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Implementing mass-scale red cell genotyping at a blood center

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Abstract

Background—When problems with compatibility beyond ABO and RhD arise, currently transfusion services search their inventories and perform time-consuming serologic testing to locate antigen-negative blood. These clinically important blood group antigens can be detected reliably by red cell genotyping, which is a technology whereby DNA-based techniques are used to evaluate gene polymorphisms that determine the expression of red cell antigens. We introduced mass-scale genotyping and measured availability of genotyped blood.

Study design and methods—All non-Caucasian donors qualified for genotyping along with Caucasian donors who had a history of repeat donation. Mass-scale red cell genotyping, performed on an electronic interfaced open array platform, was implemented to screen blood donors for 32 SNPs that predicted 42 blood group antigens. Genotype screening results were confirmed by phenotyping, when needed for antigen-negative transfusion, prior to release of the red cell unit.

Results—Approximately 22,000 donors were red cell genotyped within 4 months and a total of 43,066 donors in 4 years. There were 463 discordances (0.52% of 89,596 genotypes with a phenotype). Among the 307 resolved discordances, approximate equal numbers represented historical serological or genotyping discrepancies (n=151 and n=156, respectively). In the final year of the study, an average of 29% of the daily inventory had a genotype.

Conclusions—Red cell genotyping of blood donors using an electronic interface created a large and stable supply of red cell units with historical genotypes. The database served the needs of antigen-negative blood requests for a large regional blood center, and allowed us to abandon screening by serology.

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Statement of Disclaimer: The views expressed do not necessarily represent the view of the National Institutes of Health (NIH), the Department of Health and Human Services (DHHS), or the U.S. Federal Government.

At the time the red cell genotyping described in this article was implemented, none of the molecular immunohematology assays for red cell genotyping had been approved by the US Food and Drug Administration (FDA).

Conflict of interest disclosure: GAD is the inventor of European patents on red cell genotyping owned by Canadian Blood Services. WAF & JLG do not have a conflict of interest relevant to this article.

Authorship contribution: GAD coordinated red cell genotyping, and collected and summarized the data. JLG contributed to the content of the discussion. GAD and WAF conceptualized the study, analyzed the data, and wrote the manuscript.

Introduction

Alloimmunized transfusion recipients require antigen-negative blood for safe transfusion. Blood centers are best suited to provide antigen-negative blood because they have the entire regional blood inventory at their disposal. Current practice to provide blood for transfusion recipients relies on phenotyping to confirm that red cell units lack the cognate antigen to which the patient's alloantibody is binding. Hemagglutination is still considered the 'gold standard' method for red blood cell (RBC) antigen typing by many serologists[1] notwithstanding several important drawbacks; some clinically significant antigens cannot be identified by phenotyping and some variant antigens are known to be consistently mistyped as antigen-negative.

It is now possible to determine a far greater number of antigens by genotyping than by serology,[2-4] which if performed on a mass-scale could provide access to a larger supply of antigen-negative blood. Several groups have evaluated red cell genotyping platforms for the potential to genotype large numbers of samples. Testing volumes range from dozens[5-7] to several hundred[8-15] to thousands of samples.[16-22] Because it is considered not economically feasible to genotype all blood donors, selection criteria can be used to maximize the database. Furthermore, blood donors who have been genotyped on a previous donation have historical genotype information that may be utilized without the need for re-testing.

We developed a red cell genotyping process in 2008[14] and implemented a mass-scale program in 2010.[23] The availability of red cell units with a genotype was evaluated over the following three years, which provided genotyped inventory to support blood requests.

Methods

Blood donors

All whole blood donors who declared their ethnicity on the donation questionnaire as Asian, African American, Hispanic, or Native American qualified for red cell genotyping regardless of their ABO/Rh blood group or frequency of donation. In addition, Group O, A, and B whole blood donors qualified if they had a history of at least 3 donations in the previous 3 years, with one donation in the previous 12 months. Occasionally, group AB donors who met the historical donation criteria were included. Blood donors gave informed consent and BloodCenter of Wisconsin's Institutional Review Board approved the retrospective red cell phenotype and genotype review (BCW 12-27).

Red cell genotyping

DNA was extracted from EDTA-anticoagulated whole blood (QIAamp 96 DNA Blood Kit; Qiagen, Valencia, CA). A nanofluidic open array system using real-time PCR-fluorogenic 5' nuclease TaqMan chemistry (OpenArray Real-Time PCR System; Life Technologies Corporation, Grand Island, NY) was used as described previously.[14,23] The analysis was expanded to interrogate 32 single nucleotide polymorphisms (SNPs), which allowed for the prediction of 42 blood group antigens: C, E, c, e, V, hr^S,[24] VS, hr^B,[25] Crawford; M, N, S, s, U; Lu^a, Lu^b, Lu8, Lu14; K, k, Kp^a, Kp^b, Js^a, Js^b; Fy^a, Fy^b; Jk^a, Jk^b, Jk3; Di^a, Di^b; Yt^a, Yt^b; Sc1, Sc2; Do^a, Do^b, Hy, Jo^a; Co^a, Co^b; and Cr^a. On June 28, 2012, Polynesian/Finn Jk null

genotyping was discontinued since no Jk null donors were identified and U^{w+} testing was instituted. Genotypes were displayed using ISBT nomenclature allowing the operator to distinguish them from phenotypes (Table 1). Another 14 antigens that we encountered for antigen-negative blood requests were identified by phenotyping (Table 2).

Blood sample barcodes were used to create a traceable process from sample selection to results output. Instrument output was reviewed electronically by medical technologists. Control samples of known genotypes were tested in each run except for examples of rare homozygous genotypes. Individual results were excluded by assigning a 'no call' when values were below a defined fluorescence or were outside instrument-defined genotype clusters. The no call rate performed on 12,376 samples ranged from 0.28% to 1.60% (mean 0.63%, median 0.60%), with the exception of Yt^a/Yt^b (3.4%), Lu^a/Lu^b (3.9%), and Lu8/14 (5.3%). The instrument output file was electronically translated into ISBT genotypes using an electronic 'rules' logic table.

Red cell genotype database

Genotype results were electronically transferred to a database, and were displayed alongside historical phenotypes using the blood center computer system (LifeTrak; Mediware Information Systems, Oak Brook, IL). All genotypes were compared to the existing historical phenotypes. The output of the comparison created a report of genotype-phenotype discordances.

Antigen-negative blood requests and phenotyping

A request for antigen-negative blood was considered a single patient 'encounter' regardless of the number of red cell units desired. All genotype results were confirmed by serologic phenotyping before the release of a red cell unit, when needed for antigen-negative transfusion.

Results

Donor accrual started on January 2, 2010. Routine red cell phenotype screening was discontinued for non-Caucasian donors (C, U, and Js^b antigens) and Caucasian donors (Lu^b, Yt^a, and Co^a antigens) on July 17, 2010 because it had been replaced by mass-scale genotype screening.

Red cell genotyping of donors

Among 202,275 individuals who successfully donated a unit of whole blood from 2010 and 2013 inclusive, 79,864 donors matched the criteria for self-declared ethnicity or historical donation data, of which 43,066 (54% of qualified donors) were actually genotyped. A total of 209,540 red cell units had a red cell genotype, representing 32% of all red cell units. These donors provided an average of 2.1 red cell units per year. The database consisted of 24,332 blood donors genotyped in 2010, with 8186, 4498, and 6050 donors genotyped in 2011, 2012 and 2013, respectively (Fig. 1).

We found 463 discordant results (0.52%) among 89,596 genotypes with a historical phenotype (Table 1). The serology performed on a repeat donation sample revealed 151 (32%) data entry or serologic typing errors that included 1 E (*RHCE*03.04*), 2 Kell modifier (*KEL*02M*), and 10 Fy(b^{w+}) (*FY*02M.01*) alleles. The serology was confirmed to be correct for 156 discordant results (34%). Among them, 32 samples (7%) represented variant alleles that included 16 S-silencing (*MNS*03N.01/MNS*03N.03*), 7 S-deletional (*GYPB*01N*) and 9 e (*RHCE*ceMO*) alleles. There were another 156 discordant results (34%) awaiting resolution because the donor had not yet returned for repeat serologic typing.

Availability of red cell units with genotype

We evaluated genotype information among red cell units in 2013. The blood center collected 151,727 whole blood donations of which 45,681 had a genotype. The monthly number of donations with a red cell genotype remained stable at 29% to 32% (Fig. 2). To meet transfusion recipient needs during 2011-2013, we imported 34 rare red cell units from other blood centers for 11 of 5672 patient encounters.

Discussion

Red cell genotyping of blood donors has been shown to be feasible to screen for both common and rare blood group antigens.[19-22,26] Red cell genotyping is a technology whereby DNA-based techniques are used to evaluate genes for the particular single and multiple nucleotide substitutions, deletions, insertions, and gene conversions that determine the expression of red cell antigens. The prime utility for donor testing rests with the large number of samples and the vast number of antigens that can be genotyped in a short period. Evidence of repeat and recent donation has been used in donor screening programs to maximize antigen-negative blood inventory.[18,22,27]

We performed mass-scale red cell genotyping on over 22,000 samples during the initial 14 weeks of testing and at a lower level thereafter to maintain the number of active donors in our red cell genotype database (Fig. 1). Single determinations using TaqMan chemistry were preferred over the cost of running duplicates or triplicates to gain more accuracy, because our red cell genotyping was designed as screening tool and the phenotype was confirmed before transfusing any red cell unit. Given the low rate of discordances (0.52%), which is consistent with previously published data,[6,9,11,14,15,19,21] we had sufficient access to correctly genotyped red cell units to meet the demand for antigen-negative blood. For the 3 years following the implementation in 2010, only 34 red cell units were imported for a blood center that distributes 150,000 red cell units annually to 63 hospitals serving a population of 3.78 million. Recruitment of genotyped donors was not attempted except when we needed to import blood. It will be important to identify the number of future donations by ethnicity and to apply a recruitment strategy to address our gap to provide rare units. The vast majority of patient encounters (5661 equaling 99.8%) were quickly met from our blood center inventory by using the database and confirming the phenotype of genotyped red cell units, if needed, without the delay and expense of shipping red cell units from other blood centers. Our database was applied successfully to serve the needs of transfusion recipients for antigen-negative blood.

The genotyping program provided a steady supply of red cell units with red cell genotype data (Fig. 2). The low rate of genotype-phenotype discordances was caused by roughly equal numbers of phenotypes and genotypes (Table 1). However, a large number of *RH2* genotyping errors were observed. These and other low frequency genotyping errors may be a function of the assay chemistry used in combination with the nano-volume of DNA dispensed by the instrument as it is known for limiting dilution assays. The red cell genotyping program incorporated a fully computerized workflow process, from DNA extraction and genotyping electronic worksheets to results review, data handling and error reports. The entire process can be completed within 36 hours, consistent within the turnaround time of infectious disease testing, and the throughput capacity can handle more 700 samples in a single day. Our next step is to validate red cell genotyping as a test-of-record such that the need for serologic confirmation may be eliminated. A network of similarly mass-scale genotyped donors among US blood centers would create a large real-time repository and facilitate rapid access to antigen-negative blood nationwide.

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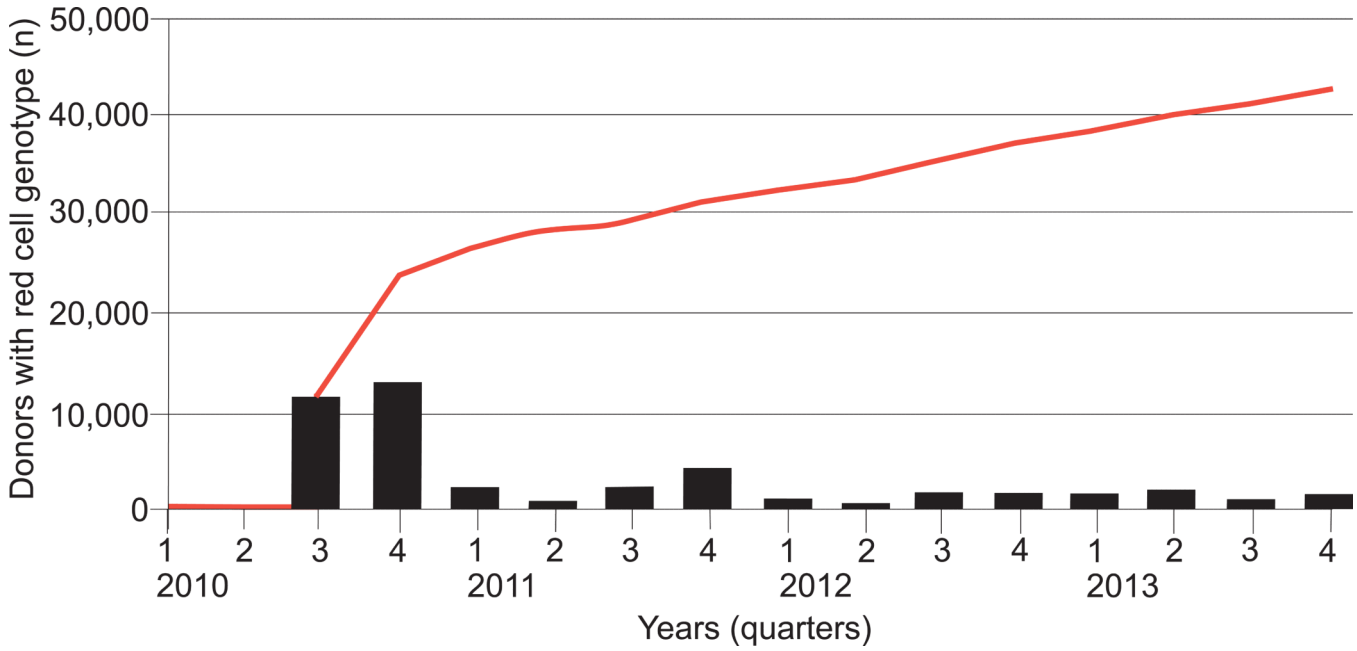

Figure 1.

Red cell genotyping of blood donors. The number of blood donors genotyped (black columns) and the cumulative number of donors with a genotype (red line) are shown from 2010 to 2013. DNA extraction started in January 2010 and genetic testing in July 2010. Red cell genotyping is continuing in monthly batches.

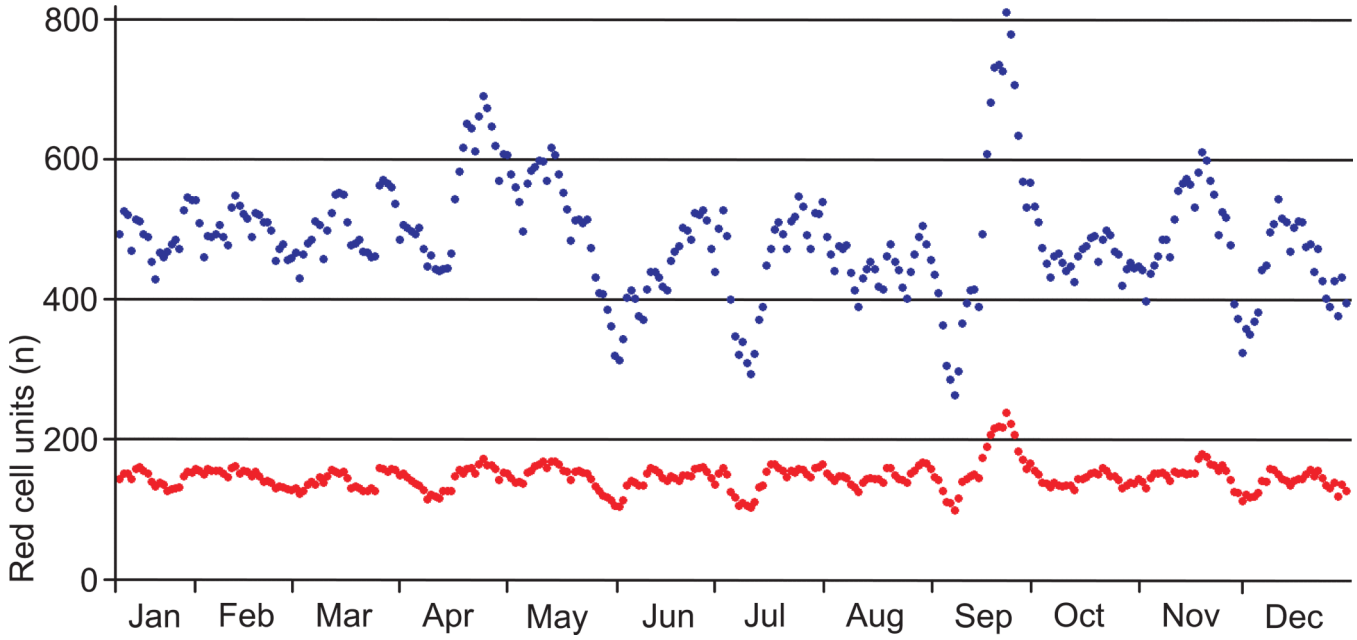

Figure 2.

Red cell units donated in the blood center in 2013. Blood was collected on 314 days. The collection numbers of a sliding window for 7 collection days are shown (mean, blue dots). The numbers of available red cell units with known genotype are indicated (mean, red dots). Overall, the percentage averaged 29.4% of the inventory throughout 2013.

Table 1

Blood group antigens by common name and ISBT terminology, which were genotyped

Antigen	ISBT terminology		Genotypes with a phenotype	
	Number	Symbol	All	Discordances
M	002.001	MNS1	924	5
N	002.002	MNS2	833	8
S	002.003	MNS3	4168	72
s	002.004	MNS4	3104	10
U	002.005	MNS5	na	na
C	004.002	RH2	8793	126
E	004.003	RH3	11534	36
c	004.004	RH4	4713	3
e	004.005	RH5	4702	30
V	004.010	RH10	77	4
hrS	004.019	RH19	na	na
VS	004.020	RH20	na	na
hrB	004.031	RH31	na	na
Crawford	004.043	RH43	na	na
Lu^a	005.001	LU1	260	8
Lu^b	005.002	LU2	3788	19
Lu8	005.008	LU8	18	0
Lu14	005.014	LU14	na	na
K	006.001	KEL1	12174	14
k	006.002	KEL2	609	0
Kp^a	006.003	KEL3	428	0
Kp^b	006.004	KEL4	4541	0
Js^a	006.006	KEL6	117	5
Js^b	006.007	KEL7	4314	5
Fy^a	008.001	FY1	4856	19
Fy^b	008.002	FY2	3376	25
Jk^a	009.001	JK1	4495	11
Jk^b	009.002	JK2	4102	23
Jk3	009.003	JK3	na	na
Di^a	010.001	DI1	1653	7
Di^b	010.002	DI2	102	1
Yt^a	011.001	YT1	3342	15
Yt^b	011.002	YT2	6	1
Sc1	013.001	SC1	1	0
Sc2	013.002	SC2	9	0
Do^a	014.001	DO1	57	11
Do^b	014.002	DO2	49	4

Antigen	ISBT terminology		Genotypes with a phenotype	
	Number	Symbol	All	Discordances
Hy	014.004	DO4	35	0
Jo	014.005	DO5	na	na
Co[a]	015.001	CO1	1820	1
Co[b]	015.002	CO2	593	0
Cr[a]	021.001	CROM1	3	0
Total	na	na	89,596	463

na, not applicable/available

Table 2

Blood group antigens by common name and ISBT terminology, which were phenotyped but not genotyped

Antigen*	ISBT Terminology	
	Number	Symbol
He	002.006	MNS6
'N'	002.030	MNS30
P1	003.001	P1PK1
P^k	003.003	P1PK3
f†	004.006	RH6
C^w	004.008	RH8
Go^a	004.030	RH30
Le^a	007.001	LE1
Le^b	007.002	LE2
Wr^a	010.003	DI3
Xg^a	012.001	XG1
H	018.001	H
Tc^a	021.002	CROM2
Vel	034.001	VEL1

*All antigens could be genotyped.

†Lack of the f antigen expression can be deduced by a negative phenotyping result for the c or e antigens.