High Throughput Blood Group Genotyping
Using Mass Spectrometry

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cooperation:
1 Blutspende Zürich, Schlieren, Switzerland
2 Sequenom GmbH, Hamburg, Germany

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Schweizerische Ver. für Transfusionsmedizin, SVTM, Freiburg, Switzerland Sept. 08 – 09, 2011
Dr. Oester, Schweizer Gen. i. Hämostase & Onkologie, SGH, Basel, Switzerland Sept. 27 – 30, 2011
International Society for Blood Transfusion, ISBT, Lisbon, Portugal Sept. 27 – 30, 2011
Hematology, University Hospital Zurich, Zurich, Switzerland Oct. 20, 2011
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Single Nucleotide Polymorphism (“SNP”) - aminoacid change – change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)
Single Nucleotide Polymorphism ("SNP")- aminoacid change – change of N-glycosylation site: Cellano (KEL2) => Kell (KEL1)

**amplification primer**

CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
KEL*02/02 (kk)

**extension primer**

CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
KEL*01/02 (Kk)

**STEP 2: ddNTP extension**

CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
CTTGGAGGCTGGGCTCCTTGGTAATGCTGACGCTTTAAGACGGAGC
KEL*01/02 (Kk)

**spectrogram of three different Js\(^a\)/Js\(^b\) (KEL6 I KEL7) DNAs**

MALDI-TOF mass spectrometry measures molecular weight (in Dalton) of extension primers (5'612) plus 1 extended base.

Heterozygous samples show 2 different molecular weights for those primers at about 5'862 and 5'898 Dalton (all indicated by arrow).

Other specific peaks are results of other bloodgroup SNPs, detected in multiplex in the same PCR.
MALDI-TOF spectrogram of a “RARE” multiplex including 19 assays (=19 SNPs)

Primer unextended ~5'100 extended ~5'830-5'920

KN’01/01 (G peak only)

Typical MassARRAY® Assay Workflow

1. Isolate DNA or RNA
2. Design assays
3. Obtain primers
4. Marker selection

~Days

~10 Hours

PCR >> Single Base Ext. or Cleavage chemistry

Transfer assay products to SpectroCHIP®

Read assay products on Mass Spec; analyze data

provided by SEQUENOM®
MassARRAY® Technology

- Genotyping
- Mutation Detection
- Methylation
- Gene Expression
- Copy Number
- Sequence Variation

up to 138'000 genotypes per day (384 format), or 1'000 DNAs with 138 SNPs!
Specificities: RHD: zygosity is detectable...

RHD

RHD

D-signal in dd samples are negative (…)
D-signal in Dd samples is approximately 50% of maximum, and
D-signal in DD samples is 100%.

RHD-CE(2-9)-D-203Cpos

RHD-CE(2-9)-D-203Cneg

D.CCee

D.CCee
current developmental status …

<table>
<thead>
<tr>
<th>RHDbroad / RHCE</th>
<th>RHDhigh</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>2</td>
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<table>
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<tr>
<th>KELL- K-F-Y</th>
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<tr>
<th>MNS</th>
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<th>Public vs RARE</th>
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<th>HPA / HNA</th>
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HPA/HNA: HPA-9 obsolete
Public vs RARE: Radin, P1/P2
KEL-L, K-F-Y, KELnull (3), Knull(1) … hence, we are confident to start soon.

projects to come … this is exemplary, not confirmed yet

<table>
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From 2011 for 3 years:
3'000 DNAs for HPA/HNA
36'000 DNAs for Public vs Rare
6'000 DNAs for RHDbroad, KELL, K-F-Y, MNS
50% (minimum) of all DNAs will be from the canton of Zurich.
Other 50% (maximum) from other Swiss areas.
risk assessment for donor typing... examples

<table>
<thead>
<tr>
<th>Specificities</th>
<th>not recognised as</th>
<th>&quot;wrongly&quot; interpreted as</th>
<th>transfused to</th>
<th>consequence</th>
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</thead>
<tbody>
<tr>
<td><strong>RHDbroad / RHCE</strong></td>
<td>RHDonull</td>
<td>RHD</td>
<td>D pos</td>
<td>no</td>
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<tr>
<td><strong>RHDhigh</strong></td>
<td>RHD weak, el</td>
<td>RHD</td>
<td>D pos</td>
<td>no</td>
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<tr>
<td><strong>KELL- K-FY</strong></td>
<td>KEL2null (R52-19pa)</td>
<td>KEL2</td>
<td>kk</td>
<td>no</td>
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<tr>
<td><strong>MNS</strong></td>
<td>MNS3/4neg</td>
<td>MNS3/4</td>
<td>Ss</td>
<td>no</td>
</tr>
<tr>
<td><strong>Public vs RARE</strong></td>
<td>Kp'</td>
<td>Kp(a-b+)</td>
<td>Kp(a-b+)</td>
<td>(yes)</td>
</tr>
<tr>
<td><strong>HPA / HNA</strong></td>
<td>HNA3a^anom/3b</td>
<td>HNA3b/3b</td>
<td>HNA3(a+b+)</td>
<td>no</td>
</tr>
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</table>

There are no risks transfusing genotyped blood to recipients, others than not having typed for an allele! Recipients on the other hand, would need highest resolution to avoid errors (e.g. unexpressed RHD, misinterpreted as D pos). Currently, recipients are "undertyped" for genetical matching (in silico) with donors! Knowing serology of the recipient (the more, the better) plus negative DAT, could circumvent need for in vitro cross-match and replace it by in silico cross-match.

Conclusions

- Overall, 85% of all specificities with control DNAs available were already operating perfectly in the first test of the first prototype. The observed success rate is highly impressive.
- Testing for RHD exon specific SNPs delivered reliable RHD gene copy number measurements.
- The presented KEL, JK and FY worked in one single multiplex PCR only! This included additional specificities for weakly and unexpressed KEL, JK and FY alleles. Module is "ready to use", waiving minor KELnull and JKnull capability.
- Success rates for prototypes of "RARE" and HPA/HNA were 92% and 93%, respectively, in the first test! Modules are "ready to use", waiving Radin, P1/P2 and HPA-S.