Non invasive Fetal *RHD* Genotyping on Plasma DNA from RHD Negative Pregnant Women Carrying the Silent *RhDPsi* Gene

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Background

Allo-immunization against the RhD red cell surface antigen is still the commonest cause of hemolytic disease of the fetus and newborn. A reliable determination of fetal *RHD* genotyping is now possible from maternal blood sample of RhD-negative women. Fetal RhD status is performed using real-time polymerase chain reaction (PCR) on fetal DNA derived from maternal plasma of RhD-negative women. This analysis implemented in the routine diagnosis allows anti-D treatment to be specifically administered only to un-sensitized pregnancies at risk of immunization (RhD+ fetus) and to save anti-D injection. Moreover this test has become a benefice for the monitoring of RHD-negative anti-D-immunized pregnant women. However this genotype analysis done from maternal plasma with « Free DNA fetal kit RHD® » CEIVD (European Community In Vitro Diagnostic) Kit (TCB, 2007 (14) :572-7) is suitable only for RHD-negative pregnant women carrying the complete deletion of *RhD* gene. For RHD-negative pregnant women carrying an intact but nonfunctional *RhD* gene, this test that uses amplification in exon 7 and exon 10 of the *RhD* gene remains inconclusive. The most frequent *RhD* silent gene is *RhDPsi*: it is carried by 66 percent of the RHD-negative pregnant woman from African population which is large in France.

Objective

Validation of a new technique and strategy for fetal *RHD* genotyping on maternal plasma in women carrying the silent *RhDPsi* gene: situation seen in 3 percent of fetal RHD genotyping analysis done in the CNRHP laboratory.

Materials and method

Plasmas DNA from 35 RHD-negative pregnant women known to carry silent *RhDPsi* gene were extracted from 500µl of plasma using QIAamp MinElute Virus Vaccum Kit Extraction (Qiagen, Courtaboeuf, France). DNAs were eluted in 35µl of water. For each patient, three real time PCR were done : one amplification of the exon 7 or 10 using the « Free DNA fetal kit RHD » as an extraction control and amplifications of the exons 5 et 6 *RHD* wildtype allele specific (Finning et al., Transfusion, 2002 (42) : 1079-1085). The results obtained were interpreted using the diagram below and then compared with the fetal *RHD* genotype determined on amniotic cell and/or the RHD phenotype of the red blood cells of the babies at birth.

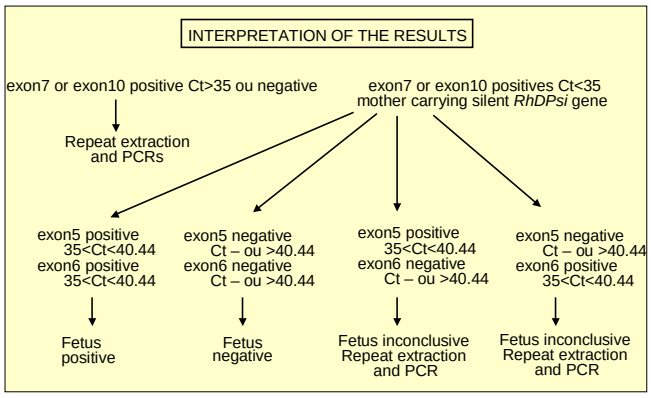

Diagram representing decision making of results for *RHD* fetal genotyping on maternal plasma carrying the silent *RhDPsi* gene

Results

	RhD+ Fetus	RhD- Fetus	Total
Test +	28	0	28
Test -	0	5	5
Inconclusive	1	1	2
Total	29	6	35

Ct_{E7m}=31.30 (±1.90)
Ct_{E10m}=32.28 (±2.43)

⇒ Inconclusive percentage =5.7%

Repeat

	RhD+ Fetus	RhD- Fetus	Total
Test +	0	0	0
Test -	0	1	1
Inconclusive	1	0	1
Total	1	1	2

⇒Inconclusive percentage=2.9%

- Sensibility =100%
- Specificity =100%
- True-positive =100%
- True-negative =100%

Conclusions

Amplifications in the exons 5 and 6, in addition with amplification in exon 7 and 10 of the *RhD* gene, are now used in our laboratory for non invasive fetal *RHD* genotype and allow the accurate diagnosis of fetal RHD status even on plasma from RHD-negative pregnant women carrying the silent *RhDPsi* gene.

Amplifications in the exon 5, 7 and 10 of the *RhD* gene are routinely done on plasma from all RHD-negative pregnant women, whereas amplification in the exon 6 is only done on plasma from RHD-negative pregnant women carrying the silent *RhDPsi* gene.