PROGRESS REPORT ON A MALDI-TOF MASS SPECTROMETRY HIGH-THROUGHPUT BLOOD GROUP GENOTYPING PLATFORM

Christoph Gassner, Blood Transfusion Service SRC, Zurich, Switzerland, c.gassner@zhbsd.ch
Beat M. Frey, Blood Transfusion Service SRC, Zurich, Switzerland, bm.frey@zhbsd.ch
Caren Vollmert, Sequenom GmbH, Hamburg, Germany, cvollmert@sequenom.de

Background: 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. Therefore, genotyping of serologically Dneg, genetically *RHD* positive individuals, detailing blood donors' antigenic profiles and screening for blood donors with rare antigenic constellations, summarized as "high-throughput-blood group genotyping (ht-bg-GT), may easily be carried out using this technique.
Methods: A set of SNPs, defining phenotypically relevant polymorphisms of *RHD*, *KEL*, *JK*, *FY, MNS* and rare alleles such as *KEL*3, *LU*1, *DI*1, *DI*3, *Y*2, *CO*2, *KN*2, and other (n total = 22), was defined and tested on two selectively compiled and partially genetically pretested donor DNA-panels. SNPs chosen were *RHD*-specific nucleotides for all 10 exons, and specific nucleotides for categories, partials, weaks, *RHD*dels and unexpressed *RHD*s (n=29), and *RHC, c, E, e* and *W* (n=5). For *KEL, JK, FY* and *MNS*, again, the major alleles and SNPs defining weakly or unexpressed alleles were considered (n=19). Two DNA panels, each including a total of 100 DNAs at a minimum, were genotyped following the standard Sequenom MassARRAY iPLEX® Pro genotyping protocol.
Results: For 10 out of 43 *RH* specificities tested, no positive control DNAs were included in the tested DNA-panel. However, PCR amplification for those alleles was still carried out in the various multiplex PCRs to emulate realistic amplification conditions. Of the remaining 33 specificities, including specificities for *RHC, c, E, e* and *W* , categories, partials, weaks, *RHD*dels and unexpressed *RHD*s, 5 (e.g. weak *RHD* type 3), did not give any results, whereas all other (n=28) were correct. Additionally, *RHD* gene copy numbers could be measured. In *KEL*, *JK*, *FY,* and *MNS* genotyping, of 19 SNPs chosen, 15 could be tested by respective control DNAs and gave correct results for 13 of them. The "rare module" gave correct results for 22 of a total of 24 SNPs tested.
Conclusions: The observed success rate of the 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 "*KEL, JK* and *FY* ", and the "*RARE* module" are currently ready for validation-testing on a serologically predetermined DNA-panel of appropriate size.