Characterizing S-s-U- and S-s-U+ variant donors using the IDCORE\textsuperscript{XT} (Progenika) and FluoGene VERYFY(Inno-train) Red Cell Genotyping Assay

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Introduction

• Haemagglutination → ‘gold standard’ for blood group phenotyping and has been used for more than a century.

• The exploration of the molecular basis of red blood cells (RBCs) antigens began in the mid-80s and still continues.

• 1-2% of all patients that receive blood transfusion develop antibodies to RBC antigens.
Introduction contin...

• Sickle cell disease - frequency of alloimmunization affects 20-30% of recipients.

• Prevention of alloimmunization:
  ❖ can be addressed by advanced red cell serological tests such as absorption/elution or extended cell phenotyping –costly and laborious
  ❖ Blood group genotyping as an alternative approach to identify antigen negative units, rare units and/or the presence of blood group variants
MNS Blood Group System

- MNS blood group system is second only to the RH blood group system in its complexity.

- Many of the alloantibodies to antigens in the MNS system are not generally clinically significant.

- Antibodies to high prevalence antigens have caused hemolytic disease of the foetus and newborn (HDFN).

- M and N – present on glycophorin A protein (GPA) encoded by gene GYP A.
MNSs contin...

- S, s and U antigen present on glycophorin B protein (GPB) encoded *GYPB*.

- The high frequency U antigen occurs almost universally in >99% of the population.

- The rare S-s- phenotype are either U negative (U-) or U+variant (U+\textsuperscript{var}) and share an African origin → when exposed to U+ blood produces anti-U.

- Anti-U is a clinically significant antibody as it causes mild to severe HDFN and hemolytic transfusion reactions (HTR).
MNS blood group system contin....

- GYPB

- GYPB deletion
  - True U negative

- Rearrangements in amino acids
  - altered GYPB
  - U variant

- S antigen is silenced – not expressed on cell surface
  - GYPB(P2) – c.270+5G>T
  - GYPB(NY) - c.230C>T

Figure 1: Distinguishing a true U negative from U variant
Methods

• **Aim:** To characterize the S-s-U- and S-s-U+var donors using molecular genotyping techniques.

• **Samples:**
  - DNA samples of 5 South African donors previously confirmed as U negative (n=2) and U+ variant (n=3) by serology.

• **Testing:**
  - IDCORE \(^{XT}\) Assay utilizing the Luminex platform – Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright and Lutheran – 10 blood groups, 29 polymorphisms.
  - FluoGene vERYfy (Inno-train) Assay utilizing the FluoVista – 6 blood groups, 6 polymorphisms.

• **Analysis**
  - Aspect SA and FluoGene software
  - BIDsXT software
Figure 2: Donor 1 and 2 Genotyping results indicating a GYPB gene deletion
## RESULTS contin...

### IDCORE XT Results

<table>
<thead>
<tr>
<th>MNS</th>
<th>Donor 3</th>
<th>Donor 4</th>
<th>Donor 5</th>
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<tbody>
<tr>
<td>GYPB’s</td>
<td>S (MNS:3)</td>
<td>S (MNS:3)</td>
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<td>GYPB’S</td>
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<td>GYPB*S_null(230T)</td>
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<td>GYPB*S_null(IVS5+5t)</td>
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### FluoGene vERYfy Results

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<td>RHD</td>
<td>Exon03/Exon05, Exon10, psi</td>
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<tr>
<td>RHCE</td>
<td>c, e</td>
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<tr>
<td>Kell</td>
<td>KEL2(k)</td>
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<tr>
<td>Kidd</td>
<td>JK1(A), JK2(B)</td>
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<tr>
<td>Duffy</td>
<td>FY2(B), FYnull</td>
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<tr>
<td>MN</td>
<td>MNS2(N)</td>
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<tr>
<td>Ss</td>
<td>MNS3(S), U+var</td>
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<tr>
<td>Dombrock</td>
<td>DO1(A), DO2(B)</td>
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+ (4): variable antigen expression

**Figure 3:** Donor 3, 4 and 5 genotyping results indicating the presence of U+var
Discussion

• Finding compatible RBC units in patients with anti-U have been found to be challenging – some anti-U reacts with U-RBCs depending on the GYPB molecular background of patient and donor.

• Study by Peyrard et al, 2012 – described that the Anti-U like made by S-s-U- individuals were reactive with the GYPB(P2) and GYPB(NY) RBCs which both express a weak/partial U-like reactivity.
Discussion

• S-s-U+\text{var} individuals produce anti-U that react with all U+ individuals and a variable proportion of U+\text{var} individuals depending on the epitopes expressed on the variant molecular (Ringressi et al, 2012).

• The expression of U antigen on U+\text{var} RBCs differs from one person to another \rightarrow dependent on antiserum used in testing (Storry et al, 2003).

• Transfusing U- patients with U- units and U+\text{var} with U+\text{var} will highly improve transfusion safety.
Conclusion

• This study confirms the importance of molecular genotyping donors with the S-s- phenotype to characterize true U- donors and the U variants.

• False positive and false negative results do exist in genotyping and serology

• Null phenotypes – show antigen negativity but genetic positivity - this poses a risk in donor genotyping if genetic approaches are exclusively applied (Meyer et al, 2016).

• Molecular genotyping testing must be used as a supplement to and not a substitute for serological testing.
References

• Meyer S, et al. MNSs genotyping by MALDI-TOF MS shows high concordance with serology, allows gene copy number testing and reveals new St(a) alleles. *British Journal of Haematology*, 2016; 174:624-636


• Saison C, et al. Family study of a Swiss patient uncovered a novel genetic basis for the S-s- U+$^\text{var}$ phenotype. Transfusion, 2014;54

• Storry JR, et al. Mutations in the GYPB exon 5 drive the S-s- U+$^\text{var}$ phenotype in persons of African descent: implications for transfusion.
Acknowledgements

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“Be the change you wish to see in the World”

Mahatma Gandhi