



ABO Next Generation Sequencing (NGS)

An Exploratory Study of Common and Rare ABO Subtypes



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BACKGROUND

- In transfusion medicine, numerous blood group red cell antigens are considered **clinically significant** as they can result in an antigen-antibody reaction in patients receiving blood from donors that have an antigen that the patient lacks.
- The resultant formation of alloantibodies called **alloimmunisation** can cause adverse events, acute or delayed haemolytic transfusion reactions (**HTRs**) or haemolytic disease of the foetus and newborn (**HDFN**).
- The most clinically significant red cell antigens are from the **ABO** and **Rh** blood group systems with ABO-incompatible blood transfusions resulting in **mild to severe** HTRs/HDFN.
- The majority (**90%**) of the red cell antigens can be easily detected by serological **phenotyping** methods which are often simple and inexpensive methods.
- The remaining **10%** of complex and/or inconclusive serology cases are resolved by red cell **genotyping** in the Immunohematology Reference laboratory.





INTRODUCTION

- ✓ Progenika/Grifols **IDCOREXT** assay utilizing the Luminex 200IS was introduced in 2015 → ability to test for **10 blood group systems and 37 red cell antigens** in one test per sample.
- ✗ ABO and RHD genes not covered in the assay – limitation.
- ✓ **Innotrain Fluogene** assay utilizing the FluoQube + PiU (pipetting) was introduced in 2018 for fully automated red cell genotyping.
- ✓ The Fluogene RBC_ABO basic kits covers **A1, A2, B and O alleles (non-A1, -A2, -B and -O)**.
- ✗ Subgroups/variant ABO alleles such as **Ax, Ael, Abantu, Bombay Oh, Parabombay** are not included in the assay – limitation.



Next Generation Sequencing (NGS)



Omixon Monotype ABO kit

- HLA NGS is currently performed on an Illumina MiSEQ sequencer at SANBS.
- Source an assay for ABO NGS that could be run on the MiSEQ – maximize use of MiSEQ
 - *Omixon Monotype ABO*



Monotype ABO



Holotype HLA



Illumina MiSeq reagents





METHODS

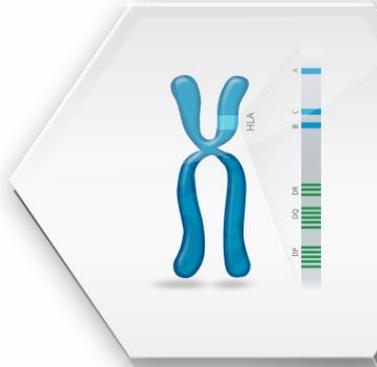
- Exploratory study – panel of standard ABO and rare/variant ABO subtypes.
- Selection included :
 - Group A₁
 - Group A₂ – homozygous and heterozygous
 - Group B
 - Group A₂B
 - Group O
 - Group O_h (Bombay)
 - Group A_{bantu}
- DNA was extracted on the Maxwell AS2000 – 50ng/ul (40mins)
- DNA + ABO primer + pre-mixed mastermix + enzyme (15 mins)





METHODS

SAMPLE PREPARATION MONOTYPE ABO™

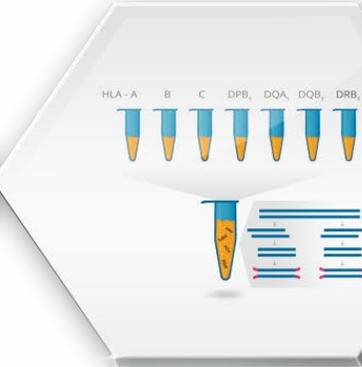


35min
HANDS-ON TIME

20min*
HANDS-ON TIME
WITH AUTOMATION

6h 45min
TOTAL TIME

LIBRARY PREPARATION MONOTYPE ABO™



2h 30min
HANDS-ON TIME

50min*
HANDS-ON TIME
WITH AUTOMATION

5h 10min
TOTAL TIME

SEQUENCING



20min
HANDS-ON TIME

17h 20min**
TOTAL TIME

DATA ANALYSIS HLA TWIN™



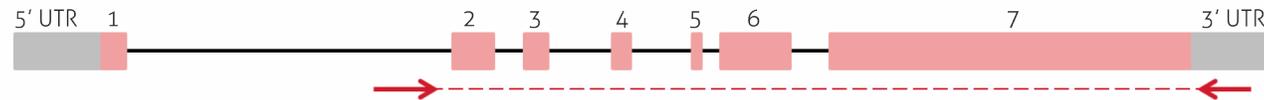
0h
HANDS-ON TIME

6h
TOTAL TIME



RESULTS/DISCUSSION

- The ABO gene is ~20kb long and is located on chromosome 9 (9q34.2).
- The Monotype ABO kit contains primers that amplifies from intron 2 to the 3' UTR (untranslated region). Allele-level genotyping results are obtained.

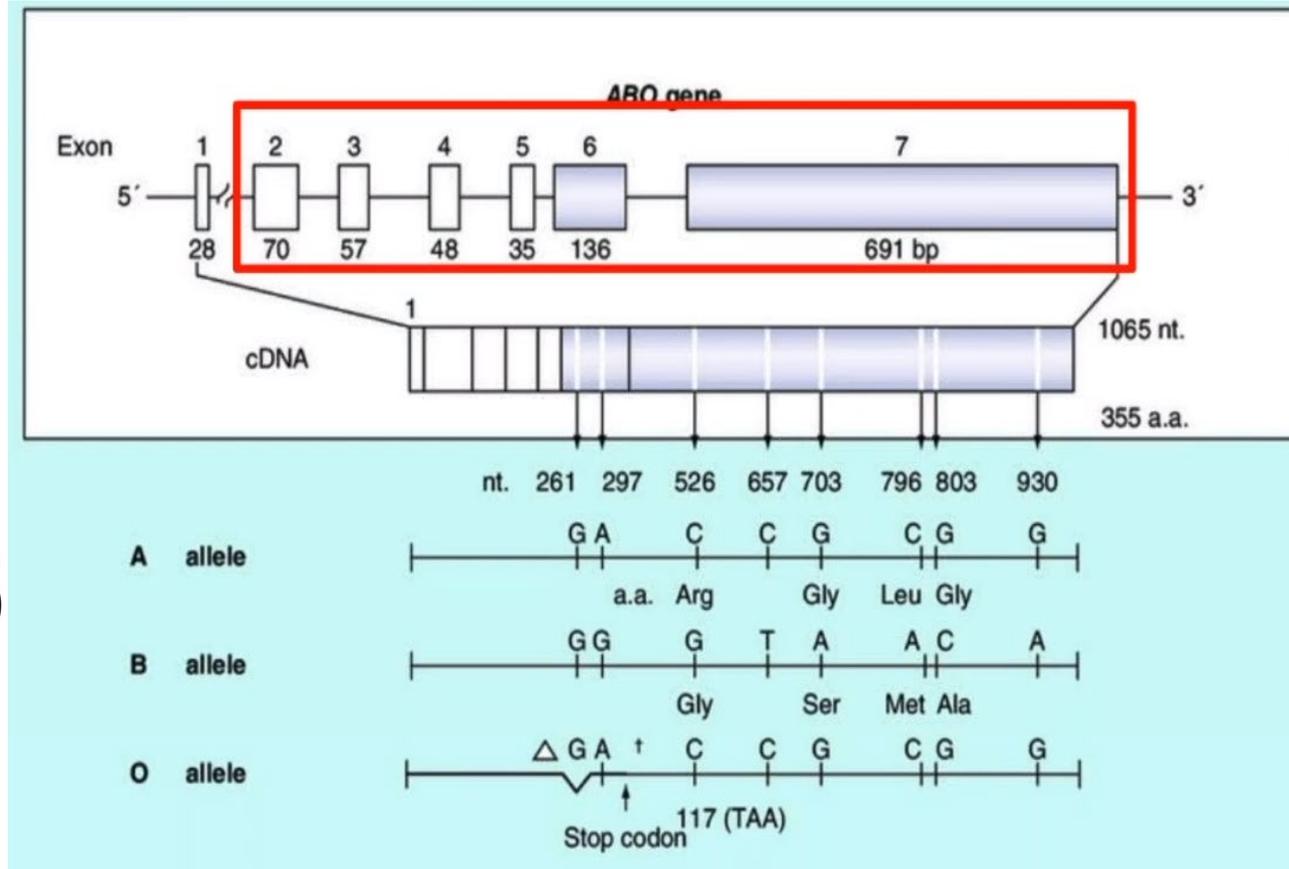


Allele	Phenotype	Genotype
Allele 1		ABO*01.01.1
		ABO*01.01.7
Allele 2		ABO*0.01.01.1
		ABO*0.01.01.7





Molecular basis of the ABO GENE



A₁ allele (ABO*A1.01)

A₂ allele (ABO*A2.01)

B allele (ABO*B.01)

O₁ allele (ABO*O.01)

O₂ allele (ABO*O.02)

297A>G, 657C>T and 930G>A

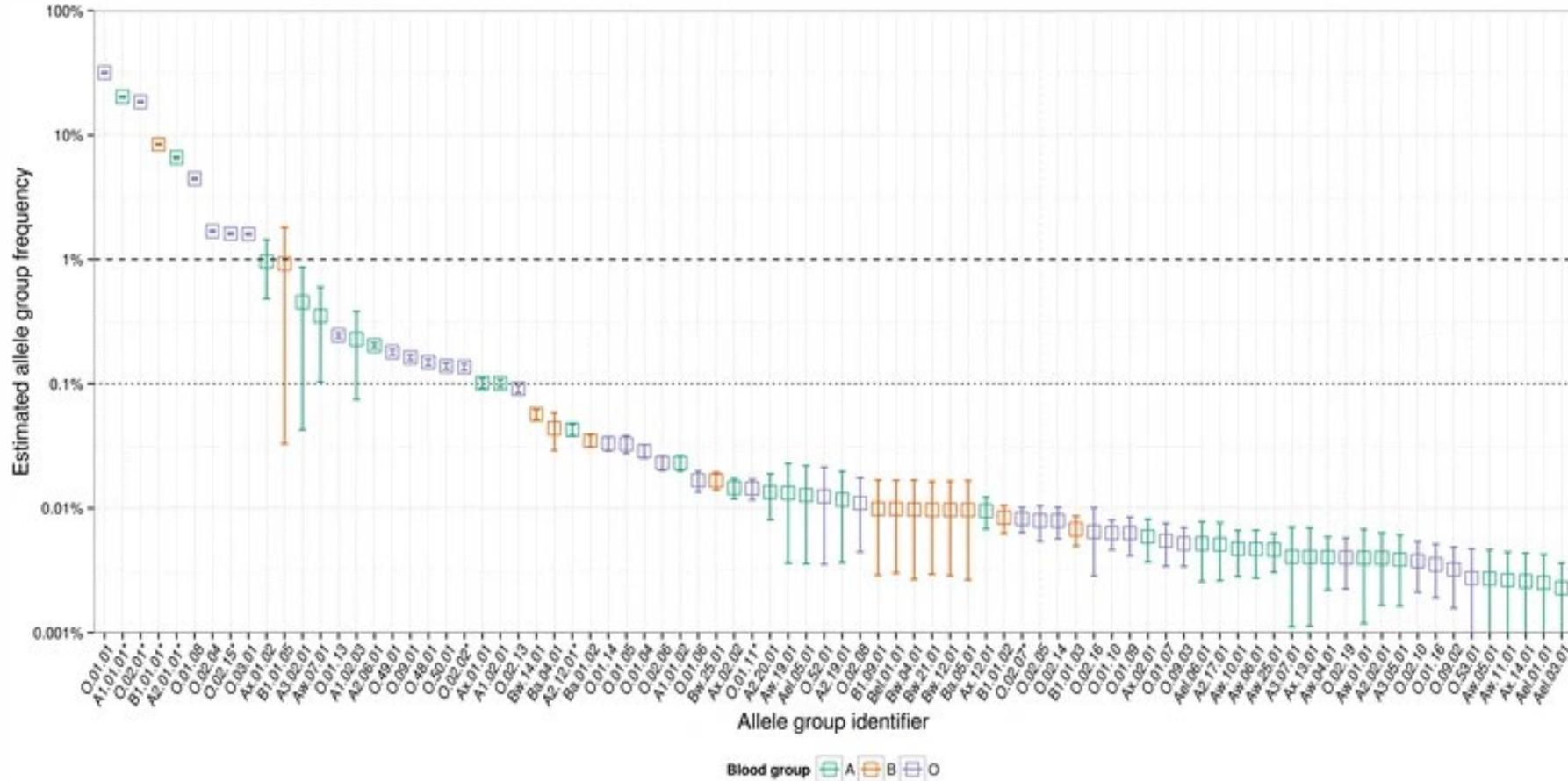
261delG aa117

no del 53G>T and 802G>A





SNP – Single Nucleotide Polymorphism





PILOT STUDY RESULTS

- HLA Twin software (used for ABO as well)
- Utilizes the dbRBC – reference sequences (blood group gene mutation-BGMUT)

	GENOTYPE FLUOGENE/**BAGENE		MONOTPYE ABO NGS		
	ALLELE 1	ALLELE 2	ALLELE 1	ALLELE 2	PHENOTYPE
SAMPLE 1	ABO*A2	ABO*A2	A2.01.01.1	A2.01.01.1	Group A ₂ A ₂
SAMPLE 2	ABO*A2	ABO*O	A2.01.01.2	O.01.01.1/7	Group A ₂ O
SAMPLE 3	ABO*B	ABO*B	B1.01.01.1	B1.01.01.1	Group B ₁ B ₁
SAMPLE 4	ABO*A2	ABO*B	Aw.22.01.1?	B1.01.01.1	Group A _w B
*SAMPLE 5	ABO*O	ABO*O	O.67.01.1#	O.67.01.1#	Group O ₁ O ₁
*SAMPLE 6	**ABO*A2	**ABO*O ^{1V}	A2.01.02.1 Aw.22.01.1?	O.02.01.1 O.02.01.4	Group A ₂ O or Group A _w O
*SAMPLE 7	**ABO*Ax	**ABO*O1	A1.01.01.1 Aw.13.01.1	O.02.01.1 O.02.01.4	GroupA ₁ O or Group A _w O

- indicates a novelty. Intron 4, position 246 was flagged as containing a deletion

*Sample 5 was genotyped by the IDCOREXT as being a hrS- rare blood type from the Rh system

*Sample 6 was a serologically known A_{bantu} DNA sample

*Sample 7 was a serologically known Bombay rare donor





CONCLUSION

- The exploratory ABO NGS study involved a new technology that shows future great benefits in the resolution of complex or unresolved ABO cases. Understanding of unusual serology results due to the patients clinical conditions such as chimerism and following haematopoietic stem cell transplant is possible.
- The results of the pilot study has revealed some uniqueness amongst the South African population → this provides an exciting prospect to develop a database of ABO gene sequences amongst the ethnicities of South Africa.
- To explore sequencing of other blood groups → RH system (kit shortly due for release).
- Experience in the analysis and interpretation of bulk sequencing data is in progress





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- Tissue Immunology laboratory, CK – assistance with the MiSEQ
- IMH Reference laboratory – provision of samples and known serology results

REFERENCES

- www.omixon.com
- Hult, Annika, *Studies of the ABO and FORS Histo-Blood Group Systems: Focus on Flow Cytometric and Genetic Analysis*, Lund University, 2013
- Olsson Martin et al, *Genomic analysis of clinical samples with serologic ABO blood grouping discrepancies: identification of 15 novel A and B subgroup alleles* BLOOD, vol 8, no.5, 2001.



Thank you