Trials of several platforms for high-throughput blood group genotyping of donors

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NHS Blood and Transplant

Background

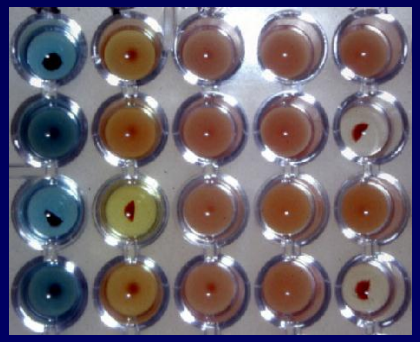
- NHSBT faces an increasing demand to provide “better matched” blood for transfusion
- Patients who are regularly transfused, e.g. sickle cell anaemia or thalassemia patients, develop multiple red cell antibodies.
- Demand for extended matching currently 38,000 patients / year
  - Identification of these antibodies
  - Identification of suitable donors
  - Units from rare donors

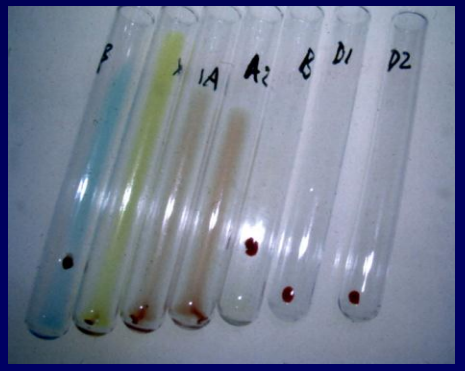
Overview

- Current status of blood group genotyping and why this needs to change.
- New technologies becoming available
- How NHSBT is evaluating them
- The future

Current blood grouping of donors

- Serological testing
- Technology not much changed in 100 years.





Current blood grouping of donors

- Full ABO, Rh and Kell phenotyping routine on all units
- Extended phenotyping performed daily on proportion of units

- Rare types are identified for further testing

- Ethnic minority donors flagged for relevant testing:
  - African/Afro Caribbean – Ss typing, if neg U typing
  - Asian – In b typing (some)

- Mass screening for various rare types:
  - Based on available anti-sera
  - e.g. Co (a-), Kp (b-); Lu (b-), Lan -; Ge -
  - 3,000 per month group O or A samples

Why embrace the use of molecular technology?

- Typing for minor blood groups
  - Reagents difficult to get, not reliable
  - Patients always have transfused cells in their blood
  - Manual process, labour intensive, skilled staff

- Identifying patient's antibodies
  - Needs panels of typed red cells
  - Needs expertise to resolve
  - Manual process
  - Difficult to automate

Molecular bases of blood groups

- The genes associated with all 29 blood groups have been sequenced
- Molecular basis for polymorphism established
- Can predict blood group phenotype from DNA with reasonable accuracy

Blood groups and number of genes

1			2	3
ABO	YT	CROM	RH	MNS
P	SC	KN	XG	
LU	DO	IN	CH/RG	
KEL	CO	OK		
LE	LW	RAPH		
FY	H	I		
JK	XK	GLOB		
DI	GE	GIL		

Blood group polymorphism

- Single nucleotide polymorphisms (SNPs)
  - amino acid change in extracellular domain of protein (e.g K/k)
  - amino acid change in transferase enzymes making carbohydrate antigens (ABO)
  - SNP in promoter region of gene (e.g. Fya-b-)
- Presence or absence of gene
  - D+ / D- phenotype

DNA based donor typing is an alternative to serological typing for some blood groups

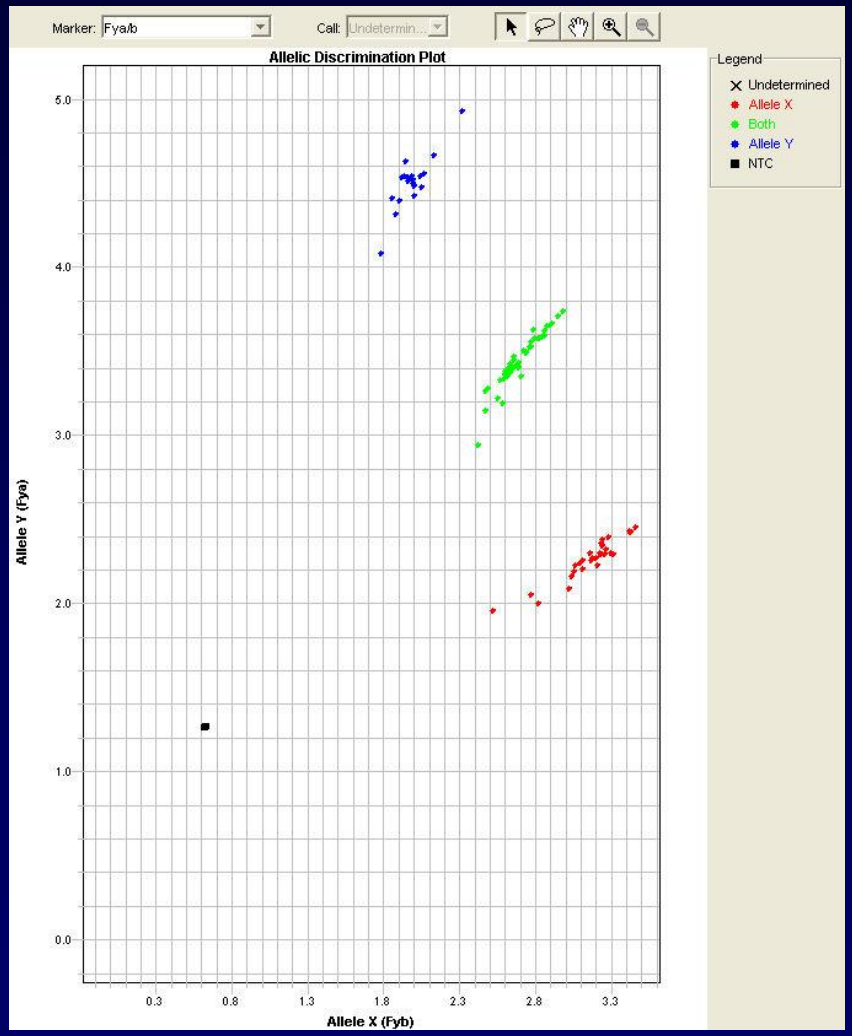
- Serological reagents are not readily available for some blood groups, e.g. Dombrock
- Serological typing may not be feasible for high numbers needed to identify rare donors
- A single molecular test may predict multiple blood group phenotypes in an individual.

Current status of blood group genotyping

- Patient genotyping in NHSBT has been available since 2003 at IBGRL.
- Routinely test for Rh, K/k, Fya/b and Fy-, Jka/b, MNS
- Can extend test for Do, Js, Kp, VS, FyX
- Current method is Taqman technology
- Reagent costs approx £5 per patient

Allelic Discrimination

Used for bi-allelic SNP investigation



200-300 routine referrals pa, mainly patients.

Result for urgent cases available on day of sample receipt.

Some automation for 384-well PCR plate set up, but results entered manually.

3000+ Fya-b- donors also tested, Fy, Js, Do, VS

Is a medium through-put assay.

New blood group genotyping platforms

- Various commercial ‘platforms’ are on the market:
  - low throughput platforms are well suited to patient typing
  - high throughput platforms may be suited to donor typing

Evaluation of platforms in NHSBT

- Evaluation of two chip based and two Luminex based genotyping platforms
- Chip based platforms
  - BloodChip from Progenika – very high coverage (ABO, Rh variants, HPA), low throughput
  - HEA BeadChip from BioArray – good coverage, includes HbS
- Luminex based platforms
  - Common technology across diagnostic labs
    - Lifecodes RBC from GenProbe
    - IDCore+ from Progenika

Genprobe Lifecodes RBC

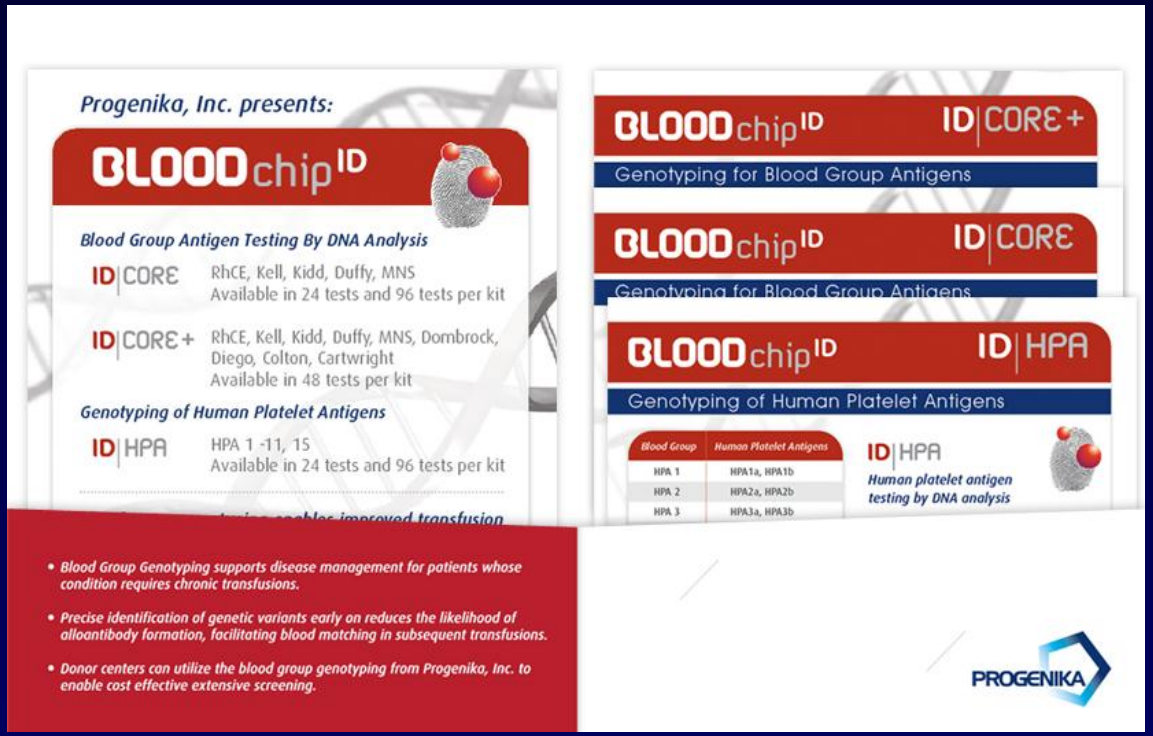
Kell – K, k, Kpa, Kpb, Kpc, Jsa, Jsb
Kidd – Jka, Jkb, Jk (Finnish null)
Duffy – Fya, Fyb, Fyx (a-b weak), FyGATAsil
MNS – M, N, S, s, S-s-Uvar (includes 2 S silencing mutations: – GPB230 and GPB intron +5)
Rh – C, c, E, e
Dombrock – Doa, Dob

Liquid (Luminex based)

Approx 5 hours post DNA extraction

Tested in RCI Newcastle and Sheffield

Progenika Bloodchip IDCore+



Luminex based

5.5-6 hours post DNA extraction

Tested at RCI / IBGRL Filton

Immucor HEA Beadchip

Rh (C,c,E,e), Kell (K, k, Kpa, Kpb, Jsa, Jsb), Duffy, Kidd, MNS, Lutheran, Dombrock (Doa, Dob, Hy, Jo), Landsteiner-Wiener, Diego, Colton, and Scianna blood group systems

Also types for HbS

Slide-based

Approx 6 hours post DNA extraction

Tested at IBGRL Filton

Methods

- Retrospective evaluation using 1034 donor DNA samples from Cambridge BioResource. Have had extended phenotyping

- Analysis
  - Compare predicted phenotype between platforms
  - Predicted phenotype compared to phenotype recorded on PULSE system.

Genotyping platforms perform well

Platform	Blood groups compared	Discrepant types	% discrepant
1	15,075	37	0.25
2	13,660	35	0.26
3	14,639	29	0.20
Interplatform	20,266	8	0.04

Interplatform comparison

Sample no	Blood group	Platform 1	Platform 2	Platform 3	Pulse phenotype
S00060	C	0	1	0	0
S00142	C	1	0	1	1
S00217	C	0	1	0	0
S00613	C	1	0	1	1
S00758	s	0	1	0	0
S00762	Dob	1	0	0	0
S00762	Fyb	1	0	0	0
S00912	E	0	1	0	0

- Platform 2 has issues with RhC and E typing which are being investigated.

Resolution of phenotype discrepancies

- Donor recall for repeat phenotype check attempted for 44 donors
- Results so far (18/44 donors) show phenotype recorded on PULSE is incorrect and the genotype was correct.
- Most donors with discrepant SNP results in this study have historical PULSE records which agree with the genotype.

Conclusions I

- Confidence that the technology works and could be deployed.
- 2 platforms may be suitable for patient genotyping in RCI labs, reducing turnaround time for patients requiring transfusion.
- Need to fit in with current lab infrastructure (advantage for Luminex-based systems).
- Patient typing needs to be complimented by donor typing to supply demand.

Conclusions II

- Current platforms may be suitable to test targeted donors (e.g. ethnic groups).
  - x10 decrease in antibody production demonstrated in sickle cell patients by matching for K, Jk and Fy in addition to ABO/Rh.
- No platform is currently suitable for high-throughput donor typing; too many manual steps, need “black box” system.
- HEA BeadChip system is being developed for donor typing...

Next generation sequencing of pooled samples

A potential game changer!

The future (10 years and beyond)

- If a situation can be attained where all donors and patients are fully genotyped, it should be possible to safely allocate blood for transfusion without further compatibility testing. This would change current practice fundamentally, removing the need for patient testing in hospital transfusion laboratories.
- All matching is done in the computer.

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