VoxSanguinis
The International Journal of Transfusion Medicine

ISBT International Society of Blood Transfusion

Vox Sanguinis (2012) **102**, 234–242

DOI: 10.1111/j.1423-0410.2011.01542.x

ORIGINAL PAPER

High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors

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Background and Objectives One to two per cent of patients in need of red cell transfusion carry irregular antibodies to red blood cell (RBC) antigens and have to be supplied with specially selected blood units. To be able to respond to those requests, blood centres have to screen a significant number of donors for a variety of antigens serologically, which is a costly and through the shortage of reagents, also limited procedure. To make this procedure more efficient, the Austrian Red Cross has developed a genotyping assay as an alternative approach for high throughput RBC typing.

Materials and Methods A multiplex polymerase chain reaction (PCR) assay was designed for typing 35 RBC antigens in six reaction mixes. The assay includes both common as well as high-frequency-alleles:

MNS1, *MNS2*, *MNS3* and *MNS4*; *LU1*, *LU2*, *LU8* and *LU14*; *KEL1*, *KEL2*, *KEL3*, *KEL4*, *KEL6*, *KEL7*, *KEL11*, *KEL17* and *KEL21*; *FY1*, *FY2*, FYB^{WK} and *FY0* (FYB^{ES}); *JK1* and *JK2*; *DI1*, *DI2*, *DI3* and *DI4*; *YT1* and *YT2*; *DO1* and *DO2*; *CO1* and *CO2*; *IN1* and *IN2*.

The assay was validated using 370 selected serologically typed samples. Subsequently 6000 individuals were screened to identify high frequency antigen (HFA)-negative donors and to facilitate the search for compatible blood for alloimmunized patients.

Results All controls showed complete concordance for the tested markers. The screening of 6000 donors revealed 57 new HFA-negative donors and the blood group database was extended by approximately 210 000 results.

Conclusion The study shows that in practice, this high-throughput genotyping assay is feasible, fast and provides reliable results. Compared to serological testing, this molecular approach is also very cost-efficient.

Received: 10 February 2011,
revised 3 July 2011,
accepted 8 August 2011,
published online 21 November 2011

Key words: blood groups, genotyping, high throughput testing, immunohematology, molecular testing, RBC antigens and antibodies.

Introduction

On average, 1–2% of the hospital based patients in Europe and North America who are in need of blood transfusions carry irregular alloantibodies to red cell antigens. Immunization is usually caused by previous transfusions or pregnancies [1–4]. The incidence of alloimmunization during pregnancy is approximately 0·24% [5]. In pre-transfused patients, the antibody prevalence is higher [1, 4]. The rate of alloimmunization and the number of antibody specificities involved correlate with the number of transfusion events in the past. Thus, in patients who are chronically transfusion-dependent the alloimmunization rate is especially high. In adult patients with sickle cell anaemia the

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alloimmunization rate was described to be 47% [6] and in Thalassemia major patients a prevalence of about 30% was observed [7, 8]. Regarding polytransfused patients, some authors also discuss an extended matching of minor antigens to prevent further alloimmunization [8, 9] for these patient groups.

While most problems in polytransfused patients arise from multiple antibody specificities directed against several antigens, antibodies to high-frequency antigens (HFAs) represent another serious problem. Approximately one-third of patients with alloantibodies to HFAs cannot be supplied with compatible blood units in time [10].

The blood supply for carriers of multiple red cell antibodies and/or antibodies against HFAs with antigen-negative blood is a challenge for blood centres. Hundreds to thousands of samples have to be screened to identify one appropriate donor or blood unit.

To be able to provide compatible blood units within short term, blood centres usually serologically type a subset of donors for relevant minor antigens beside ABO, RhD and Kell.

In practice, this 'extended' phenotyping is limited by availability and costs of CE-certified serological reagents. Monoclonal antibodies are accessible for phenotyping of several antigens. For other clinically significant antigens only limited or insufficient amounts of quality assured polyclonal sera are commercially available.

To resolve these restrictions of serology, new approaches for extended antigen typing and the identification of HFA-negative blood donors are sought. The key requirements for such alternatives are independence from availability of serological reagents, the possibility of automated high-throughput screening and cost-efficiency.

In principle, all antigens which correspond to known DNA-polymorphisms are accessible for genotyping. This applies for most RBC antigens. In contrast to serology, there is no shortage of reagents in molecular typing.

Recently, several working groups have demonstrated the feasibility and the advantages of different technical methods and platforms for high-throughput genotyping of RBC antigens [11–18]. However, these approaches also have several disadvantages, where perhaps the most important are the high testing costs, which still prevent a routine application in blood donor screening.

From this background, the aim of our work was to develop a reliable low-cost, high-throughput molecular testing design tailored to the needs of blood centres for large-scale donor screening. In addition, such testing might also be applicable in patient testing and screening for cellular reagents for antibody identification.

Material and methods

Target polymorphism selection

As a result of our assessment of antibody prevalence, clinical significance of the antibody and antigen frequency, the following 35 alleles were included into the multiplex screening panel: in the MNS blood group system, the genotypes for *MNS1* ('M'), *MNS2* ('N'), *MNS3* ('S') and *MNS4* ('s') [19, 20]; in the Lutheran system *LU1* ('Lu^a'), *LU2* ('Lu^b'), *LU8* and *LU14* [21]; in the Kell system *KEL1* ('K'), *KEL2* ('k'), *KEL3* ('Kp^a'), *KEL4* ('Kp^b'), *KEL6* ('Js^a'), *KEL7* ('Js^b'), *KEL11*, *KEL17* and *KEL21* [22, 23]; in the Duffy system *FY1* ('Fy^a'), *FY2* ('Fy^b') [24], one *FyX* allele [25, 26] and a *Fy0* allele (−33 promoter silencing polymorphism) [27, 28]; in the Kidd system *JK1* ('Jk^a') and *JK2* ('Jk^b') [29]; in the Diego system *DI1* ('Di^a'), *DI2* ('Di^b'), *DI3* ('Wr^a') and *DI4* ('Wr^b') [30, 31]; in the YT ('Cartwright') system *YT1* ('Yt^a') and *YT2* ('Yt^b') [32]; in the Dombrock system *DO1* ('Do^a') and *DO2* ('Do^b') [33]; in the Colton system *CO1* ('Co^a') and *CO2* ('Co^b') [34] and the *IN1* ('In^a') and *IN2* ('In^b') polymorphism of the Indian blood group system [35].

ABO and Rh antigens were not included in the assay because most blood centres routinely perform automated serological testing for ABO and RhD as well as for C, c, E and e.

Samples

Ethylenediaminetetraacetate (EDTA)-anticoagulated blood samples or buffy coats of donors or patients were used consecutively to routine testing consistent with our institutions' guidelines. Genomic DNA extraction was performed on a Tecan RSP150 platform using the QIAamp® DNA Blood BioRobot 9604 Kit (Qiagen, Hilden, Germany) in 96-well microplate format. Alternatively, DNA was extracted manually with QIAamp® DNA Blood Mini Kit (Qiagen). DNA concentration in eluate was 34·8 ng/μl on average (n = 84, standard deviation 18·2). For validation of the assay, a number of DNA samples of individuals with rare phenotypes provided by other centres were included.

Phenotyping

Serological typing was performed with CE-certified reagents (DiaMed AG, Cressier; Ortho-Clinical Diagnostics, Neckargemuend; and Medion Diagnostics GmbH, Duedingen) according to the manufacturers' instructions. Rare blood types were partly typed with sera from patients with antibodies of confirmed specificities. Wherever possible, antigen specificities were confirmed by at least two different reagents.

Table 1 PCR primers used in the 35-allele multiplex PCR

Allele	Polymorphism-specific primer	Sequence (5′–3′)[a]	Reverse primer	Sequence (5′–3′)
MNS1	GYPA-M-72T-f1	CAGCATCAAGTACCACTGGT	GYPA-i2-r1	TTCAGAGGCAAGAATTCCTCCA
MNS2	GYPA-N-72G-f1	TCAGCATTAAGTACCACTGAG	GYPA-i2-r1	TTCAGAGGCAAGAATTCCTCCA
MNS3	GYPB-S-127T-r1	CGATGGACAAGTTGTCCCA	GYPB-i2-f1	GGAGTAATGGCTCCATATGCC
MNS4	GYPB-s-127C-r1	CGATGGACAAGTTGTCCCG	GYPB-i2-f1	GGAGTAATGGCTCCATATGCC
LU1	LU1-230A-f1	CGGGAGCTCGCCCgCA	LU-x4-r1	GACAGTGTCCCTTTGTTGGGG
LU2	LU2-230G-f1	CGGGAGCTCGCCCgCG	LU-x4-r1	GACAGTGTCCCTTTGTTGGGG
LU8	LU8-611T-f1	CTCTCCCAGAGGGCTACAT	LU-i6-r2	GCTTGTGCGACCAATTGAGG
LU14	LU14-611A-f1	CTCTCCCAGAGGGCTACAA	LU-i6-r2	GCTTGTGCGACCAATTGAGG
KEL1	KEL1-578T-r3	ACTCATCAGAAGTCTCAGCA	KEL-i5-f1	CTAGAGGGTGGGTCTTCTTCC
KEL2	KEL2-578C-r2	CTCATCAGAAGTCTCAGCG	KEL-i5-f1	CTAGAGGGTGGGTCTTCTTCC
KEL3	KEL3-841T-f3	TGTCAATCTCCATCACTTCAT	KEL-i9-r2	CTGCCCGCACAGGTGGC
KEL4	KEL4-841C-842G-f5	CAATCTCCATCACTTCACG	KEL-i9-r2	CTGCCCGCACAGGTGGC
KEL6	KEL6-1790C-f4	GCCTGGGGGCTGCCC	KEL-i18-r1	CTTGCTCACTGGTTCTGC
KEL7	KEL7-1790T-f9	GGGGGCTGCCT	KEL-i18-r1	CTTGCTCACTGGTTCTGC
KEL11	KEL11-905T-r4	GAGCTGGTCGATAGTGA	KEL-i7-f3	CTCTTCTTCTCATGCCCCTC
KEL17	KEL17-905C-r5	AGCTGGTCGATAGTGG	KEL-i7-f3	CTCTTCTTCTCATGCCCCTC
KEL21	KEL21-842A-f5	CAATCTCCATCACTTCACA	KEL-i9-r2	CTGCCCGCACAGGTGGC
FY1	FY1-42(1)-as	CAGCTGCTTCCAGGTTGGgAC	FYES-p-46T-f1	CTCATTAGTCCTTGGCTCTTAT
FY2	FY2-42(1)-as	CAGCTGCTTCCAGGTTGGgAT	FYES-p-46T-f2	TCATTAGTCCTTGGCTCTTAT
FY0	FYES-p-46C-f1	CTCATTAGTCCTTGGCTCTTAC	FY2-42(1)-as	CAGCTGCTTCCAGGTTGGgAT
FYX	FYX-265T-f2	GCTTTTCAGACCTCTCTTCT	FY-x2-r3	CAAATTCCCACAGTGAGC
JK1	JK1-838G-f1	GTCTTTCAGCCCCATTTGcGG	JK-i8-r3	CCAAGGCCAAGTGTCAGTGC
JK2	JK2-838A-f2	AGTCTTTCAGCCCCATTTGcGA	JK-i8-r3	CCAAGGCCAAGTGTCAGTGC
DI1	DI1-2561T-r4	GGGCCAGGGAGGCCA	DI-i17-f3	TGGCTCCATATGGTGCCTG
DI2	DI2-2561C-r6	CCAGGGAGGCCG	DI-i17-f3	TGGCTCCATATGGTGCCTG
DI3	DI3-1972A-r4	CATCATCCAGATGGGAAACTT	DI-x16-f1	GGACTACCTCTACCCCATCC
DI4	DI4-1972G-r5	ATCATCCAGATGGGAAACTC	DI-x16-f1	GGACTACCTCTACCCCATCC
YT1	YT1-1151(1)-s	CTCATCAACGCGGGAGACTaCC	YT-1409-as	GAGCCAGAGAGATGAACAGTT
YT2	YT2-1151(1)-s	CTCATCAACGCGGGAGACTaCA	YT-1409-as	GAGCCAGAGAGATGAACAGTT
DO1	DO1-624T-f1	ATTCGATTTGGCCAATTCCTT	DO1-793A-r1	TGACCTCAACTGCAACCAGTT
DO2	DO2-624C-f1	ATTCGATTTGGCCAATTCCTC	DO2-793G-r1	GACCTCAACTGCAACCAGTC
CO1	CO1-134C-f4	GAACAACCAGACGGC	CO-x1-r1	CTGAGAGGATGGCGGTGG
CO2	CO2-134T-f3	GGGAACAACCAGACGGT	CO-x1-r1	CTGAGAGGATGGCGGTGG
IN1	IN1-137C-f2	GTCGCTACAGCATCTCTCC	IN-i2-r1	CCATTCAGCTGTGGGAAAGGAGC
IN2	IN2-137G-f3	CGCTACAGCATCTCTCG	IN-i2-r1	CCATTCAGCTGTGGGAAAGGAGC

[a]Nucleotides written in small letters mark mismatches to the complementary genomic DNA sequence.

Multiplex PCR

With regard to cost-efficiency, we choose a conventional qualitative PCR. The test for 35 genotypes is carried out in six multiplex reaction mixes consisting of up to seven different amplification targets per mix. The PCR products are analysed subsequently using agarose gel electrophoresis. The assay also detects genotypes related to 12 HFAs. The high-incidence SNPs are included in every PCR reaction and serve as internal controls. By combining two or three HFA-related alleles with alleles of average or low frequency in one reaction mix, the results on the gel are clearly separated by the lines or grid of the high-frequency bands and can easily be interpreted.

The test system, including the extraction step, was set up end-to-end in 96-well microplate format to enable scalability and high throughput and to minimize the use of consumables. Sixteen individuals per microplate can be typed for 35 genotypes each. This is equivalent to 560 single tests on one microplate.

Oligonucleotide design and assortment of the specific genetic polymorphisms were carried out using the 'Blood Group Antigen Gene Mutation Database' (NCBI dbRBC; http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home) and the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore), both hosted by the National Center for Biotechnology Information (Bethesda, MD, USA) [36].

Table 2 PCR reaction mixes and product sizes

Oligo mix	Target allele	Polymorphism specific primer	Reverse primer	Product size (bp)	Primer conc. (nmol)
1	KEL21	KEL21-842A-f5	KEL-i9-r2	623	1
	DI4	DI4-1972G-r5	DI-x16-f1	538	1
	DI1	DI1-2561T-r4	DI-i17-f3	460	1
	LU2	LU2-230G-f1	LU-x4-r1	351	1
	FYX	FYX-265T-f2	FY-x2-r3	276	1
	IN2	IN2-137G-f3	IN-i2-r1	209	1
2	FY0	FYES-p-46C-f1	FY2-42(1)-as	713	1
	DI3	DI3-1972A-r4	DI-x16-f1	539	1
	DI2	DI2-2561C-r6	DI-i17-f3	457	1
	LU1	LU1-230A-f1	LU-x4-r1	351	1
	IN1	IN1-137C-f2	IN-i2-r1	211	1
3	KEL11	KEL11-905T-r4	KEL-i7-f2	522	1
	KEL6	KEL6-1790C-f4	KEL-i18-r2	422	1
	LU8	LU8-611T-f1	LU-i6-r2	364	1
	YT2	YT2-1151(1)-s	YT-1409-as	300	1
	CO1	CO1-134C-f4	CO-x1-r1	221	1
4	KEL17	KEL17-905C-r5	KEL-i7-f2	521	1
	KEL7	KEL7-1790T-f9	KEL-i18-r2	418	1
	LU14	LU14-611A-f1	LU-i6-r2	364	1
	YT1	YT1-1151(1)-s	YT-1409-as	300	1
	CO2	CO2-134T-f3	CO-x1-r1	223	1
5	FY1	FYES-p-46T-f1	FY1-42(1)-as	713	2
	KEL4	KEL4-841C-842G-f5	KEL-i9-r2	623	2
	JK1	JK1-838G-f1	JK-i8-r3	528	2
	MNS4	GYPB-s-127C-r1	GYPB-i2-f1	397	1
	KEL1	KEL1-578T-r3	KEL-i5-f1	322	1
	MNS1	GYPA-M-72T-f1	GYPA-i2-r1	259	1
	DO1	DO1-624T-f1[a]	DO1-793A-r1[a]	210	1
6	FY2	FYES-p-46T-f2	FY2-42(1)-as	712	2
	KEL3	KEL3-841T-f4	KEL-i9-r2	625	2
	JK2	JK2-838A-f2	JK-i8-r3	529	2
	MNS3	GYPB-S-127T-r1	GYPB-i2-f1	397	1
	KEL2	KEL2-578C-r2	KEL-i5-f1	321	1
	MNS2	GYPA-N-72G-f1	GYPA-i2-r1	260	1
	DO2	DO2-624C-f1[a]	DO2-793G-r1[a]	209	1

[a]Both primers are polymorphism specific.

Primer pairs, consisting of at least one sequence-specific primer (SSP) for the determination of the 35 selected polymorphisms, are shown in Table 1. PCR products lengths range from 209 to 713 bp.

The arrangement of multiplex PCR assay in six reaction mixes, containing five to seven primer pairs per mix, is displayed in Table 2. The oligonucleotide mixes were aliquoted in ready-to-use concentrations into 96-well PCR plates. The plates were stored at −20 °C until use.

The PCR was performed using the GoTaq® kit (Promega, Mannheim, Germany). Each reaction tube contained 0·75 U Taq, 3 μl of 5× PCR buffer, 2·5 μl of 25 mM $MgCl_2$ solution (4·17 nmol/μl), 0,33 mM desoxynucleoside triphosphates (Applichem, Darmstadt, Germany), oligonucleotide primers (Table 2), 35 μg of gDNA and PCR-grade water in a final reaction volume of 15 μl.

GeneAmp® dual-head PCR 9700 Systems (Applied Biosystems, Foster City, California, USA) were used for the PCR. The temperature profile started with five minutes at 94 °C followed by six cycles at 94 °C for 30 s, 67 °C for 40 s (touchdown for 0·5 °C per cycle) and 72 °C for 50 s. Subsequently, 27 cycles at 94 °C for 30 s, 64 °C for 40 s and 72 °C for 50 s were added. The protocol ended with a final step at 72 °C for two minutes.

Thereafter, the PCR products were separated on ethidium bromide-stained 1·5% agarose gels and subsequently analysed by two operators.

Assay validation

For validation of the multiplex PCR assay, DNA was extracted from 371 selected blood samples of blood donors. The red cells were serologically phenotyped for 13 antigens for which regulated reagents were available (MNS1, MNS2, MNS3, MNS4, LU1, LU2, KEL1, KEL2, KEL3, FY1, FY2, JK1, JK2). For the other antigens, the number of phenotyped controls was lower.

In addition, 99 selected samples with a KEL2-negative phenotype, two U-negative samples (MNS -2,-3), eight LU 1,-2 samples and one KEL 3,-4 sample from our local pool were included.

Several samples were typed serologically for KEL6, KEL7, KEL11, KEL17, DI1, DI2, DI3, DI4, YT1, YT2, DO1, DO2, CO1 and CO2.

DNA samples for the rare KEL genotypes *KEL*6/6* and *KEL*17/17* were supplied by S. Lee (New York Blood Center, Staten Island, NY, USA). DNA samples of the *LU*14/14*, *KEL*6/6*, *DI*1/1* and *CO*2/2* were given by J. Poole and G. Daniels (International Blood Group Reference Laboratory, Bristol, UK). Several rare LU8 and LU14 samples were serologically confirmed by the IBGRL. DNA *MNS-3,-4* were sent by M. de Haas (Sanquin, Amsterdam, the Netherlands). A DHARSHI cell line homozygous for *IN*2/2* was provided by M. Telen (Duke University, North Carolina, USA). Three FYX positive samples where sequenced for verification of the 271C>T and 304G>A SNP changes in the DARC-gene.

Unfortunately, no control samples for KEL21 were available.

Donor screening

DNA was extracted from EDTA-anticoagulated blood of 6000 blood donors, predominantly of ABO group O RhD-negative phenotype. The aim was to identify new HFA-

negative donors and to add all antigen typing results to our database. The screening character of genotyping data is documented and distinguished from the data produced by regulated reagents.

Cost calculation

Costs were calculated for typing 5500 donors per year with manual and automated serological approaches vs. the molecular typing assay described in this work. For each of the methods, the test costs (reagents and disposables), the personnel costs, the depreciation on investments (e.g., cyclers, pipettors and electrophoresis devices), the costs for occupancy and the facility infrastructure were summarized. Only 20 antigens, for which serological typing reagents are commonly available, were included in the calculation.

Results

Assay validation

For all samples and markers tested, there was complete concordance with the reference typing results. Detailed data are displayed in Table 3. A typing example is shown in Fig. 1.

Donor screening

The results of the donor genotyping of 6000 samples provided 209 650 acceptable results. In 0·014% of the reactions, the initial result was indeterminable for at least one allele and a rerun was applied. In ten cases, the result remained indeterminable for the *FyX* allele. We did not refer these donors to be Fy^b-negative and it was not further investigated.

The screening revealed 55 donors with a rare blood type. These individuals were lacking one of the 12 high-incidence antigens tested. We identified nine donors that are LU2-negative, five LU8-negative, five KEL2-negative, one KEL4-negative, 24 YT1-negative and eleven CO1-negative individuals. The results were always confirmed by serology. In all cases, there was concordance between serological testing and the PCR result. Most of these donors have been filed to the International Rare Donor Panel [37].

Twelve red cell units originating from these donors have been issued for transfusion to alloimmunized patients so far. In all of these instances, the phenotype was confirmed by serology and a red cell cross-match was performed.

Cost

It is difficult to compare costs for serologically typing 20 antigens to the 35 antigen genotyping assay. The cost substantially differs depending on infrastructure, overhead cost and hands-on time. In our case the cost for serological typing for 20 minor RBC antigens (M, N, S, s, P1, C^w, Lu^a, Lu^b, K, k, Le^a, Le^b, Kp^a, Kp^b, Fy^a, Fy^b, Jk^a, Jk^b, Wr^a, Co^b) related to reagents and disposables were 28·0 EUR. For the automated phenotyping approach the personnel costs were 5·0 EUR, and the depreciation and occupancy costs were 2·0 EUR per donor. Manual phenotyping causes personnel costs of 10·0 EUR, and a depreciation of less than one EUR per donor. Hence, the total cost for serological typing varied from 35·0 to 39·0 EUR per donor.

For the multiplex PCR assay costs for typing a single donor for 35 RBC antigens were 5·0 EUR for reagents and disposables (including costs for DNA extraction), 5·0 EUR for personnel costs, and further 5·0 EUR for depreciation on investments and occupancy. Our total cost for the genotyping approach is 15.0 EUR per donor.

For one single minor red cell antigen, this results in a total of 1·75 or 1·90 EUR for serological typing

Table 3 Controls tested serologically and by genotyping

	MNS1	MNS2	MNS3	MNS4	LU1	LU2	LU8	LU14	KEL1	KEL2	KEL3	KEL4	KEL6	KEL7	KEL11	KEL17	KEL21
Homozygous positive	133	69	52	171	348	8	4	1	89	332	1	365	3	13	0	1	0
Heterozygous	170	170	147	147	22	22	3	3	48	48	5	5	3	0	0	0	0
Negative	69	133	171	52	8	348	1	4	332	89	365	1	13	3	1	0	0
Total count	372	372	370	370	378	378	8	8	469	469	371	371	19	16	1	1	0
Concordance to reference typing (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	–

	FY1	FY2	FY0	FYX	JK1	JK2	DI1	DI2	DI3	DI4	YT1	YT2	DO1	DO2	CO1	CO2	IN1	IN2
Homozygous positive	77	124	7	0	101	91	2	13	0	3	4	12	0	0	13	5	1	0
Heterozygous	169	169	0	3	178	178	2	0	0	0	0	12	1	1	0	5	0	0
Negative	124	77	0	0	91	101	13	2	3	0	12	4	0	0	5	13	0	1
Total count	370	370	7	3	370	370	17	17	3	3	16	28	1	1	18	23	1	1
Concordance to reference typing (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

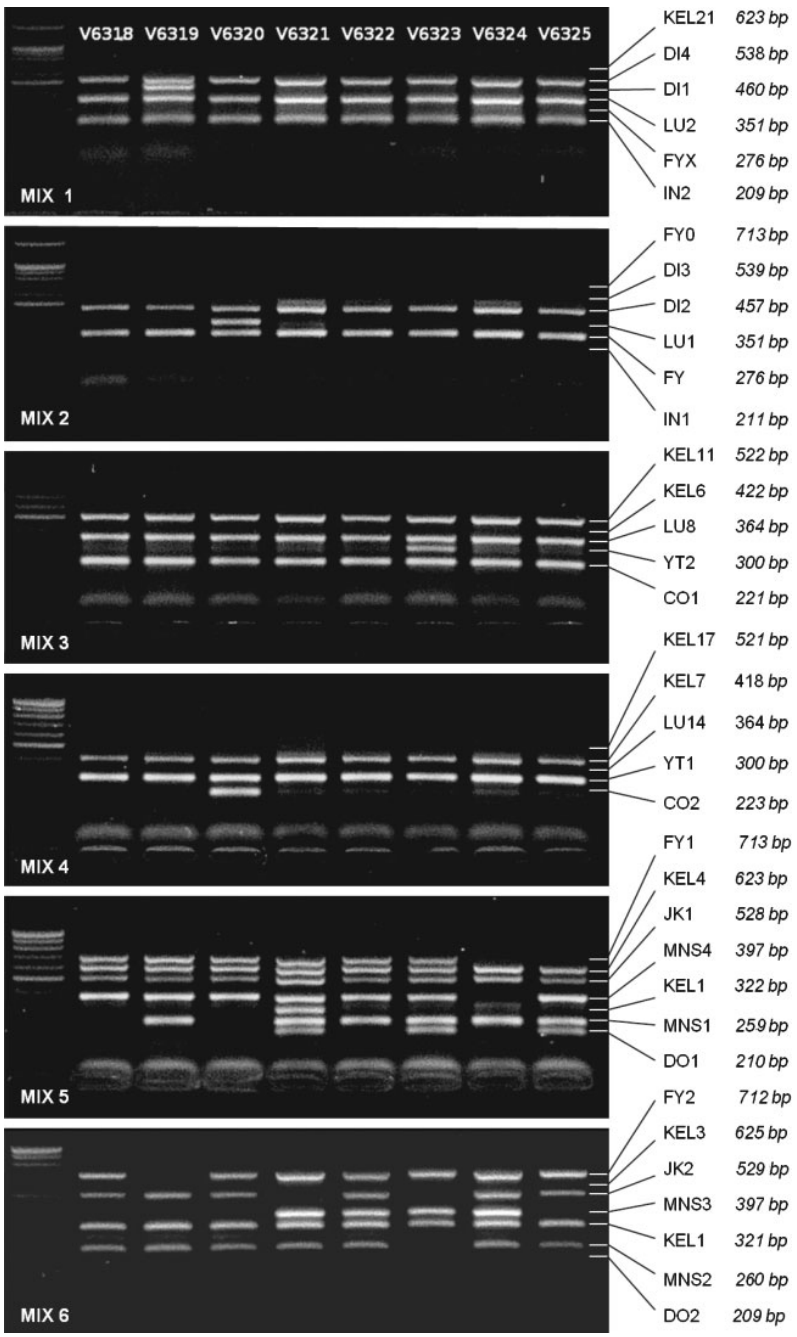

Fig. 1 Multiplex PCR RBC genotyping: ethidium bromide stained agarose gel. Example for typing results of eight individuals (columns: V6318-V6326). Each of the six reaction mixes (MIX1-MIX6) is designed to detect five to seven alleles. Designations of the alleles and the sizes of the PCR-products are indicated on the right border. A size marker is running in the left lane. As an example, the predicted phenotype of V6318 is: MNS:-1,2,-3,4; LU:-1,2,8,-14; KEL:-1,2,-3,4,-6,7,11,-17,-21; FY: -0,1,2; JK:1,2; DI:-1,2,-3,4; YT:1,-2; DO:-1,2; CO:1,-2; IN:-1,2.

(automated vs. manual) compared to 0·43 EUR for genotyping. The genotyping programme was, in our case, the most cost efficient way to generate a high number of antigen typing results for the donor database.

Discussion

Serological screening of blood donors for minor red cell antigens is an important but costly and, by shortage of reagents, also limited procedure. Several authors have

already published alternative procedures on the basis of molecular genetic typing techniques [11–16, 18, 38]. There are advantages and disadvantages to be considered with all alternatives.

Microarray applications enable simultaneous testing of thousands of polymorphisms in a single individual while recent applications only target a lower number (approximately 100 or less) of alleles. The costs of this technique are still unattainable. A further method is the real-time PCR, which also has the advantage of simple detection and analysis of the results.

In contrast to the above, conventional qualitative PCR enables testing at a comparably reasonable cost, but requires post-PCR steps which are difficult to automate. A crucial point is, that if testing and transfer of the results is not automated, transcription errors are likely to occur. In this case it is important to implement processes that compensate these potential errors. We currently follow the policy that double confirmation testing using regulated reagents, if available, is required if the antigen should be displayed on the label of a red cell unit or if a unit is to be released for a patient carrying RBC-alloantibodies. In the latter case a serological crossmatch is indispensible.

Furthermore an adequate IT solution is necessary to support the full integration of large-scale donor screening into the blood bank operation [39].

This genotyping project focused on applicability and cost efficiency. Disadvantages of qualitative PCR, such as limitations in the automation of the post-PCR detection, were accepted. The typing costs could be reduced to 23% or 25% for a single allele/antigen compared to automated or manual serological procedures. Furthermore, there are only approximately 20 minor antigens for which serological typing reagents are available on a regular base. An advantage of DNA based typing methods is the possibility to routinely screen for a broader spectrum of antigens than by phenotyping.

The continuous set-up and workflow in a 96-well format facilitates high-throughput testing in manual, as well as in automated platforms. Manually, approximately 100 individuals (equivalent to 3500 antigens) can be genotyped per day by one technician.

The result of assay validation was comparatively good [40]. Genotyping does not provide the immediacy of the antigen-antibody reaction. In the setting for donor screening, genotyping is focusing on one or a small number of SNPs to deduce a certain phenotype. The accuracy is dependant on the alleles included in the assay. Less frequent alleles that were not included, or any mutations beyond the primer binding sites that affect the gene expression, cannot be detected in this setting. Thus, 'mistypings', i.e. incorrect predictions of the red cell phenotype are possible.

The PCR-assay is also useful in patient typing. We already utilize it routinely as a complementary technique for antigen-typing in certain individuals with a positive direct antiglobulin test (DAT) as autoantibodies can constrain the usage of the indirect antiglobulin test (IAT) for antigen phenotyping when using polyclonal antisera.

The assay and typing results were also used for identification of suitable cells for antibody-differentiation and absorption procedures in patients with multiple antibody specificities. In case of an antibody to a HFA, the 12 HFAs included in the assay can give the clue to the corresponding antibody specificity if the patient is negative for a HFA.

Approaches for extensive phenotype matching in patients who are long-time transfusion dependent, that would effectively decrease alloimmunizations, were so far restricted by missing preconditions. High numbers of typed donors and adequate software solutions would be needed. Today, some standard IT solutions for blood centres already support extensive phenotype matching. In this context, the benefit seems to be accessible with reasonable effort.

Another possible application of the method described above is identifying suitable panel cells for serological antibody differentiation. Particularly HFA-negative or low-frequency antigen (LFA)-positive RBCs are useful for reference centres for immunohaematology to clarify uncommon antibody specificities.

To summarize, the method for blood donor red cell genotyping presented in this paper is a feasible alternative to other high-throughput donor RBC typing approaches. Despite some limitations, it can be deployed in most blood centres with reasonable investments. Using this method, could be a strategy to bridge the gap until higher resolutions techniques, such as microarray technology, are available at an affordable price.

Acknowledgements

We posthumously like to thank Peter Kirsten Rabitsch for his outstanding contribution to this project. We are grateful to the IBGRL, Bristol for their support with samples, and we also thank Martin Gollowitzer for programming the Tecan Genesis RSP150 platform for automated gDNA purification.

Conflict of interest

C.J. declares conflicting interests (EP10153395.8).

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