Identification of High-Frequency Antigen-negative blood donors using the Universal Blood Donor Typing genotyping array

Blood transfusion Genomics Consortium

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Poster 602

Background

High-Frequency Antigen-negative (HFA-negative) is defined as an antigen absent from the red blood cells of less than 1 in 1000 donors. On rare occasions, an HFA-negative patient may form an alloantibody after transfusion or in pregnancy. To be able to supply compatible units, blood services make immense efforts to identify HFA-negative donors. Extended genotyping will significantly simplify the identification of rare donors. The Blood transfusion Genomics Consortium (BGC) has developed the Axiom Universal Blood Donor Typing (UBDT_PC1, also known as BloodGenomiX) array to genotype donors for human erythrocyte, platelet and leukocyte antigens (HEA, HPA and HLA, respectively).

Aim

To identify HFA-negative donors in the five main ancestry groups in an international multi-centre validation study using the UBDT_PC1 genotyping array (*the 17 HFA included in this analysis are listed in* ***Table 1***).

The Blood transfusion Genomics Consortium

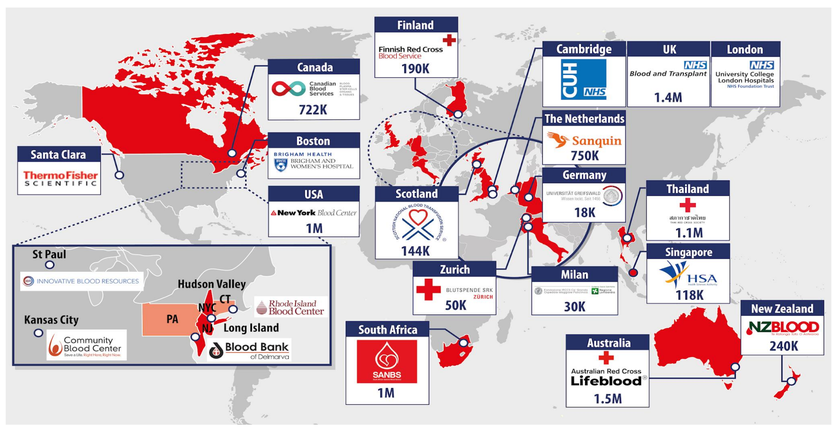

Figure 1: The BGC is an international partnership between blood services, research institutions and Thermo Fisher.

Identification of HFA-negative blood donors for 17 selected HEA phenotypes among 6,679 samples

- The call plots of all predicted HFA-negative samples were visually inspected. Additionally, the 'no calls' were examined. If the signal of the probe was closer to the minor allele than the major allele, the sample was sequenced to confirm the HFA phenotype (n=9).
- **157 HFA-negative donors** were identified:
  4 U-, 8 Lu(b)-, 36 k-, 1 Kp(b)-, 8 Js(b)-, 1 Di(b)-, 12 Yt(a)-, 2 Hy-, 7 Jo(a)-, 17 Co(a)-, 1 LW(a)-, 4 Kn(a)-, 55 McC(a)-, and 1 Vel-. No Wr(b)-, Sc1-, and Cr(a)-.
  - The HFA-negativity of **29** samples were already registered in electronic donor records:
    4 U-, 4 Lu(b)-, 8 k-, 5 Js(b)-, 2 Yt(a)-, 1 Hy-, 3 Jo(a)-, 1 Co(a)-, and 1 LW(a)-.
  - **128** samples with an HFA-negative result were newly identified by the UBDT_PC1 array. These samples were confirmed by Sanger sequencing.
- HFA-negative donors were from AFR (n=72), AMR (n=9), EAS (n=1), EUR (n=73), and SAS (n=2) ancestry.

International multi-centre validation study

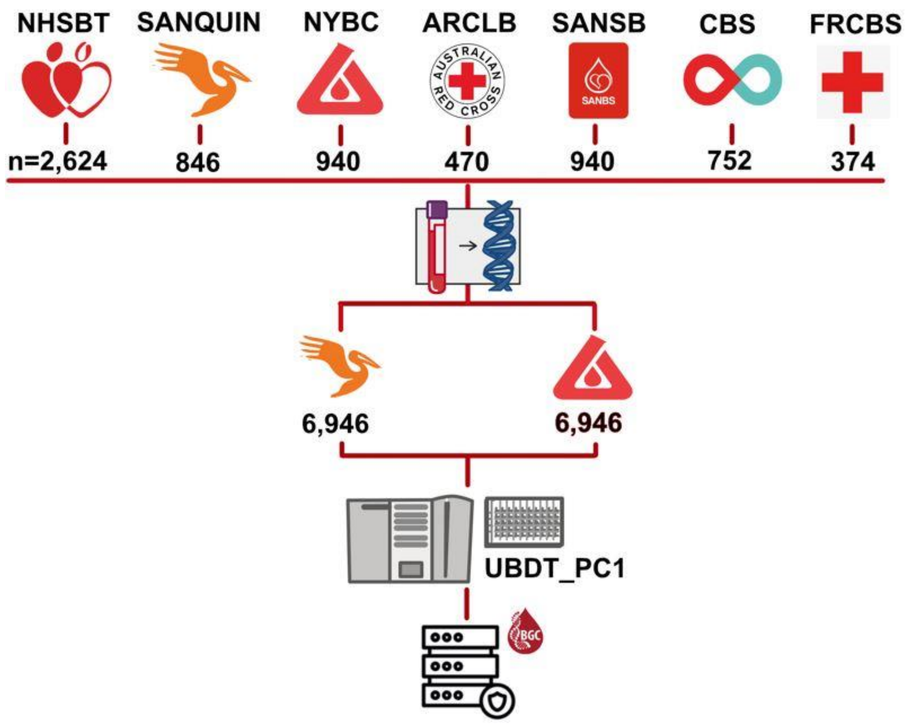

Figure 2: DNA samples of 6,946 blood donors were provided by blood services from the United Kingdom, the Netherlands, the United States of America, Australia, South Africa, Canada and Finland, respectively. The same cohort of DNA samples was genotyped with the UBDT_PC1 array on the GeneTitan (Thermo Fisher) at the New York Blood Center (NYBC, Kansas City, USA) and Sanquin (SQ, Amsterdam, the Netherlands). To increase the number of HFA-negative samples, an additional set of rare HEA phenotypes was tested in either SQ (n=88), NYBC (n=87) or NHSBT (n=146). The inferred HEA phenotypes by the bloodTyper module of the Axiom™ Total Blood Typing Solution were compared to HEA types retrieved from electronic donor records.

Ancestry distribution of the unified donor cohort

After the QC, an unified dataset was created (n=6,679) for which results were produced by both NYBC and SQ.

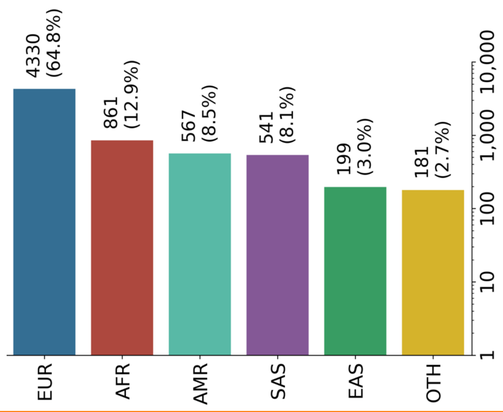

Figure 3: The genotypes were used to infer ancestry, showing that 35.2% of the samples were from non-European blood donors.

Specificity of the Axiom™ Total Blood Typing Solution

The UBDT_PC1 array and accompanying integrated analysis package (IAP) demonstrate high specificity in typing HFA phenotypes both at NYBC and SQ. If the array predicted that the donor was positive for HFA, this was a correct result in 99.988% and 99.999%, respectively. The reproducibility of the Axiom™ Total Blood Typing Solution is 99.72%.

6,679 samples x 17 HFAs = 113,543 comparisons

113,386 positive comparisons for 17 HFAs

SQ	• 113,283 correctly typed • 102 no calls • **1 FN (false-negative):** *Vel*
NYBC	• 113,174 correctly typed • 199 no calls • **13 FN:** *U (n=1) and Co(a)* (n=12)* **due to lower intensities*

157 negative comparisons for 17 HFAs

SQ	• 151 correctly typed • 6 no calls • **0 FP (false-positives)**
NYBC	• 151 correctly typed • 5 no calls • **1 FP:** *Lu(b)*

Sensitivity of the Axiom™ Total Blood Typing Solution

The sensitivity of identifying HFA-negative samples is 93.7% (269 out of 287) (Table 1). In 3.8% of the samples (n=11), the donor was falsely typed as HFA-positive, and in 2.4% (n=7) there was no genotyping result. For Kp(b), Co(a), Cr(a), and Kn(a), the observed false HFA-positives were explained either by rare variants not included on the array or in the algorithm, or alternatively by incorrect clinical typing results.

Table 1: Results of 287 HFA-negative samples.

System	Negative for HFA	Detected in unified dataset (n=…)	Known and tested from additional dataset (n=…)	Total (n=…)	Concordant (n=…)	Discordant (n=…)	No result (n=...)
MNS	U	4	16	20	20	0	0
LU	Lu(b)	8	6	14	13 SQ / 12 NY	0 SQ / 1 NY	1
KEL	k	36	2	38	38	0	0
KEL	Kp(b)	1	31	32	26	6	0
KEL	Js(b)	8	5	13	13	0	0
DI	Di(b)	1	3	4	3	0	1
DI	Wr(b)	0	6	6	6	0	0
YT	Yt(a)	12	2	14	14	0	0
SC	Sc1	0	0	0	0	0	0
DO	Hy	2	5	7	7	0	0
DO	Jo(a)	7	6	13	12	0	1
CO	Co(a)	17	9	26	25	1	0
LW	LW(a)	1	6	7	5	0	2
CROM	Cr(a)	0	5	5	3	2	0
KN	Kn(a)	4	5	9	7	2	0
KN	McC(a)	55	7	62	62	0	0
VEL	Vel	1	16	17	15	0	2
		157	130	287	269	11	7

Conclusions

- The Axiom™ Total Blood Typing Solution is capable of identifying HFA-negative blood donors, represented in the five main ancestry groups.
- In this multi-ethnic donor cohort, 81.5% of the identified HFA-negative blood donors lacked prior registration in electronic donor records, demonstrating their status as newly identified HFA-negative donors. The HFA-negativity of these donors needs to be confirmed with a serological test as long as this test is the gold standard.
- The Axiom™ Total Blood Typing Solution exhibits 99.9% specificity and a robust sensitivity of 93.7%. Although the sensitivity for Kp(b), Cr(a), and Kn(a) is currently lower, efforts to improve this are ongoing. No Sc1-negative samples have been tested yet.