VALIDATION OF *KEL* (Kell), *SLC14A1* (Kidd) AND *DARC* (Duffy) MALDI-TOF MS HIGH-THROUGHPUT BLOOD GROUP GENOTYPING USING >3.100 SEROLOGICALLY PRE-TYPED DONOR SAMPLES

Stefan Meyer[1], Caren Vollmert[2], Nadine Trost[1], Andreas Buser[3], Beat M. Frey[1], Christoph Gassner[1]

[1] Blood Transfusion Service Zurich, Swiss Red Cross, Department (Dept.) of Molecular Diagnostics & Cytometry (MOC), Schlieren, Switzerland
[2] Sequenom GmbH, Hamburg, Germany
[3] Blood Transfusion Center, Swiss Red Cross, Basel, Switzerland

Background: Optimized for the detection of nucleic acids, matrix-assisted laser desorption/ ionisation, time-of-flight mass spectrometry (MALDI-TOF MS) represents an ideal tool for SNP based, high throughput-blood group genotyping. Following *ABO*(ABO) and *RH*(Rh), *KEL*(Kell), *SLC14A1*(Kidd) and *DARC*(Duffy), are the next important blood groups relevant to routine donor typing.

Aims: In order to validate the method for routine purpose, a total of >3.100 serologically pretyped donors from the cantons of Zurich and Basel were genotyped using MALDI-TOF MS, and results were compared for *KEL*(Kell), *SLC14A1*(Kidd) and *DARC*(Duffy).

Methods: Amplification of the relevant sequences was carried out in one single multiplex PCR per sample using 30 ng genomic DNA. Subsequent primer extension was capable to generate allele-specific analytes representative of K, k, Kpa, Kpb, Jsa, Jsb, K0 (7 prevalent null-, 1 mod-allele), Jka, Jkb, Jk0 (2 prevalent null-alleles) and Fya, Fyb, Fyx, and Fy0(-67t>c"GATA"). In total 15 SNPs for total material costs of about EUR 10.00 (without DNA preparation, hardware and handling costs). Batches of 384 donor samples each (theoretically up to 10 per day), were analysed using MALDI-TOF MS.

Results: With respect to K, k, Kpa and Kpb no discrepancies between pheno- and genotypes were observed for all >3.100 samples. Jsa positive individuals among the investigated Swiss population were rare (allele-frequency = 0.0031) and validation using Js phenotypes is pending. No *KEL*null-alleles, but 2 *KEL*2(1719C>T)mod were observed. Among all >3.100 samples, three discrepancies between serology and genotyping for *SLC14A1* (Kidd) were observed. Of these, two serological pre-values were wrong, the third was a new *JK*Bnull allele, which is currently being sequenced. One *JK*A(Y194X)null positive individual was detected. With respect to *DARC*(Duffy) typing, 24 of a total of 43 (56%!) *FY*A/X heterozygous individuals were serologically misinterpreted as Fya homozygous, two discrepancies were due to false Fy serology and one represented a new *FY*B(781G>A)null allele. Allele-frequencies specific for Fyx and Fy0(-67t>c"GATA") were 0.016 and 0.007, respectively, among all >3.100 Swiss donors investigated.

Conclusion: As shown by comparison of >3.100 donor data sets, genotyping accuracy of the major alleles of *KEL*(Kell), *SLC14A1*(Kidd) and *DARC*(Duffy) using MALDI-TOF MS, reached 100% for K, k, Kpa, and Kpb and 99.984% (1 error of 6.080 alleles investigated) for both Jka and Jkb, and Fya, Fyb, Fyx, and Fy0(-67t>c"GATA"). Full concordance was only prohibited by two new alleles, *JK*Bnull (sequence pending) and *FY*B(781G>A)null. Both alleles were in fact correctly genotyped, but risked to be wrongly translated into phenotypes if unrecognized as specific null-alleles. In comparison to error rates observed in serology, in this study genotyping error rates were much lower for *DARC*(Duffy) typing due to *FY*X and *SLC14A1*(Kidd)! In other words, genotyping the major alleles of the human blood groups Kell, Kidd and Duffy, works distinctively better and to lower costs than serology! Therefore, MALDI-TOF MS based blood group genotyping for the major alleles of *KEL*(Kell), *SLC14A1*(Kidd) and *DARC*(Duffy) seems to be superior to confirm phenotype data as compared to a second round of serological typing as required by current Swiss Red Cross prescriptions.