Non-invasive prenatal diagnosis on the fetal RhD-status from maternal plasma - a feasibility study

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Methods

- 15ml EDTA-anticoagulated blood/pregnant women
- Transport at room temperature 0 – 8 days (median 2 days)
- Storage of plasma and buffy coat at -80°C
- D-status via indirect anti-globulin test

QIAamp DSP Virus Kit (Qiagen, Hilden, Germany) spin-protocol, “spin columns"

1x 1.0ml chemagic Magnetic Separation Module 1 (Chemagen, Baesweiler, Germany) “magnetic tips"

RHD exons 5/7
2 replicates/sample
control: β-globin PCR
Subjects

1113 women, gestational week 6-32 (median 25), serologically D-negative

5 carriers of RHD ex5/7:
- weak D type 1
- RHD (M295I), DEL
- RHD (frame shift) 2x
- RHD wild type (RHD unexpressed)

16 hemolytic, 7 serum samples

1084 RHD ex5/7 deletions, 1 RHDΨ

1022 (94.2%) cord blood serology ↔ real-time PCR results
## Results

1022 samples

12 discrepancies

<table>
<thead>
<tr>
<th>n samples</th>
<th>false negative</th>
<th>false positive</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>spin columns</td>
<td>magnetic tips</td>
<td>week 25, <strong>transport 6 days</strong>, repeat tests (14pg/ml) and <strong>RHD</strong> sequencing pos.</td>
</tr>
<tr>
<td>1</td>
<td>spin columns</td>
<td></td>
<td>week 22, repeat test and magnetic tips pos., but yield of fetal DNA below average</td>
</tr>
</tbody>
</table>
| 3         | serology       |                | 1 normal **RHD**, repeat serology pos.  
2 weak D type 2 |
| 3         | spin columns   | magnetic tips  | repeat tests and **RHD** sequencing neg. |
| 4         | magnetic tips  |                | repeat tests neg. |

**662 (64.8%)** D-positive  
**360 (35.2%)** D-negative
### Sensitivity – Specificity - Accuracy

<table>
<thead>
<tr>
<th></th>
<th>Spin Columns (Manual Extraction)</th>
<th>Magnetic Tips (Automated Extraction)</th>
<th>Postnatal Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>99.7%</td>
<td>99.8%</td>
<td>99.5%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>99.2%</td>
<td>98.1%</td>
<td>&gt; 99.7%</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>99.5%</td>
<td>99.2%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>
Conclusion

Antenatal real-time PCR is at least as sensitive as postnatal serology for the determination of the child’s D-status, serology is more specific.

Determination of the fetal RhD-status from maternal plasma for decision making on Rh-prophylaxis is feasible.

There seems to be a risk-benefit balance between this approach and the established routine antenatal Rh-prophylaxis.
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Participating Obstetricians and patients

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Thank you for your attention!