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

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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 € 10 (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.