identification of those patients who are at increased risk of forming antibodies after transfusion but also to improve molecular testing platforms, so genotype-matching programs can be studied and integrated into cost-effective patient management strategies.

Gehrie EA, et al. Medical and economic implications of strategies to prevent alloimmunization in sickle cell disease. *Transfusion*, 2017;57: 2267–2276

An antibody missed and a discussion of antibody detection and identification techniques

Antibody detection and, when positive, antibody identification testing must be performed prior to transfusion to ensure compatibility. Presented in this report is a case where an anti-E antibody was detected by gel testing but not by tube method. This resulted in transfusion of incompatible red cells and a subsequent hemolytic transfusion reaction.

Gel and tube methods are acceptable for antibody detection and identification testing with each having advantages and disadvantages. The tube method is considered the “gold standard” and offers the flexibility of using different potentiators (PEG, LISS, etc.), incubation times, and temperatures to enhance antibody reactivity, but has also been shown to detect more clinically insignificant antibodies than gel, particularly cold autoantibodies. Gel testing allows for automation of pretransfusion testing, which is extremely attractive as more transfusion service laboratories are faced with staffing and budgetary constraints. Gel testing also offers stability of reactions, allowing for review of results for up to 24 hours after testing is initially performed. This can be particularly useful to enable less experienced technologists to have questionable reactions evaluated by a senior staff member or a more experienced technologist. However, gel has also been associated with increased nonspecific reactivity, particularly in patients with abnormally high serum protein concentrations such as multiple myeloma patients. Many transfusion service laboratories utilize gel for the initial antibody detection or screening as well as antibody identification. A lot of these laboratories, including the laboratory in this case, use the tube method to confirm unclear reactions.

No matter which method or combination of methods is ultimately chosen for antibody detection and identification, it is of utmost importance to understand the limitations of any

testing method used. Since gel is considered to be more sensitive than tube, if testing is performed by both methods it would be wise to weight the results based on this knowledge. In this case, that would have resulted in the identification of a possible allogeneic anti-E in gel and selection of E negative RBCs for compatibility testing and transfusion, rather than assuming a rule-out of anti-E by the tube method. If there is doubt or if results seem to indicate a pattern of reactivity associated with an antibody, a safe approach would be to crossmatch and issue antigen-negative blood. A consult with more experienced staff could have been helpful in this situation as well: a retrospective review of the initial gel testing results was consistent with the presence of an anti-E. In the end, the goal of antibody detection and identification is to provide the patient with the safest possible transfusion even when methods offer conflicting results.

Shmookler A, et al. Acute hemolytic transfusion reaction caused by a red cell antibody that was missed by pretransfusion testing using tube method. *Laboratory Medicine*, 2017;48(3):258–261.



Alloimmunization rates among pediatric sickle cell disease patients in Paris

Alloimmunization is the development of antibodies to antigens present on donor cells following transfusion. This complication is especially important for patient populations such as those with sickle cell disease (SCD), who require chronic transfusions beginning at a young age. Studies have shown that SCD patients have an increased risk for alloimmunization but there is very little data with respect to alloimmunization rates and risk factors specifically among pediatric SCD patients.

The transfusion records of 175 SCD patients from a French university hospital ≤ 18 years of age who were treated in 2014 were analyzed retrospectively to identify potential risk factors for alloantibody formation. Among this particular population of SCD patients who had received at least one RBC transfusion in their lifetime, the alloimmunization rate was almost 14%. This included possible natural occurring antibodies (e.g., anti-M, Lewis antibodies) and when these were excluded, the confirmed alloimmunization rate was found to be 7.4%. The most frequent alloantibodies formed by these patients were anti-Kpa, anti-S, and anti-D. Other antibodies formed by these patients were Rh (C, Ce) Duffy (Fya, Fy3), and anti-U. Primary risk factors for alloimmunization appeared to be: an increased number of RBC transfusions and a presence of one or more RBC autoantibodies. The study also compared SCD patients who were chronically transfused to prevent complications in a manual exchange program to SCD patients who were transfused episodically.

Those who were chronically transfused received more RBCs and were more likely to form alloantibodies, and the rate was significantly greater than for episodically transfused (0.24 vs. 1.43/100 RBC units received, $p < 0.001$). The authors conjecture that this may be due to the episodic transfusions more likely occurring when the recipients are in an inflammatory state related to SCD complications such as acute chest syndrome, vaso-occlusive crisis, *etc.*

Overall the alloimmunization rate observed within this patient population is significantly lower than the 20% alloimmunization rate observed in other studies, possibly because the patients in this study received Rh and K matched units exclusively: there is a large number of donors of African-Caribbean origin in Paris. As a result, it is probable that these particular patients received units of RBCs from ethnically similar donors, which increases the probability of antigen matching among Duffy, Kidd and MNS as well as the Rh and K systems. Although additional larger studies are needed, the data presented here confirm that receipt of RBCs from ethnically similar donors is a significant factor in the reduction of alloimmunization among chronically transfused patients and underscores the importance of increased recruitment and retention of these donors.

Gavva C, Yates SG, Rambally S, Sarode R. Transfusion management of Factor V deficiency: three case reports and a review of the literature. *Transfusion* 2016; 56:1745-1749.

Barriers to minority donation in the United States

Volunteering is crucial to supporting civic base needs, with research demonstrating that minorities are more likely to be involved. Such programs have included mentoring programs in college and involvement in church activities, which have been pivotal to assisting members of the community. Minorities are active participants in social movements, for instance during the civil resistance of the 1950s and 1960s, many African Americans volunteered both their resources and time to help in the fight for equality. So why is it that this philanthropy does not extend to regular blood donation? The question has been explored by various studies that consistently show the rate of blood donation among those who identify as African American, Asians, or Hispanics is less than 50% than that of Caucasians.

This report presents several reasons for this underrepresentation in the blood donor base, including deferrals. For instance, iron deficiency is significantly lower among minorities, especially

women, accounting for as much as 30% of deferrals. Although this ineligibility status is temporary, as it can be corrected through appropriate dietary measures, the return rate is 29% less, when compared to a donor who was not deferred. Possible mitigation strategies for decreasing these deferrals include: instituting iron supplementation and educational programs, lowering the hemoglobin requirement for female donors, decreasing collection volume, and/or increasing the required interval between donations.

The lack of understanding of different motivators and barriers that minorities have can contribute to the challenges in recruitment. For instance, historical events, such as the Tuskegee syphilis study, contributes to a sense of distrust in the medical system and uncertainty regarding the safety of the blood donation process and supply. A survey of African American blood donors

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indicated that they were more likely to donate to save a life versus Caucasians, who felt it was the right thing to do. Such subtleties in verbiage can influence marketing strategies. To manage this, recruitment activities must be modified to address such concerns through enhanced educational programs. In addition, focusing on conditions, such as sickle cell disease, where their blood can be demonstrated to have a significant impact should enhance donation among minorities.

The demographic characteristics in the United States population are shifting, with a significant increase in the minority base. However, like organ transplants, the genetic

makeup between the recipient and donor does matter. There is sufficient scientific and clinical support to show a more positive response rate to transfusions when antigen-matched blood from donors of the same ethnicity are given. For this reason, it is critical to expand recruitment efforts in focusing on minority donors such as African Americans, Asians, and Hispanics. Through the development of such targeted recruitment and education programs, availability of an adequate blood supply can be achieved.

Shaz BH, Hillyer CD. Minority Donation in the United States: Challenges and Needs. *Current opinion in hematology.* 2010;17(6):544-549.

Current state of HLA and RBC genotyping



It is truly amazing what has been accomplished in the areas of transplantation and transfusion since the first successful kidney transplant in the early 1950s. Fasano and colleagues present current clinical applications of HLA and RBC genotyping in a well-researched historical context, showing how new technologies over the decades have facilitated new opportunities for clinical implementation. Molecular methods have allowed for more accurate and reliable typing of hematopoietic progenitor cell donor and recipients, thus leading to better patient outcomes. When molecular antigen typing techniques are combined with advanced solid phase antibody testing, it is possible to perform a virtual crossmatch between recipient and potential donors; decreasing the time to transplant for these patients. Also as a result of more advanced HLA typing methods, databases and registries of millions of potential donors have been established, increasing the odds of finding a better HLA-matched donor.

In an even shorter span of time, genotyping in transfusion medicine has built upon the strides made in HLA genotyping to make similarly large gains. Human platelet antigen typing allows for more comprehensive assessment of fetal risk in obstetric patients with platelet antibodies and, together with HLA typing, aids in the identification and selection of

compatible products for patients who have become refractory to platelet transfusions. Red blood cell (RBC) genotyping has made it possible to more comprehensively characterize donors, including those with uncommon or exceedingly rare types which are typically not detected using serologic methods. The management of chronically transfused patients, such as those with thalassemia or sickle cell disease (SCD), is one area where RBC genotyping has shown particular utility. Molecular genotyping methods offer a more detailed characterization of the patient and donor RBC phenotypes and antigen-matched units may be provided prophylactically to reduce the risk of alloimmunization. In addition to providing a handy literature review of matching strategies for RBC antigens, these authors offer their institution's practice and extensive experience with molecular testing, offering an excellent model of clinical implementation of these programs to hospitals moving in that direction.

As information systems become more sophisticated and more donor genotyping is performed, the ability to use a patient antigen profile to search and compare against a database of donor profiles seems to be a next logical step. Such programs, which require a close blood center and hospital partnership, have the potential to provide faster, more reliable, and more extensive antigen-matching for these transfusion-dependent patients, especially those with SCD. Although the cost-effectiveness of matching programs is unclear, expanded use of molecular methods in transfusion medicine is an important tool that is gaining momentum as a means to continue to improve patient safety.

Fasano RM, et al. Genotyping applications for transplantation and transfusion management: the Emory experience. *Archives of Pathology & Laboratory Medicine.* 2017;141:329-340.

Gehrie EA, et al. CMedical and economic implications of strategies to prevent alloimmunization in sickle cell disease. *Transfusion.* 2017;57: 2267-2276



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Red Cross HLA Laboratories



American Red Cross HLA laboratories provide comprehensive and state-of-the-art HLA services supporting hematopoietic stem cell transplantation (HSCT), organ transplantation and HLA-matched platelet transfusions to support physicians and their patients. HLA laboratories also provide DNA-based typing for genetic polymorphisms of cytokine genes, minor histocompatibility antigens and natural killer cell immunoglobulin-like receptor (KIR) genes, and for genetic monitoring of engraftment post-HSCT transplantations using short tandem repeat (STR) markers.

The five Red Cross HLA laboratories are CLIA-certified and accredited by agencies such as the American Society for Histocompatibility and Immunogenetics (ASHI), the New York Department of Health and the United Network of Organ Sharing.

HLA services include both allelic level and intermediate resolution level Class I (HLA-A,B,C) and Class II (HLADRB1, DRB345, DQA1, DQB1, DPA1, DPB1) and KIR gene typings. Fragment analysis of STR markers is utilized for genetic monitoring of engraftment post-HSCT. Microarray-based technology is used for customized quantification of various cytokines, chemokines, growth/ differentiation factors, cell signal transduction and apoptosis proteins.

The laboratories also use various approaches to identify unacceptable antigens for highly sensitized patients receiving organ transplantations or to identify acceptable antigens for highly immunized patients receiving platelet transfusions.

Publications Corner

Recent publications by American Red Cross scientists and physicians:

Enhancement of Zika virus pathogenesis by preexisting ant flavivirus immunity. Bardina SV, Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown JA, Nachbagauer R, Foster GA, Krysztof D, Tortorella D, Stramer SL, Garcia-Sastre A, Krammer F, Lim JK. *Science*, 2017 356(6334):175-180.

Temperature cycling during platelet cold storage improves in vivo recovery and survival in healthy volunteers. Vostal JG, Gelderman MP, Skripchenko A, Xu F, Li Y, Ryan J, Cheng C, Whitley P, Wellington M, Sawyer S, Hanley S, Wagner SJ. *Transfusion*, 2017 Nov 8. doi: 10.1111/trf.14392. [Epub ahead of print]

Effect on gene expression of three allelic variants in GATA motifs of ABO, RHD, and RHCE regulatory elements. Fennell K, Hoffman R, Yoshida K, Iwamoto S, Govender L, Vather K, Sookraj A, Jentsch U, Pambrun C, McAuley C, Keller MA, Ochoa-Garay G. *Transfusion*, 2017 57(11):2804-2808.

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