A Microsphere-Based Suspension Array for Blood Group Molecular Typing: An Update

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Keywords

Genotyping · Red blood cell antigens · Red blood cells

Summary

Background In a previous publication we described a method for Jk^a/Jk^b, Fy^a/Fy^b, S/s, K/k, Kp^a/Kp^b, Js^a/Js^b, Co^a/Co^b, and Lu^a/Lu^b genotyping based on a microsphere suspension array. Here, an improved version of the assay is presented. **Methods:** Two multiplex polymerase chain reactions (PCR) were developed: one for amplification of samples routinely tested and the other for those systems that are tested less frequently. Each biotinylated PCR product is hybridized in a single multiplex assay. A total of 2,020 samples were analyzed, and the genotypes were compared to the blood group phenotypes. **Results:** There have been no discrepancies with the serology results other than null and/or weak phenotypes. **Conclusion:** In its present form, the method presented here has the capacity to genotype hundreds of a samples in few hours with a high concordance rate with serology.

Schlüsselwörter

Genotypisierung · Erythrozytenantigene · Erythrozyten

Zusammenfassung

Hintergrund: In einer vorangegangenen Publikation beschrieben wir eine Methode für die Jk^a/Jk^b-, Fy^a/Fy^b-, S/s-, K/k-, Kp^a/Kp^b-, Js^a/Js^b-, Coa/Cob- und Lu^a/Lu^b-Genotypisierung basierend auf einem Mikrosphären-Suspensions-Array. An dieser Stelle präsentieren wir nun eine verbesserte Version dieses Assays. **Methoden:** Zwei Multiplex-Polymerase-Kettenreaktionen (Multiplex-PCR) wurden entwickelt: eine für die Amplifizierung der routinemäßig getesteten Proben, die andere für solche Systeme, die weniger häufig untersucht werden. Jedes biotiniliertes PCR-Produkt wird in einem einzelnen Multiplex-Assay hybridisiert. Insgesamt wurden 2020 Proben analysiert und deren Genotypen mit den Blutgruppenphänotypen verglichen. **Ergebnisse:** Außer Null- und/oder schwachen Phänotypen gab es keine Abweichungen zwischen den Genotypuntersuchungen und den serologischen Resultaten. **Schlussfolgerung:** In seiner derzeitigen Form hat die hier präsentierte Methode die Kapazität, hunderte von Proben in wenigen Stunden zu genotypisieren und dabei eine hohe Übereinstimmungsrate mit den serologischen Ergebnissen aufzuweisen.

Introduction

The molecular biology of red blood cell antigens and the benefits of molecular immunohematology, as compared to important limitations in hemagglutination-based testing, have been explained and described extensively in the last couple of years

by several authors [1–6]. Blood group genotyping is rapidly and successfully making its way into transfusion medicine. Various laboratories such as reference laboratories, donor centers and transfusion services are evaluating how to implement the newly emerging technologies and, most importantly, which molecular technique is best suited to their specific needs [4–6].

In a previous publication [4] we described the use of Luminex® xMAP™ system (Multi Analyte Profiling; Luminex Corporation, Austin, TX, USA) in genotyping red blood cell antigens. Here we present an improved version of the assay for Jk^a/Jk^b, Fy^a/Fy^b, S/s, K/k, Kp^a/Kp^b, Js^a/Js^b, Co^a/Co^b, and Lu^a/Lu^b genotyping.

Material and Methods

DNA Isolation

Genomic DNA was isolated from peripheral blood samples with a DNA blood kit (QIAamp 96, Qiagen GmbH, Hilden, Germany) or by automated extraction (GenoM-6, Qiagen GmbH).

DNA Amplification by Polymerase Chain Reaction (PCR)

Two multiplex PCR reactions were set up. The PCRs were divided on the basis of the frequency of use in our laboratory and, therefore, the utility of the reactions. PCR (I) amplified the systems for which samples were tested on a routine basis and PCR (II) amplified those systems that were tested less frequently (table 1). Both PCR reactions were carried out using the same PCR parameters as for the single PCRs. The amplification reaction consisted of 0.3 µmol/l of each primer where at least one primer of each primer pair was biotinylated at the 5' end, 4 µl of genomic DNA (100–400 ng), 0.2 mmol/l of dNTPs, 1.5 mmol/l of MgCl₂, 1.5 X PCR buffer (Applied Biosystem, Foster City, CA, USA) and 4 U of Taq polymerase (GoTaq, Promega, Madison, WI, USA). The final reaction

Table 1. Target alleles and primer sequences of multiplex PCR (I) and PCR (II)

Target alleles	Primer sequence (5'–3')
K/k*	Fw: TTTAgTCCTCACTCCCATgCTTCC Rw: TATCACACAggTgTCCTCTCTTCC
S/s*	Fw: AAgACTgACACATTACCTCA Rw: AACATACCTggTACAgTgAA
Fy ^a /Fy ^b *	Fw: CTTCCggTgTAACCTCTgATgg Rw: CATCCAgCAggTTACAggAgT
Jk ^a /Jk ^b *	Fw: CATgCTgCCATAggATCATTgC Rw: gAgCCAaggAggTgggTTTgC
Kp ^a /Kp ^b †	Fw: TgAggCCAggAgAAAAgCA Rw: TgACCATCTggAAgAgCTTgC
Js ^a /Js ^b †	Fw: AACTTTgCCATgCTCCTgg Rw: gCCCTTgACACTTgCATACCT
Lu ^a /Lu ^b †	Fw: CTgAggAgCgCTgggACACCCgg Rw: CCCCgggTgTgTgCATT
Co ^a /Co ^b †	Fw: TATAAATAggCCCAgCCCAg Rw: CCAgCgACACCTTCACgTT
*PCR (I).	
†PCR (II).	

Table 2. Target SNPs and probe sequences of multiplex hybridization mixtures of Hyb(I) and (II)

Gene name*	Target SNP*	Probe set	Probe name	Sequence (5'–3')
<i>GYPB</i>	143C>T	Ss	s(143C) S(143T) SsNC	TAGGAGAAAACGGGACAACCTT AGGAGAAAATGGGACAACCTTG TCGGATAAAAAGAGACCACTG
<i>DARC</i>	125A>G	Fy	Fyb Fya Duffy-NC	TGGAGACTATGATGCCAACCC GAGACTATGGTGCCAACCTG GAGGCTATCCTGACAAGCTT
<i>KEL</i>	578C>T	Kk	k K- NC6-KEL	TTAACCGAACGCTGAGAC TAACCGAAATGCTGAGAC CTATCCCAAAGCTAAGGC
<i>SLC14A1</i>	838G>A	Jk	Jka Jkb JKNC	AGTAGATGTCTCAAATGGG GTAGATGTCTCAAATGGGG CGTGGATTCTTTCAGAGG
<i>KEL</i>	841C/T	Kpa/Kpb	Kpa Kpb NC	TCACTTCATGGCTGTTCCA ATCACTTCATGGCTGTTCCAG AACTCTACAGGGCTCTTCGA
	1790T/C	Jsa/Jsb	Jsa Jsb NC	GGCTGCCCCGCCTGTGACAA GGCTGCCTCGCCTGTGACAA GCCAGCCACGCGTGTCACTA
<i>AQPI</i>	134C/T	Coa/Cob	Coa Cob NC	AACCAGACGGCGGTCCAGGA CAACCAGACGGTGGTCCAGG AGCCACACTGGGGACCTGGA
<i>LU</i>	230G/A	Lua/Lub	Lua Lub NC	TCGCCCCCGCCTAGCCTCGG TCGCCCCACCTAGCCTCGG TAGCCTCCTCCAAGACTA

*From the Blood Group Antigen Gene Mutation Database (BGMUT).

volume was 50 µl. PCR was performed with a Mastercycler egradient S (Eppendorf AG, Hamburg, Germany) with the following program: 94 °C for 2 min, 35 amplification cycles at 94 °C for 20 s, 60 °C for 20 s and 72 °C for 30 s as well as the final elongation step at 72 °C for 5 min.

Hybridization and Analysis of Fluorescence Emission

5 µl of each biotinylated amplicon of PCR (I) and (II) was transferred to 96-well plates (96-well PCR Thermo-Fast® 96, low profile ABgene; Epsom, UK) and hybridized in a single multiplex assay at 52 °C (Hyb (I)) and at 58 °C (Hyb (II)) (table 2) with the corresponding probe mixture for 15 min. Hybridization was performed according to the procedure described by Karpasitou et al. [4]. Each probe was covalently coupled to a different set of fluorescent polystyrene carboxylated microspheres, purchased internally labeled and ready to couple from the Luminex Corporation, by a carbodiimide method in separate reactions [8, 9].

In order to optimize hybridization conditions with respect to what has been previously published [4], probes were modified in their length (probes specific for Jk^a, Jk^b from 18 to 20 nucleotides, and for K from 18 to 17 nucleotides). Moreover, probes for Lu^a, Lu^b were extended from 18 to 20 nucleotides.

Detection and analysis of the fluorescence emission was performed using the Luminex 100 analyzer. In the present formulation of our typing system, the allelic ratios were recalculated, and the cut-off ranges were reset [4].

Samples of 2,020 subjects (blood donors and patients) were typed.

Results

Samples of 2,020 subjects were successfully typed. The concordance between the method presented here and serology was 100% for all alleles phenotypically expressed. One Co (a–b–), 74 Fy^b+^{weak} and 56 Fy(a–b–) phenotypes were detected only with serology.

Discussion

In this paper, we present an updated version of a previously published method [4, 5] for identifying blood group single nucleotide polymorphisms.

The number of PCR reactions was reduced by setting up 2 multiplex reactions in order to minimize laboriousness and hands-on time. The PCRs were divided on the basis of the frequency of use in our laboratory and, therefore, the utility of the reactions.

With current assay conditions we can genotype 96 samples (one 96-well plate) per blood group in approximately 4 h with about 1 h of operator time, DNA isolation time excluded. With two 96-well plates, 192 samples may be typed simultaneously without causing a significant increase in total time required. The use of 384-well plates could further increase productivity.

In the last 2 years, we successfully typed more than 2,000 subjects. One limitation of the method, in its present version, is the inability to detect null and/or weak phenotypes. As a matter of fact, in the series of subjects tested we have found 1 Co(a–b–) [10], 74 Fy^b+^{weak}, 54 Fy(a–b–) phenotypes which would remain undetected using Luminex alone.

Although multiplexing greatly increases throughput, the use of multiplexing, in our opinion, is not advisable when a donor cohort must be screened for a specific phenotype. Nevertheless, due to the flexibility of this typing approach, multiplexed or separate PCR reactions can be chosen, depending on the specific requirements of the laboratory, in order to rapidly screen a large number of donors.

Disclosure

The authors certify that they have no competing financial interest.

F.D., K.K. and F.P. have applied for a patent related to the work that is described in the present study.

References

- Hillyer CD, Shaz BH, Winkler AM, Reid M: Integrating molecular technologies for red blood cell typing and compatibility testing into blood centers and transfusion services. *Transfus Med Rev* 2008; 22:117–132.
- Daniels G: The molecular genetics of blood group polymorphism. *Transpl Immunol* 2005;14:143–153.
- Avent ND, Martinez A, Flegel WA, Olsson ML, Scott ML, Nogués N, Písacka M, Daniels G, van der Schoot E, Muñiz-Díaz E, Madgett TE, Storry JR, Beiboer SH, Maaskant-van Wijk PA, von Zabern I, Jiménez E, Tejedor D, López M, Camacho E, Cheroutre G, Hacker A, Jinoch P, Svobodova I, de Haas M: The BloodGen project: toward mass-scale comprehensive genotyping of blood donors in the European Union and beyond. *Transfusion* 2007;47: 40S–46S.
- Karpasitou K, Drago F, Crespiatico L, Paccapelo C, Truglio F, Frison S, Scalapogna M, Poli F: Blood group genotyping for Jk(a)/Jk(b), Fy(a)/Fy(b), S/s, K/k, Kp(a)/Kp(b), Js(a)/Js(b), Co(a)/Co(b), and Lu(a)/Lu(b) with microarray beads. *Transfusion* 2008;48:505–512.
- Drago F, Karpasitou K, Poli F: Microarray Beads for identifying blood group single nucleotide polymorphisms. *Transfus Med Hemother* 2009;36:157–160.
- Veldhuisen B, van der Schoot CE, de Haas M: Blood group genotyping: from patient to high-throughput donor screening. *Vox Sang* 2009;97:198–206.
- Hashmi G, Shariff T, Zhang Y, Cristobal J, Chau C, Seul M, Vissavajhala P, Baldwin C, Hue-Roye K, Charles-Pierre D, Lomas-Francis C, Reid ME: Determination of 24 minor red blood cell antigens for more than 2000 blood donors by high-throughput DNA analysis. *Transfusion* 2007;47:736–747.
- Colinas RJ, Bellisario R, Pass KA: Multiplexed genotyping of beta-globin variants from PCR-amplified newborn blood spot DNA by hybridization with allele-specific oligodeoxynucleotides coupled to an array of fluorescent microspheres. *Clin Chem* 2000;46:996–998.
- Dunbar SA, Jacobson JW: Application of the Luminex LabMAP in rapid screening for mutations in the cystic fibrosis transmembrane conductance regulator gene: a pilot study. *Clin Chem* 2000;46: 1498–1500.
- Karpasitou K, Frison S, Longhi E, Drago F, Lopa R, Truglio F, Marini M, Bresciani S, Scalapogna M, Poli F: A silenced allele in the Colton blood group system. *Vox Sang* 2010;99:158–162.