High-throughput blood group genotyping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), report on a prototype

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Background. A rapidly growing number of reports are focusing on the feasibility of high-throughput blood group genotyping (ht-bg-gt) approaches for routine purposes. Genotyping of serologically Dneg, genetically *RHD* positive individuals, detailing blood donors' antigenic profiles and screening for blood donors with (very) rare antigenic constellations are just some examples reasoning these efforts. MALDI-TOF MS is an accurate, highly automatable and fast technology with the capacity of genotyping more than 150.000 single nucleotide polymorphisms (SNPs) per day.
Aims. In order to proof the principal potential for ht-bg-gt, a set of SNPs, defining phenotypically relevant polymorphisms of *RHD*, *KEL*, *JK*, *FY* and *MNS* was defined and planned to be made detectable by MALDI-TOF MS. This prototype was then intended to be tested on a selectively compiled and genetically pretested donor DNA-panel.
Methods. SNPs chosen were to define *RHD*-specific nucleotides for all 10 exons, category and other partials (n=7), weak *RHD* types (n=11), *RHD*dels (n=4), unexpressed *RHD*s (n=7), and *RHC, c, E, e* and *W* (n=4). For *KEL, JK, FY* and *MNS*, again, the major antigenic counterparts and SNPs defining weakly or unexpressed alleles were considered (n=19). All SNP data were analyzed for optimal PCR-multiplex conditions using Sequenoms TYPER 4.0 software. A total of 100 DNAs including (very) rare alleles such as *RHD* category VII, weak *RHD* type 15, *RHD*(IVS3+1G>A)del, *RHD*psi(null), *KEL*2(IVS3+1G>A)null, or (very) rare genotypes such as *KEL*2, or *FY**X homozygotes, were genotyped following the standard Sequenom MassARRAY iPLEX® Pro genotyping protocol.
Results. For 10 out of 43 *RH* specificities, no positive control DNAs were included in the tested DNA-panel. However, PCR amplification for those (10) alleles were still carried out in the various multiplex PCRs to emulate realistic amplification conditions. Of the remaining 33 specificities, 5 (weak *RHD* type 3 and 5, partial *RHD* DAU, discrimination of genotypes *RHEE* vs. Ee and ee and *RHD*-CE(2-9)-D-i2+268Anull), did not give any results, whereas all other (n=28) gave correct results. Additionally, testing for *RHD* exon specific SNPs also delivered reliable *RHD* gene copy number measurements. With respect to the detection of *KEL*, *JK*, *FY* and *MNS*, 4 out of 19 specificities were not detectable due to the lack of specific positive control DNAs. Still, beside specificity for *KEL*2(Q348X)null and MNS34(Ss) genotyping, all other SNPs (n=13) were correctly typed. All *KEL*, *JK* and *FY* specificities could be reliably multiplexed in one single multiplex PCR. Overall, 85% of all specificities with control DNAs available (28 plus 13 of 48) were already operating perfectly in the first test of the described prototype.
Conclusions. The observed success rate of a prototype using MALDI-TOF MS for ht-bg-gt, is highly impressive. The described method is independent of fixed formats like DNA-chips, and users are therefore free to choose and configure modules for their needs. E.g., the presented *KEL, JK* and *FY* could represent such a module. This included additional specificities for weakly and unexpressed *KEL, JK* and *FY* alleles, which were all prepared for pre-analytical amplification in one single multiplex PCR only! Further experiments and prototypes are planned.