A model for integrating molecular-based testing in transfusion services

S. Gerald Sandler[1], Trina Horn[2], Jessica Keller[2], Al Langeberg[1], Margaret A. Keller[2]

[1]*Department of Pathology and Laboratory Medicine, MedStar Georgetown University Hospital, Washington, DC;* [2]*National Molecular Laboratory, American Red Cross Biomedical Services, Philadelphia, PA, United States of America*

Background. Molecular-based laboratory tests can predict blood group antigens and supplement serological methods, adding a unique technology to assist in resolving discrepant or incomplete blood group typing or antibody identification. Hospital transfusion services have options for integrating molecular-based methods in their routine operations. We describe here the model of a hospital-reference laboratory partnership.

Materials and methods. Blood samples for compatibility testing were obtained from patients in a 609-bed hospital serving an urban multiethnic and multiracial population. When results of blood group phenotyping by serological methods were inconclusive, samples were referred for molecular-based testing. The reference laboratory used several methods for genotyping, including polymerase chain reaction followed by restriction enzyme-linked polymorphism analysis, sequence-specific primer polymerase chain reaction and array-based approaches. Human erythrocyte antigen, *RHCE* and *RHD* single nucleotide polymorphism arrays were integrated into the laboratory as they became commercially available.

Results. The hospital-reference laboratory model made it possible to integrate blood group genotyping promptly by current technology without the expense of new laboratory equipment or adding personnel with technical expertise. We describe ten cases that illustrate the categories of serological problems that were resolved by molecular methods.

Discussion. In-hospital molecular testing for transfusion services has logistical advantages, but is financially impractical for most hospitals. Our model demonstrates the advantages of a hospital-reference laboratory partnership. In conclusion, hospital transfusion services can integrate molecular-based testing in their routine services without delay by establishing a partnership with a molecular blood group reference laboratory. The hospital reference-laboratory model promotes genomic medicine without the expense of new equipment and skilled personnel, while supporting the economy of centralised large-scale laboratory operations.

Keywords: immunohaematology, blood group genotyping, blood transfusion.

© SIMTI Servizi Srl

Introduction

Advances in genomic medicine have increased the options available to hospital transfusion services for resolving incomplete or inconclusive results for serological compatibility testing[1-5]. Molecular-based laboratory methods are available and capable of resolving discrepancies in serological blood typing when antigens are expressed weakly or have missing or altered epitopes, identifying blood group antigens when reagent anti-sera are not available, predicting blood group phenotypes in recently transfused patients with a positive direct antiglobulin test result, and predicting extended phenotypes by high-volume automated analysers by genotyping arrays. There are at least three options for transfusion services that decide to add molecular-based testing to existing serological services. The transfusion service may add molecular-based testing to its conventional in-hospital serological testing services, as has been described by Sapatnekar and Figueroa for the Cleveland Clinic[6]. In this model, the transfusion service added molecular-based blood typing services by establishing a new laboratory, purchasing an automated extractor for DNA, polymerase chain reaction (PCR) work stations, centrifuges, a thermal cycler, hybridisation oven, imaging system and computer which were relocated in a newly configured laboratory space[6]. We consider this model to be financially unrealistic for most hospitals in the current cost-conscious environment. A second model was described in a four-hospital study in which transfusion recipients' blood samples and units of donors' red blood cells (RBC) were matched by extended molecular-based blood

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other use without premission

group genotypes and electronically crossmatched[7]. The investigators did not provide estimates for the cost of implementing this model, but concluded that there was a "theoretical feasibility" of establishing an inventory of donor RBC that had been genotyped for extended blood group phenotypes and could fill a substantial portion of requirements for patients requiring that service. We do not consider that option to be financially practical as a model for most transfusion services.

In our report, we describe the third option, using the time-proven paradigm of a hospital-reference laboratory partnership, integrating molecular-based testing in our transfusion service by outsourcing to a reference laboratory. Through this partnership, the hospital has been able to offer patients the benefits of genomic medicine in a timely and cost-effective programme. We recognise the appeal and logistical advantages of establishing in-hospital molecular testing for compatibility testing. However, we propose that the hospital-reference laboratory partnership is a more realistic alternative for most hospital laboratories in the current cost-conscious healthcare environment. This report shares our experience, describing how we selected cases for referral for molecular-based testing, resolved complex serological problems by up-to-date genomic medicine, and did so without purchasing expensive new equipment or adding highly-skilled molecular biologists to the hospital's payroll.

Materials and methods

Samples

Beginning in January 2011, blood samples were collected from patients whose serological test results were inconclusive and, based on published studies of blood group genotyping, were considered to be promising candidates for resolution by molecular-based methods. Patients were served by MedStar Georgetown University Hospital, a 609-bed acute care and teaching hospital providing care for an inner-city multiethnic and multiracial population.

Serological compatibility testing

All samples were tested initially for ABO/Rh and antibody screened using an automated analyser, either Galileo (Immucor, Norcross, GA, USA) or Tango Optima (Bio-Rad, Hercules, CA, USA). Inconclusive antibody screens were repeated by testing using either Capture-R Ready-ID Panoscreen I & II (Immucor) or Biotestcell 1&2 (Bio-Rad) and, if unresolved, were tested by manual serological methods according to the AABB Technical Manual[8].

Molecular-based test methods

EDTA-anticoagulated blood samples were received at the American Red Cross National Molecular Laboratory (NML; Philadelphia, PA, USA). The NML isolates DNA using a DNA Blood MiniKit (QIAGEN, Carlsbad, CA, USA) extraction from buffy coat. Genotyping for blood group antigens is performed using several methods. These include PCR followed by restriction enzyme-linked polymorphism (RFLP) analysis and sequence-specific primer (SSP) PCR with custom-made primers (Life Technologies, Carlsbad, CA, USA). DNA fragment sizes are determined by agarose gel electrophoresis and imaging. Human erythrocyte panel (HEA), *RHCE* and *RHD* BeadChip™ tests (Immucor) were used according to the manufacturer's instructions[9]. *RHD* and *RHCE* BeadChips became commercially-available during the course of this study and replaced the use of most gel-based genotyping assays, including multiplex PCR, PCR-RFLP and SSP-PCR. Sanger DNA sequencing was performed using BigDye Terminator chemistry (Life Technologies) and alignment with reference sequences using Sequencher (GeneCodes, Ann Arbor, MI, USA). These and other commonly used methods for RBC genotyping with their test types, resolution and throughput times are listed in Table I. Test types include laboratory-developed tests, also known as "home brews", commercially-available genotyping kits sold for research use only and Food and Drug Admnistration-licensed *in vitro* diagnostics. Test resolution is classified as "low" if one or a few genetic variants are interrogated, "medium" if many (≥20) genetic variants are interrogated and "high" if the sequence of a portion of a gene or cDNA is obtained in its entirety. Throughput was classified as "low" if samples are tested and analysed individually, "medium" if small batches of samples (<20) are typically tested simultaneously, and "high" if large batches of samples (48-96) are tested simultaneously. Turnaround times for RBC genotyping vary based on the type and number of tests performed. For example, HEA BeadChip is a single test that can be performed in a single work day. Since it is a batch test, the turnaround time from sample receipt to report will depend on the test volume of the laboratory. In contrast, an investigation of a null phenotype, such as in our Case 8, involved gene-specific exonic sequencing which may take a week or more. The approach to such a case may differ from laboratory to laboratory and often the laboratory will use the patient's race/ethnicity to target testing to gene regions with known variants that could explain the reported phenotype. If a sample is found to be a compound heterozygote, such that alleles cannot be assigned, cloning of cDNA PCR products into plasmid vectors followed by sequence analysis can be used to assign alleles. This process can take several weeks.

Results

During a 3-year period, the hospital transfusion service performed an average of 17,500 antibody screens

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other use without premission

Table I - Resolution and throughput of common RBC genotyping assays.

Method	Example	Test type	Resolution	Throughput	Cases
Gel-based genotyping (PCR-RFLP, SSP-PCR, multiplex PCR)	Rh multiplex (Singleton, *et al.*[30])	LDT	Low	Medium	1, 2, 3, 4, 5, 6, 9
	BAGgene (GenTrak, Inc., Liberty, NC, USA)	RUO	Low	Medium	N/A
DNA Sequencing analysis (Sanger)	Exon-specific	LDT	High	Low	3, 6, 8
Array-based genotyping	HEA BeadChip (Immucor, Norcross, GA, USA)	IVD (previously RUO)	Low	Medium (8, slides) or High (96, plates)	1, 7, 9
	ID Core XT (Grifols, Los Angeles, CA, USA)	RUO	Medium	High (up to 48)	N/A
	RHCE and RHD BeadChips (Immucor)	RUO	Medium	Medium (8, slides)	2, 4, 6
MALDI-TOF	HemoID (Agena, San Diego, CA, USA)	RUO	Medium	High (96 or 384)	N/A
cDNA analysis	*RHD* or *RHCE* cDNA analysis	LDT	High	Low	N/A

RBC: red blood cell; PCR: polymerase chain reaction; RFLP: restriction enzyme-linked polymorphism; SSP: sequence-specific primer; LDT: laboratory-developed test; RUO: research use only; N/A: not applicable; IVD: *in vitro* diagnostic; MALDI-TOF: matrix-assisted laser desorption ionisation time-of-flight.

and transfused approximately 12,500 units of red blood cells annually. The number of blood samples encountered by a transfusion service which are suitable for molecular testing will vary depending on the racial and ethnic composition of the hospital's population. In this study, the transfusion service encountered an average of ten samples each year which were selected for molecular testing. The results of our hospital-reference laboratory partnership are illustrated by ten cases that we selected from our 3-year experience. These cases illustrate categories of problems encountered by the hospital's transfusion service which could not be resolved by standard serological methods and were resolved by molecular-based testing. The cases also demonstrate that adding molecular-based testing requires expertise and technology for a scientific discipline that is significantly different from the serological basis of conventional compatibility testing.

© SIMTI Servizi Srl

Case 1: genotyping after recent transfusions

A 15-year old African-American male with sickle cell disease was transferred from another hospital with anaemia, a negative antibody screen and a weakly reactive (1+) direct antiglobulin test. He had been transfused with multiple RBC units within the preceding 2 weeks, preventing reliable blood group phenotyping by standard serological methods. Serological approaches involving hypotonic washing of RBC from patients with sickle cell disease who have been recently transfused can lead to false typings[10]. His newly-acquired physicians requested that RBC for transfusion be matched for C, E and K, according to our transfusion service's policy for limited phenotype matching. A sample was sent for blood group genotyping to facilitate matching for C, E and K antigens. HEA genotyping predicted his blood group phenotype to be c+ C (0)* e+ E− K− k+ Kp(a−b+) Js(a− b+) Jk(a+b+) Fy(a−b−) M+ N− S+ s+ Lu(a−b+) Di(a−b+) Co(a+b−) Do(a−b+) Jo(a+) Hy+ LW(a+b−) Sc(1+ 2−), where C (0)* indicates the potential presence of an r'S haplotype that expresses an altered C antigen. Based on this result, RH characterisation was performed and determined that the patient carried one *RHD*DIIIa-CE(4-7)-D* hybrid gene and one deleted *RHD* gene *(RHD*01N.01)*. Individuals with the *RHD*DIIIa-CE(4-7)-D* hybrid gene can type C+ or C+weak but are at risk of alloimmunisation to RhC. Based on these results, the RBC supplied for transfusion were C− E− K−. Subsequent serological cross-matches have been negative.

Case 2: mimicking anti-e

A 70-year old African-American female with warm (IgG)-type autoimmune haemolytic anaemia presented with a positive direct antiglobulin test result (3+) using anti-IgG antihuman globulin reagents. The patient's plasma also reacted 3+ in the antibody screen and identification panels with e+ reagent RBC and 1+ to 2+ with e− reagent RBC. The patient's RBC typed as e+ with multiple anti-e reagents. Since the patient had received multiple transfusions previously, a sample was referred for *RHCE* genotyping to determine e variant status and the potential for allo-anti-e stimulation versus a mimicking auto-anti-e[11]. The patient's *RHCE* genotype was determined to be *RHCE*Ce/RHCE*ce48C*. Thus, the patient did have an e− variant *RHCE*ce48C* allele, but since it was *in trans* with a conventional *RHCE*Ce* allele, the patient was not at risk of forming allo-anti-e. However, the laboratory has seen this genotype associated with formation of anti-ce(f). Molecular-based testing established that

All rights reserved - For personal use only
No other use without premission

the patient does not require uncommon e− RBC for transfusions. Subsequently, she has been transfused on multiple occasions with "least incompatible" e+ ABO/Rh-compatible RBC without evidence of overt haemolysis or shortened RBC survival.

Case 3: serological weak D phenotype, resolved as RhD-positive

Routine antenatal RhD typing for a 36-year old Caucasian female identified a serological weak D phenotype, that is, her RBC typed weakly positive (≤2+) by automated testing on the Galileo and by manual tube tests, using anti-D monoclonal reagents. Three months previously, an RhD type performed by a commercial reference laboratory was reported to be RhD-negative. That laboratory does not perform a weak D test (indirect antiglobulin test) if initial typing with an anti-D reagent is negative. Her blood sample was referred for molecular-based testing to resolve the discrepancy in her serological RhD typing results. Her *RHD* genotype was determined to be *RHD*weak D type 1/RHD*01N.01* such that the predicted phenotype is D+^{weak}. For purposes of blood transfusion and Rh immunoprophylaxis her weak D type 1 classifies her as RhD-positive[12]. Molecular-based testing established that she may be transfused safely with RhD-positive RBC and that she does not require RhD-negative RBC[12]. We provided the patient with a letter informing her that her RBC expressed a serological weak D phenotype and that, if she is RhD typed in the future by another laboratory, she may be told that she has a different RhD type, depending on that laboratory's procedures.

Case 4: serological weak D phenotype, resolved as RhD-negative

A 64-year old Caucasian man was typed as RhD-negative by automated testing on the Galileo (Immucor), but interpreted to be RhD-positive when a weak D test was microscopically positive using a monoclonal anti-D reagent (Monoclonal Blend Blood Grouping Reagent Anti-D Series 4, Immucor). One month later, the Galileo result was RhD-negative and a weak D test result using a different anti-D reagent (Monoclonal Blend Blood Grouping Reagent Anti-D Series 5, Immucor) was also RhD-negative. The patient's sample was referred for genotyping to resolve the discrepant serological RhD typing results. The patient's *RHD* genotype is *RHD*01N.01/RHD*01N.01,* that is, he does not have an intact *RHD* gene, is phenotypically D antigen-negative and should be managed as RhD-negative[11]. His predicted phenotype is D− C− E− c+ e+. The laboratory ruled out *RHCE*ce* variants that express D-like epitopes and can type as RhD-positive with some monoclonal reagents (*RHCE*ceCF* and *RHCE*ceHAR*), and those that are associated with a weak false-positive RhD typing (*RHCE*ceRT* and *RHCE*ceSL* alleles). The patient was transfused with RhD-negative RBC without incident.

Case 5: anti-e in an e+ patient

A plasma sample from a 72-year old African-American female agglutinated all e+ reagent RBC when tested by two antibody identification panels, but her RBC typed as e+ by multiple anti-e reagents. The sample was referred for genotyping to determine whether she had an auto-anti-e or an allo-anti-e associated with an e variant. The *RHCE* genotype of the patient is RHCE**ce733G/ RHCE*ce733G*. Her predicted phenotype is D+, C−, E−, partial c+, partial e+, VS+V+, and hr^{B}+^{weak}/−. The hr^{B} status is equivocal according to the ISBT Working Party[28] due to the reactivity of some unlicensed antisera. Homozygosity for this partial *RHCE*ce* allele is associated with the presence of allo-anti-e or -ce(f). At the time of the testing, in 2012, the American Rare Donor Program did not have any potentially-compatible donors in their registry. The patient declined to become an autologous blood donor. Molecular testing established the rarity of the patient's Rh phenotype and alerted us to the likelihood of an alloimmune haemolytic transfusion reaction if she were to be transfused with e+ red blood cells. Currently, the American Rare Donor Program has more than 130 donors of this *RHCE* genotype (Cindy Flickinger, personal communication, January 2015).

Case 6: partial D presenting as serological weak D

RBC from a 40-year old African-American male typed RhD-negative by automated testing on the Galileo and RhD-positive by a weak D test. His antibody screen was negative, although he had been transfused with RBC previously. The patient's sample was referred for *RHD* genotyping to resolve the discrepant RhD typing results. Molecular testing established that his *RHD* genotype is *RHD*DAU2/RHD*01N.01*. His *RHCE* genotype is *RHCE*ce48C/RHCE*ce*. His predicted Rh phenotype is partial D+ C− E− c+ e+ VS−V− hr^{B}+. *RHD*DAU2* encodes a partial D antigen and is associated with allo-anti-D. The patient was given a letter informing him of his genotyping results. He was also informed that if he requires transfusion of RBC, they must be RhD-negative, because he may form allo-anti-D if transfused with RhD-positive RBC.

Case 7: matching red blood cells for anti-U

Plasma from a 31-year old African-American female with a missed abortion agglutinated all reagent RBC on an antibody screen and identification panels, except 3 S−s−U− reagent RBC. Her direct antiglobulin test was w+ with anti-IgG antihuman globulin reagents. Recognising that some patients with these serological

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other use without premission

findings may have an auto-anti-U or a U variant phenotype[13], the transfusion service referred a sample for molecular-based testing to verify that, if needed, she had an absolute requirement for rare U− RBC. Her extended blood group phenotype results were predicted by genotyping with HEA BeadChip™: c+ C− e+ E+ K− k+ Kp(a−b+) Js(a−b+) Jk(a+b+) Fy(a+b−) M−N+ S"LS" s"LS" U− Lu(a−b+) Di(a−b+) Co(a+b−) Do(a+b−) Jo(a+) Hy+ LW(a+b−) Sc(1+2−). The "LS" (low signal) results for the S and s antigens is indicative of a *GYPB* gene deletion with a predicted phenotype of S−s−U− and an absolute requirement for U− RBC, if needed. An incidental finding was that the patient had a GATA mutation in the promoter of the *DARC* gene that codes for Fy antigens. This variant silences the gene in RBC, but not in other cell types[14]. This is a useful finding as the patient typed serologically as Fy(b−), and based on this information, would be considered at risk of forming anti-Fy^b. The incidental finding of a GATA mutation indicates that the patient could be transfused with Fy(b+) RBC without the risk of forming anti-Fy^b [15].

Case 8: K_0 presenting as anti-Kp^b

Routine pre-operative compatibility testing identified anti-Kp^b in the plasma of a female of Native American, African and German descent. When her RBC typed negative for three Kell system high-prevalence antigens [k−, Kp(b−), Js(a−)], her blood sample was referred for genotyping for Kell (*KEL*) variants. The laboratory amplified and sequenced *KEL* exons 1 to 6, 8 to15, 17 and 18 and associated splice sites, as K_0 has been associated with variants in these gene regions[28]. Sequence analysis identified *KEL* exon 4 c.382T predicted to encode a premature stop codon at amino acid 128 (R128X), exon 6 c.578C associated with *KEL*02*, exon 8 c.841C and c.842G associated with *KEL*04* (Kp^b+), and exon 17 c.1790C associated with KEL**07* (Js^a+). The stop codon at amino acid R128X is associated with a null phenotype in the Kell blood group system. Based on these findings, the predicted phenotype is K_0. The patient declined to be an autologous donor. Her two siblings were genotyped and were each found to carry one copy of the null allele and one copy of a conventional allele and, therefore, neither was compatible with the patient.

Case 9: RhD-positive with anti-D

A 40-year old Caucasian woman presented for antenatal testing for her third pregnancy. During her second pregnancy, she was typed at another hospital as weak D-positive. She did not receive Rh immune globulin after delivery of a RhD-positive neonate. A blood sample tested by a commercial reference laboratory reported her to be RhD-negative with anti-D. A sample was referred for *RHD* genotyping to determine whether her RBC were expressing a D variant phenotype. Molecular testing demonstrated that *RHD* intron 4 failed to amplify by PCR, consistent with *RHD*DVI*. She was informed that she had inherited an uncommon Rh blood type and that she should inform her sister, who was pregnant, that she may be at risk of RhD alloimmunisation and should have *RHD* genotyping. The sister declined and we informed her that she should receive RhD-negative RBC and be managed as RhD-negative with reference to Rh immune globulin.

Case 10: anti-e, auto- or allo-antibody?

A 13-year-old African-American female with sickle cell disease and β-thalassemia had a history of anti-C, -E, -Jk^b and a warm-reacting autoantibody. Her RBC typed as D+ C− E− c+ and e+. A current sample showed possible auto-e and panagglutinin. The sample was referred for molecular-based testing for a possible e variant. The patient was found to have two variant *RHCE* alleles: *RHCE*ce48C, 733G, 941C,1006T*[16]/ *RHCE*48C, 733G*. Both these alleles express e variants that lack the hr^B antigen. These findings are consistent with the patient having made an anti-e-like antibody. This finding, along with the presence of anti-E, would make finding compatible RBC for transfusion very challenging. The monocyte monolayer assay is used in our transfusion service to determine if an antibody mediates opsonisation of RBC as a surrogate for clinical significance[17]. However, the patient was discharged without transfusion and temporarily lost to follow up before a monocyte monolayer assay could be performed. Use of hr^B− blood is another option. There are multiple *RHCE* variants predicted to express an hr^B negative phenotype, however, there is some evidence that these variants are not cross-compatible[18]. *RH* genotype matching is an option in very difficult cases. This involves matching donors and patients based on *RH* alleles[18-20].

Discussion

These case reports illustrate how the hospital's transfusion service partnered with a reference laboratory to provide the benefits of genomic medicine to patients in a timely and comprehensive programme, using the latest technology and without the expense of new equipment or personnel. Depending on the racial and ethnic composition of a hospital's patient population, the transfusion service will encounter a varying number of cases requiring molecular-based testing. For example, as many as 1% of Caucasians will have a serological weak D phenotype[21]. Of these, an estimated 80% of non-Hispanic Caucasians with a serological weak D phenotype will have a weak D type 1, 2 or 3[22]. If women of child-bearing age with a serological weak

All rights reserved - For personal use only
No other use without premission

D phenotype had *RHD* genotyping performed, and if they were to be identified to be weak D type 1, 2 or 3, they could be managed safely as RhD-positive and, if pregnant, would not require injections of Rh immune globulin[22]. Women with other racial and ethnic backgrounds have lower prevalence of weak D types 1, 2 or 3[22]. Genotyping potential transfusion recipients with a serological weak D will identify approximately 80% of non-Hispanic Caucasians to have a weak D type 1, 2 or 3 and these individuals can be transfused safely with RhD-positive RBC. The benefits of matching RBC transfusions for patients with sickle cell disease using molecular-based testing has been reported and recommended by others[23-27].

The key to the success of our programme for integrating molecular-based tests in the transfusion service was an early decision to partner with a reference laboratory performing molecular immunohaematology testing and not to attempt to establish a new in-hospital service. This model has been proposed by others[3,5] although without reporting transfusion service experience or specific case studies. Before we committed to our hospital-reference laboratory partnership, we considered adding blood group genotyping to the services offered by one of our hospital's other molecular-based testing laboratories. After careful analysis, we came to appreciate that the requirements for a molecular-based testing laboratory for blood groups had relatively few commonalities with molecular laboratories serving other disciplines. This is due in part to the nature of the testing. Until recently, all molecular tests for blood group antigens were "research use only" and many are laboratory-developed tests. In many cases, especially those involving *RHCE* and *RHD* genotyping, interpretation of multiple test results may be needed to generate antigen predictions. In addition, the field of blood group antigen variants is growing rapidly, with many new alleles identified each year, as evidenced by scanning the web pages of the ISBT Working Party on Allele Terminology and on Red Cell Immunogenetics and Blood Group Terminology[28]. We decided to base our transfusion service's molecular testing programme on the time-proven paradigm of a hospital transfusion service partnering with a reference laboratory to perform the more complex laboratory services. This model supports standardisation of the evolving molecular-based test methods, sharing the cost for skilled personnel, and avoids an up-front investment for expensive molecular analysers and ongoing costs of maintaining proficiency and validation programmes.

One of the first challenges to our decision to outsource molecular testing services was how would the reference laboratory's charges for genotyping be reimbursed to the hospital. There are fiscal and logistical impediments to advocacy for new technology in the current cost-conscious healthcare environment. The American Medical Association and the Center for Medicare and Medicaid Services have approved a CPT code for HEA panel and *RHD* genotyping (81403, Tier 2 MoPath) and reimbursement rates for *RHD* genotyping are being established. A recent study compared the financial implications of *RHD* genotyping pregnant women with a serological weak D to the cost of the current practice of serological testing and Rh immune globulin use in obstetrics patients versus the cost of *RHD* genotyping all pregnant women with a serological weak D phenotype[29]. The conclusion of this analysis was that incorporating *RHD* genotyping for pregnant women with a serological weak D would be expected to reduce costs marginally, provided that the additional cost of genotyping was less than $ 256. Cost savings would be highest for non-Hispanic Caucasian women (–$ 0.17/pregnancy), because of their higher prevalence of weak D types 1, 2 or 3. Net costs would increase marginally for non-Hispanic African American women (+$ 0.51/pregnancy) and Hispanic women (+$ 0.37/pregnancy), because of their lower prevalence of weak D types 1, 2 or 3[29].

Conclusions

Molecular-based testing can supplement traditional serological methods for resolving discrepant or incomplete blood group typing or antibody identification. Transfusion services have options for integrating molecular-based tests and genomic medicine to improve blood donor-recipient matching for transfusions. The option of adding molecular-based testing to in-hospital laboratory services has logistical advantages, but the disadvantage of high cost for evolving technology and skilled personnel. Our model of a hospital-reference laboratory partnership offers the advantage of prompt integration of up-to-date genomic medicine while supporting the economy of centralised, large-scale reference laboratory operations. Our cases illustrate categories of problems for which molecular-based testing can supplement serological methods for the resolution and more precise compatibility testing.

Authorship contributions

SGS authored those portions of the manuscript describing patients and serological testing. MAK authored those portions of the manuscript describing molecular testing. AL performed tests using serological methods. TH and JK oversaw the molecular-based testing and assigned alleles. All Authors reviewed, edited and approved the final manuscript.

Disclosure of conflicts of interest

SGS, TH, JK and AL have no conflicts of interest. MAK has served on Immucor's Speakers Bureau.

© SIMTI Servizi Srl

All rights reserved - For personal use only
No other use without premission

References

1) Wagner FF, Frohmajer A, Ladewig B, et al. Weak D alleles express specific phenotypes. Blood 2000; **95**: 2699-708.
2) Flegel WA, Wagner FF, Müller TH, Gassner C. Rh phenotype prediction by DNA typing and its application to practice. Transfus Med 1998; **8**: 281-302.
3) Westhoff CM. The potential of blood group genotyping for transfusion medicine practice. Immunohematology 2008; **24**: 190-5.
4) Denomme GA, Flegel WA. Applying molecular hematology discoveries to standards of practice in blood banks: now is the time. Transfusion 2008; **48**: 2461-75.
5) Hillyer CD, Shaz BH, Winkler AM, Reid M. Integrating molecular technologies for red blood cell typing and compatibility testing into blood centers and transfusion centers. Transfus Med Rev 2008; **22**: 117-32.
6) Sapatnekar S, Figueroa PI. How do we use molecular red cell antigen typing to supplement pretransfusion testing? Transfusion 2014; **54**: 1453-58.
7) Klapper E, Zhang Y, Figueroa P, et al. Toward extended phenotype matching: a new operational paradigm for the transfusion service. Transfusion 2010; **50**: 536-46.
8) Fung MK, Grossman BJ, Hillyer CD, et al., editors. *Technical Manual*. 18th ed. Bethesda: American Association of Blood Banks; 2014.
9) Hashmi G, Shariff T, Zhang Y, et al. Determination of 24 minor red blood cell antigens for more than 2000 blood donors by high-throughput DNA analysis. Transfusion 2007; **47**: 736-74. Erratum in: Transfusion 2007; **47**: 952.
10) Horn T, Hamilton JR, Hare V, et al. Ability of commonly used RBC phenotyping pretreatments to yield an accurate antigen profile [abstract]. Transfusion 2014; **54** (Suppl S2): 165A.
11) Issitt PD, Pavone BG. Critical re-examination of the specificity of auto-anti-Rh antibodies in patients with a positive direct antiglobulin test. Br J Haematol 1978; **38**: 63-74.
12) Flegel WA. How I manage donors and patients with a weak D phenotype. Curr Opin Hematol 2006; **13**: 476-83.
13) Peyrard T, Lam Y, Daison C, et al. Anti-U-like as an alloantibody in S-s-U- and S-s-U+^{var} black people. Transfusion 2011; **52**: 622-8.
14) 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.
15) Mota MA, Aravechia MG, Dezan MR, et al. Patients with -33C mutation can safely receive Fy(b+) blood units [abstract]. Transfusion 2007; **47** (Suppl S3): 162A.
16) Hue-Roye K, Hipsky CH, Velliquette RW, et al. A novel RHCE*ce 48C, 733G allele with nucleotide 941C in exon 7 encodes an altered red blood cell e antigen. Transfusion 2011; **51**: 32-5.
17) Nance SJ, Arndt P, Garratty G. Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay. Transfusion 1987; **27**: 449-52.
18) Fasano RM, Monaco A, Meier ER, et al. RH genotyping in a sickle cell disease patient contributing to hematopoietic stem cell transplantation donor selection and management.Blood 2010; **116**: 2836-8.
19) Keller MA, Horn, T, Crowley, J, et al. Genotype compatibility tables for matching patients and donors for Rh variants [abstract]. Transfusion 2013; **53** (Suppl S2): 174A.
20) Castilho L, Sippert EA, Gaspardi, AC, et al. Matching sickle cell disease patients and donors for Rh variants [abstract]. Vox Sang 2014; **107** (Suppl S1): 192.
21) Garratty G. Do we need to be more concerned about weak D antigen? Transfusion 2005; **45**: 1547-51.
22) Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype. Transfusion 2015; **55**: 680-89.
23) da Costa DC, Pellegrino J Jr, Guelsin GA, et al. Molecular matching of red blood cells is superior to serological matching in sickle cell disease patients. Rev Bras Hematol Hemoter 2013; **35**: 35-8.
24) Sesok-Pizzini DA, Friedman DF, Smith-Whitley K, Nance SJ. Transfusion support of patients with sickle cell disease at the Children's Hospital of Philadelphia. Immunohematology 2006; **22**: 121-5.
25) Hillyer KL, Hare VW, Josephson CD, et al. Partners for life: the transfusion program for patients with sickle cell disease offered at the American Red Cross Blood Services, Southern Region, Atlanta, Georgia. Immunohematology 2006; **22**: 108-11.
26) Isaak EJ, LeChien B, Lindsey T, Debaun MR. The Charles Drew program in Missouri: a description of a partnership among a blood center and several hospitals to address the care of patients with sickle cell disease. Immunohematology 2006; **22**: 112-6.
27) Nance ST, Keller MA. Comments on: molecular matching of red blood cells is superior to serological matching in sickle cell disease patients. Rev Bras Haematol Hemoter 2013; **35**: 9-11.
28) International Society of Blood Transfusion Working Party on red cell immunogenetics and blood group terminology. Available at: http://www.isbtweb.org/nc/. Accessed on 12/03/2015.
29) Kacker S, Vassallo R, Keller MA, et al. Financial implications of *RHD* genotyping of pregnant women with a serologic weak D phenotype. Transfusion 2015; **55**: 2095-103.
30) Singleton BK, Green CA, Avent ND, et al. The presence of an RHD pseudogene containing 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group. Blood 2000; **95**: 12-8.

Arrived: 16 March 2015 - Revision accepted: 22 June 2015
Correspondence: S. Gerald Sandler
Department of Pathology and Laboratory Medicine
MedStar Georgetown University Hospital
3800 Reservoir Road, NW
Washington, DC 20007, USA
e-mail: Sandlerg@gunet.georgetown.edu

All rights reserved - For personal use only
No other use without premission