North East Thames Regional Genetics Service

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Rapid, efficient, non-invasive fetal sex determination direct from maternal plasma

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Background

Many European countries determine fetal sex by analysis of cell free DNA (cfDNA) in maternal plasma to triage pregnancies at high risk of sex-linked disorders for invasive testing. This has significantly reduced the invasive testing rate in this high risk group by targeting male bearing pregnancies for X-linked disorders. In the UK this test was approved in our public sector National Health Service (NHS) in 2011 and is now delivered by five NHS Regional Genetics laboratories. Current approaches require cfDNA extraction from plasma prior to analysis. Here we present validation of a method analysing cfDNA direct from maternal plasma.

Objective

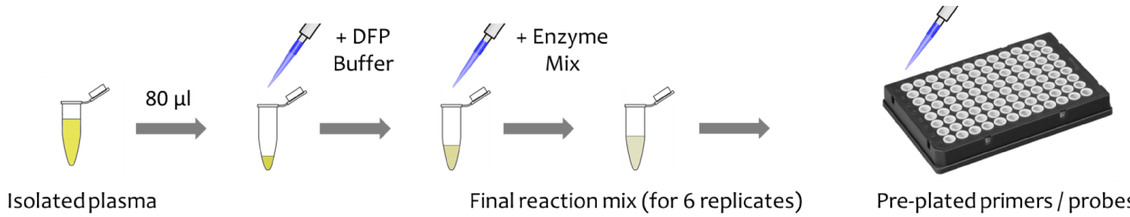
To assess the first fetal sexing method analysing cfDNA direct from maternal plasma in a diagnostic laboratory.

Method

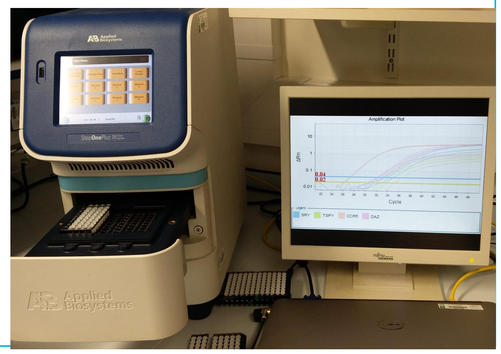
We identified stored plasma samples taken from 50 male and 50 female high risk pregnancies from 7+1 to 9+6 weeks gestation with fetal sex tested between January 2013 and March 2016 and clinically confirmed.

Our current protocol involves duplicate cfDNA extractions (QIAsymphony SP, 2hrs duration) from 2ml of double-spun plasma followed by detection of the *SRY* and *CCR5* genes by quantitative real-time PCR (Q/RT-PCR) (Taqman, ABI 7300). 44 samples had been reported male, 43 female and 13 inconclusive (e.g. SRY amplification detected below threshold and a repeat maternal blood sample requested). One pregnancy is tested per run with a minimum turnaround of 2 days.

Up to thirteen 80ul plasma samples with controls were tested simultaneously using the Cell3™Direct: Fetal Sex Determination kit (Nonacus, UK). Triplicate testing with male and female cfDNA controls was performed on single maternal plasma samples by real-time qPCR (ABI7300 and StepOnePlus PCR systems) using multiplexed TaqMan assays targeting *SRY, DAZ* and *TSPY* on the Y-chromosome and *CCR5*, an autosomal house-keeping gene to control for the presence of total cfDNA. *TSPY* and *DAZ* are both Y-chromosome specific multicopy genes, as the more targets the more specific and reliable the test can be. If the result was not conclusive the test was repeated. The kit can also be used with extracted cfDNA.



Direct from plasma (DFP) buffer counteracts PCR inhibitors in maternal plasma to allow PCR amplification of cfDNA in a pre-plated Cell3™Direct: Fetal Sex Determination kit (Nonacus, UK). Triplicate testing amplifies targets *SRY, DAZ* and *TSPY* on the Y-chromosome and *CCR5* in a flexible break-apart plate with 1-4 separate runs with controls possible per plate.

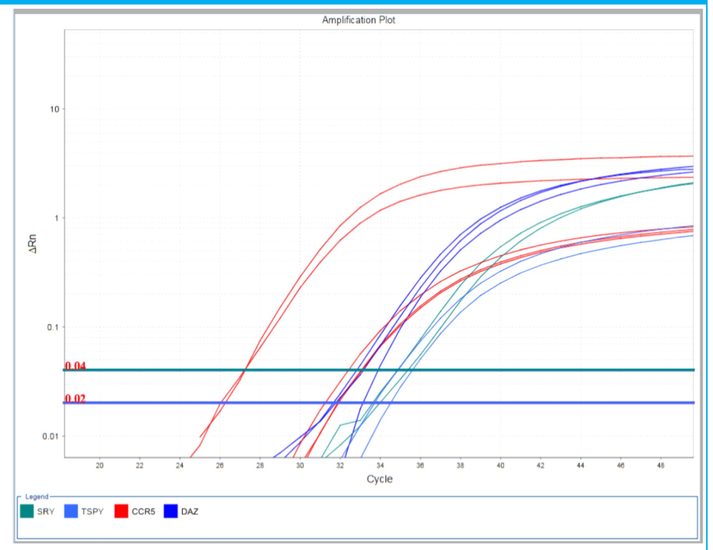


Results

Triplicate direct from plasma qPCR plots of *SRY, DAZ, TSPY* and *CCR5* targets of a male pregnancy taken under 10 weeks gestation.

The graph shows female cfDNA control sample (left-hand *CCR5* curve) and male plasma sample (right), with all 4 genes amplified.

Thresholds for classification of male or female were established: A pregnancy is predicted to be female if only CCR5 amplifies, or male if a minimum of 1/3 replicates show positive amplification (Ct <40) for each gene. Plasma samples falling below this threshold but with evidence of *SRY* amplification were repeated.



From the cohort of 50 male and 50 female-bearing high risk pregnancies including 13 previously reported as inconclusive using the standard protocol, 89 plasma samples tested showed conclusive concordant results first time. The remaining 11 were repeated once and showed conclusive results second time, therefore we show this technique to be more sensitive than the current protocol.

All 3 female and 2 male samples from pregnancies under 9 weeks gestation were concordant first time.

Conclusion

- This is the first direct from plasma, non-invasive prenatal test to determine fetal sex.
- Removal of the 2-hour extraction step streamlines the protocol and reduces consumables and labour costs.
- The pre-plated assay design allows a rapid result turnaround of approximately 3 hours from sample receipt
- The potential for same-day reporting would improve the current turnaround time.
- 1-13 samples can be tested per run, increasing throughput and reducing hands-on time.
- Reduced plasma volume required allows for a smaller blood collection or for further testing if required.
- Further validation is required to define precise sensitivity with confidence before any diagnostic analysis.

Reference

Hill M, Lewis C, Jenkins L, Allen S, Elles RG, Chitty LS. Implementing noninvasive prenatal fetal sex determination using cell-free fetal DNA in the United Kingdom. Expert Opin Biol Ther. 2012 Jun;12 Suppl 1:S119-26.

Acknowledgements

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