

MALDI-TOF MS BASED BLOOD GROUP GENOTYPING – PROGRESS OF AN ALTERNATIVE APPROACH

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Introduction and Method: Matrix-Assisted Laser Desorption/Ionisation, Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) is an ideal tool for high-throughput blood group genotyping. Using this technique, this Swiss (BTS Zurich) – German (Sequenom GmbH, Hamburg) cooperation aims to genotype Swiss blood donors for blood groups Rh, Kell, Kidd, Duffy, MNSs (n = 6,000), HPA and HNA (n = 3,000) and high frequency antigens (HFA, n = 36,000) within three years. Specificities are grouped into single multiplex reactions (MPX, n = 10) with up to 17 SNPs tested simultaneously in one tube.

Results: Using the *KEL-JK-FY* MPX, more than 4,000 donor DNAs have been tested and compared to serological pre-values. Concordance between geno- and phenotypes reached 100% for K/k, 99.98% for Kp^{a/b}, 99.93% for Jk^{a/b} and 99.23% for Fy^{a/b/x⁰}. Only one discrepancy each for Kp, Jk and Fy could be attributed to genetics, the others were erroneous serotypes revealed by genotyping. Genetic discrepancies were three new variants: *KEL*02.03(R700G)null* - a Kp^a relative, *JK*B(mutation n.d.)* and *FY*B(G261R)null*. Serology for Js^{a/b} of some few *KEL*02.06* positives confirmed validity of genotyping. Numbers of detected known *KEL*mod* and *null*, *JK*null* and *FY*null(GATA)* alleles were 3, 2 and 51, respectively. Call failures (no result) were observed in less than 2% of all MPXs.

Genotyping for RHD, RHCE (5 MPX), GYPA and B (MNSs, 1 MPX) on more than 4,000 samples, delivered results with discrepancy rates for MNSs comparable to above, and better rates for *RHDCE*. Call failure were at approximately 2% again. Reproducibility, robustness and analytical accuracy of the technique allowed measurement of gene copy numbers with relevance for *RHD* zygosity estimation and detection of the *GYPB* deletion in U negatives. *RHD* category, partial, weak, DEL and null alleles and the genetic correspondents of Vw, Mg, Mi(a), He, and Uvar were observed.

Genotyping for HPA and HNA (1 MPX) showed expected results among 2,300 samples with the exception of HNA-1a, b and c, where frequent duplications and deletions of *FCGR11B* pose difficulties for all genotyping approaches in general. In **HFA (“RARE”) genotyping (2 MPX)**, currently, more than 13,000 blood donors were analyzed, and delivered: 29 KK, 2 Kp(a+b-), 48 Js(a+b-), 41 KEL11+KEL17+, 20 Lu(a+b-), 3 LU14+LU08-, 52 Yt(a-b+), 17 Co(a-b+), 11 Kn(a-b+), 98 LW(a+b+), and >10 others. Specificities for Vel negativity and Scianna have been included into HFA typing, recently.

Conclusion: Analysis for Kell, Kidd and Duffy showed that genotyping worked qualitatively better and to costs comparable to serology. Consequently, genotyping Kell, Kidd and Duffy instead of routinely performing a second round of serotyping as mandatory for donors in Switzerland, is recommended. Ahead of comparable suggestions with regard to Rh and MNSs, a more detailed statistical analysis of existing raw data is needed. However, genetically identified donors with rare blood phenotypes, e.g. such as Yt(a-b+), are already selected for respective transfusions and are a strong indicator for the value of the presented project.

References: Transfusion Medicine, Vol. 23, Supplement 2, October 2013, 128, 1-29