Fetal \textit{RHD} genotyping in maternal plasma: from validation to management of a non-invasive prenatal test

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OMIC’s, 3rd International Conference and Exhibition on Clinical and Cellular Immunology, 29/09/2014-01/10/2014, Baltimore
fetal *RHD* genotyping on maternal plasma

- *RHD* gene: many variant forms
- analysis of 3 regions of the *RHD* gene:
  - Sensibility +++, specificity +++, Cut off values
  - Validation of the method

⇒ False negative=0

2 steps
- DNA extraction
- real time PCR
RHD gene particularities

RHD gene

RHCE gene

D positive

D negative
Other cases

From gene

Presence of the RHD gene
Expected phenotype: RH:1 (positive)

Absence of the RHD gene
Expected phenotype: RH:-1 (negative)

Presence of an abnormal RHD gene
Phenotype can not be determined

….to expression of RH1 antigen

Erythrocyte’s surface
## Known RHD gene variant forms tested by the method used in 2010-2011 in our laboratory

*(poster, congress SFBC, Lyon 2011)*

<table>
<thead>
<tr>
<th>EXON4</th>
<th>EXON5</th>
<th>EXON10</th>
<th>CHARACTERISTICS</th>
<th>DENOMINATION</th>
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</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>P</td>
<td>RH1 partial DVI type 2</td>
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<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>P</td>
<td>D variant type VI type?</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>P</td>
<td>African origin pseudo gene ψ or r’s?</td>
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<td>-</td>
<td>+</td>
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<td>P</td>
<td>DHAR (*)</td>
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<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>P immunogenicity+++</td>
<td>DVI type 3</td>
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<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>P?</td>
<td>D partial III type 4+ deletion exons 4 to 7</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>P</td>
<td>DNB(*)</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak type 10</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>Initially known as D- then D weak type 1</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak type 5 without allo-immunisation</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>RH1 weak type 11</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak type?</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>F</td>
<td>D weak type?</td>
</tr>
</tbody>
</table>

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SENSIBILITY: detection of fetal DNA (5%)

SPECIFICITY: detection of *RHD* gene

**RHD** gene structure

Currently studied exons

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fetal *RHD* genotyping

3 levels of specificity

*RHD* gene detection

1) SPECIFIC probes

2) SPECIFIC primers

DNA amplification

Fluorescence emission

Probe’s hydrolysis

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DNA amplification

Fluorescence Intensity

Obtained value = FETAL or VARIANT CARRYING MOTHER?

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SENSIBILITY: detection of fetal DNA (5%)

SPECIFICITY: detection of RHD gene

Detection of fetal RHD gene only?

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Necessity to define cut off values to guarantee fetal specificity

=> 3rd level of specificity

Cut off valuel Ct = FETAL SPECIFICITY

Earlier amplification = lower Ct value
MATERNAL origin
=> Maternal variant form of RHD gene
Control samples: OK (known D+, D- samples, blank)
- Yes
  - Control gene (CCR5) for each patient’s sample: OK
    - Yes
      - Analysis of each sample’s result:
        - Amplification of each tested exons
          - In normal range
            - Presence of the RHD gene (always verified on a second sample)
        - Absence of amplification
          - Absence of the RHD gene
        - Abnormal amplification
          - Amplification of 1 or 2 of the 3 tested exons:
            - Fetal RHD variant form
          - Early amplification of 1, 2 or the 3 tested exons:
            - Maternal RHD variant form
    - No
      - New run for all the samples
- No
  - New run for possible inconclusive sample

Inconclusive sample: RHD gene sequencing
Method with high **SENSIBILITY** and **SPECIFICITY**

**Conclusions:** validated method
Decision to set up the test routinely

Fetal **SPECIFICITY**
Laboratory

-Pre-PCR area, post-PCR area: are geographically separated

-Access limited to staff

-No manipulation of the post-PCR products

=>in order to LIMIT the risk of DNA contamination
Fetal *RHD* Genotyping in maternal plasma

- Non Invasive Prenatal Diagnosis:
- Tested from maternal plasma sample
- Can be performed starting at 10 weeks of pregnancy
Fetal *RHD* Genotyping in maternal plasma

- Indications of this test:
  - anti-D immunised patients: to increase medical supervision during the pregnancy (if the result is positive)
  - Prevention of anti-D immunisation
Fetal *RHD* Genotyping in maternal plasma

Continuous improvement and innovation

- **Scientific knowledge**
  - specialized articles in newspapers
  - internet (for example: http://www.uni-ulm.de)

- **Laboratory management**: Yearly review
  - Performances analysis
  - Procedures review
  - Key Indicators analysis
  - Customers’s expectations
  - Continuous improvements
  - Risk management

- **How to maintain the dynamism of the laboratory?**
  - Biologists participate in weekly antenatal meetings
  - The laboratory is part of a national group

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Fetal RHD Genotyping in maternal plasma
Continuous improvement and innovation

Quality management: continuous quality improvement is symbolized by the Deming wheel:
Cooperation between Hospital, Laboratory, Physicians

The different healthcare actors

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Thank you for your attention!

Any questions?

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