

Non-invasive prenatal testing: Technical strategies to achieve testing of cell free fetal DNA (cffDNA) RHD genotype in a clinical lab

Stephen Brown M.D., University of Vermont; Leonard H Kellner M.S., Lenetix MSL Inc.; Marsha Munson B.S., Lenetix MSL, Inc.; Yanfeng Yang Ph.D., Sequenom, Inc.; Brandy Klotzle M.S., Sequenom, Inc.; Allan T Bombard M.D., Sharp Mary Birch Hospital, San Diego CA

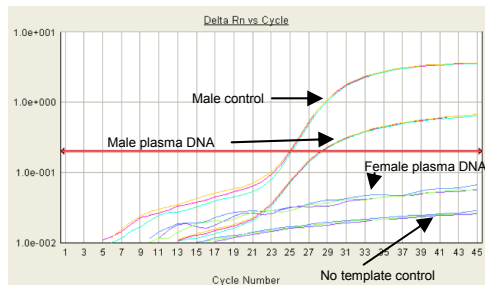
Overview:

Non-invasive determination of fetal Rh genotype based on cffDNA has been available for routine clinical use in Europe for several years; however, this testing has not been available in the US. In order to provide such testing in the US clinical market, it must be extensively validated. We set out to establish and validate non-invasive testing of fetal Rh genotype as a routine clinical test. This has proceeded as follows:

- 1) Establishment of rtPCR assays for the detection of SRY and for RhD gene sequences using samples of banked blood.
- 2) Assay validation for use on cffDNA by making use of stored second trimester serum samples from pregnancies where fetal sex was known. These studies showed that correct determination of fetal sex was possible in those samples where sufficient DNA was present.
- 3) Prospective validation of assays on prospectively obtained samples from ongoing pregnancies.
- 4) Securing of New York State approval to offer routine clinical testing fetal Rh genotype. Non-invasive testing of fetal Rh genotype is now commercially available in the US.

Results: Assay Development

rtPCR assays for SRY and for exons 4, 5, 7 and 10 of the RhD gene were based on previously described Taqman[®] assays. Importantly, the exon 4 and 5 assays were designed to detect the ψ allele that is common in people of African descent. These were optimized by testing a variety of primer and probe concentrations and PCR instruments. Serial dilutions of a standard DNA were used to demonstrate sufficient sensitivity to detect 1 genome equivalent of DNA. Samples of banked blood of known RhD serotype were then used to demonstrate that the correct RhD serotype could be predicted.



An example of rtPCR results for the SRY locus. Shown are results from plasma DNA from pregnancies where the fetus was male and others where the fetus was female. In addition, there is a "no template control" and a male genomic DNA control.

Results: Stored Serum Samples

Stored second trimester serum samples were provided by Women and Infants' Hospital; Providence, RI.

In a series of 78 samples of second trimester stored serum, 32/60 (53%) male samples were correctly identified and, importantly, 0 of 18 females were identified as male.

Following assay optimization, a second series of 50 paired serum and amniotic fluid samples was tested. 28/35 (80%) male samples were correctly identified and no females were identified as male. In those samples where the fetus was male, Rh genotype was consistent with the newborn serotype.

Conclusion: These results proved that when DNA was present, it could be amplified and that sex could be correctly determined.

Results: Prospectively Collected Samples

We obtained IRB approval to collect blood and AF samples from women who had elected to have second trimester amniocentesis. Plasma DNA was prepared within 24 hours of blood collection.

This work is ongoing, but demonstrates that in 39 of 40 samples, the sex was correctly obtained. Moreover, in all of these samples, the maternal Rh genotype was consistent with the serotype and the fetal Rh genotype from cffDNA was consistent with the fetal genotype as determined from the AF sample.

Conclusion: Fetal sex and Rh genotype can be determined from cffDNA.

Conclusions and Future Directions

- 1) Adequate efficiency, sensitivity and specificity for detection of fetal Rh genotype has been achieved.
- 2) NY State approval has been obtained; as a result, non-invasive testing of fetal Rh is now commercially available in the US.
- 3) We are developing methodology to improve detection of cffDNA when the fetus is an Rh negative female.