Evaluation of a validated array platform for blood group antigen and allele prediction using routine and special samples pre-genotyped by MALDI-TOF mass spectrometry

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Background: Comprehensive and accurate blood donor genotyping is crucial for reducing the risk of alloimmunisation in patients. MALDI-TOF mass spectrometry (MS) is a high-throughput genotyping platform, which has been used in our transfusion center for donor genotyping since 2012. Depending on the SNV panel in use, it has been allowing the prediction of up to 64 antigens on erythrocytes (HEA) and platelets (HPA). The high-throughput Axiom Universal Blood Donor Typing (UBDT_PC1) array, a platform tailored to the needs of blood services, may represent an affordable alternative in a more centralised and automated setting.

Aims: The goal of this work was to assess blood group antigen prediction on the UBDT_PC1 array with selected routine donor samples genotyped on a MALDI-TOF MS system. Beyond routine typing, particular samples with rare blood group alleles, often of non-European ethnicity, were used to inspect the array's performance.

Methods: Up to 70 antigens were assessed in 569 routine donor samples either serologically (N=10) or inferred by MALDI-TOF MS genotyping. A further 191 samples, in which MALDI-TOF MS, PCR-SSP or Sanger sequencing revealed unusual allele combinations, were added and collectively analysed on the UBDT_PC1 array in an accredited laboratory with a GeneTitan instrument. The array predicted 199 HEA and 54 HPA based on ~400 genetic variants processed by the bloodTyper software. Predictions for presence of particular blood group alleles (N=17) representing RHD, GYPB, JK and FY systems were also available.

Results: 753 samples (99.1%) passed quality control. Of all provided antigens, only 10 (14%) were not predicted on the array (A, B, MNS-6, 9, 10, 11, 15, Xg(a), HPA-3a, 3b). More than 30,000 comparisons of antigen presence vs. absence revealed no discordance in routine samples. In the group of challenging samples, concordance was also high (99.6%) with 23 discrepancies restricted to RH (n=8), MNS (n=5), JK (n=8) and DI (n=2) blood group systems. Discordant results were attributable to 4 rare hemizygous *RHD* null alleles, two of which were detectable with in-house MALDI-TOF MS or PCR-SSP (*RHD*01N.06*/*07*, *RHD*01N.08*). One sample was interpreted as a weak C due to an alleged *RHD*/*RHCE* hybrid gene on MALDI-TOF MS, which proved wrong by subsequent Nanopore sequencing. Specific alleles on the homologous genes could potentially explain two discrepancies resulting from the array compared to serological results, a non-inferred N in a *GYPA*M*/*N* sample with *GYPB*03N.04*, and a non-inferred e in a *RHCE*E*/*e* sample with an additional *RHD*06.01*. Furthermore, all samples with pre-typed *GYPB*04N.01* alleles (n=2) caused missing calls in the underlying variant on the array. JK discrepancies were attributable to rare null alleles, only one of which (*JK*01N.03*) was detected by the

MALDI-TOF MS system. The discrepancies in DI resulted from a translation error in the antigen data of the pre-typed samples.

Summary/Conclusions: Results from the validated UBDT_PC1 array platform showed perfect concordance with previously in-house typed routine samples and still very high agreement with challenging allele constellations. While the array is much more comprehensive, the two very challenging blood group systems RHD and MNS show a larger panel of genetic variants for MALDI-TOF MS allowing more refined allele and antigen prediction, respectively. Only a handful of discordances might need further investigation to optimize the array's prior settings for genotype calling or algorithmic inference.