Extraction protocol to increase the yield of circulating fetal DNA in maternal plasma

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Background

Compare to the current invasive prenatal diagnosis methods, non-invasive approach is an ideal way to perform diagnostic test in much earlier stage of pregnancy while reducing the chance of miscarriage to a minimum.
However, the nature of low concentration of cell free fetal DNA (cffDNA) in maternal circulation is the major technical challenge to achieve effective non-invasive prenatal diagnosis. We present here a sample collection and extraction workflow generating substantial increase in extraction yield and total amount of fetal DNA in maternal plasma, increasing the feasibility for clinical application of non-invasive prenatal diagnosis.

Materials and Methods

Ten milliliters of maternal blood samples were collected with Cell-Free DNA™ BCT (Streck ®, Inc., USA) and processed with the following protocol within 24 hours after collection.
Blood samples were first centrifuged at ambient temperature with 700 x g for 10 min. The plasma layer was carefully collected and centrifuged at 16000 x g for another 5 min. The cell-free plasma was carefully removed and DNA was extracted using QIAamp Circulating Nucleic Acid kit (Qiagen, Germany) with manufacturer's protocol. The plasma DNA was eluted in 100ul AVE buffer. Cell-free fetal DNA to maternal DNA ratio was determined by real-time PCR against Y-specific sequence [1,3].

Results and Discussion

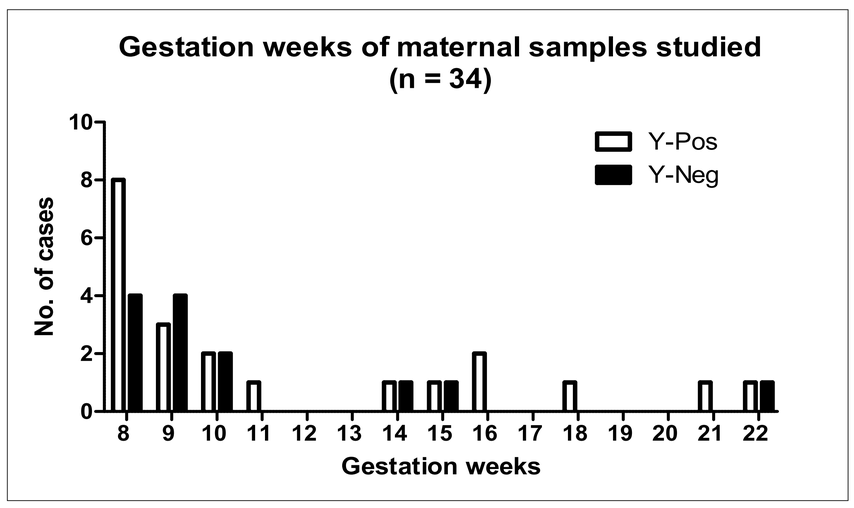


Figure 1. Gestation weeks of maternal blood samples in the study. Total 34 maternal blood samples were studied (ranged from 8 to 22 weeks). Real-time PCR with Y-specific sequence was used for fetal DNA detection, in which 21 samples were Y-positive and 13 samples were Y-negative.

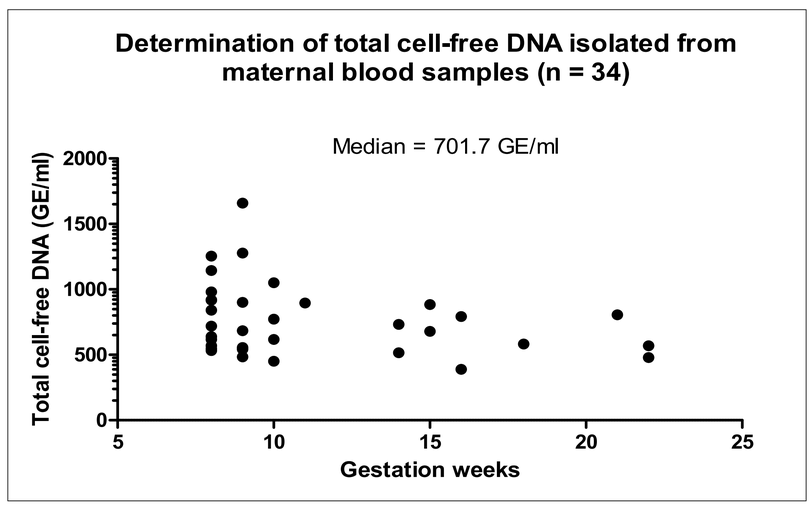
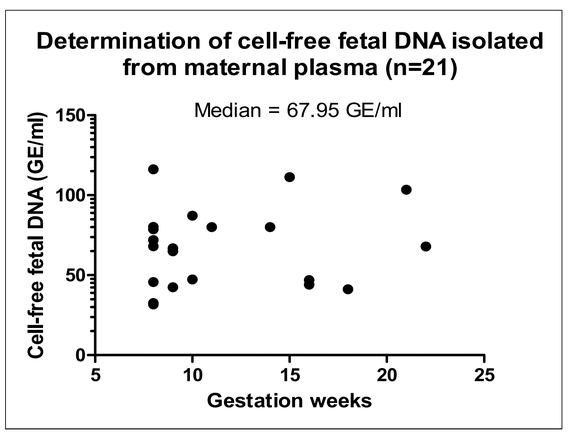


Figure 2. Determination of total cell-free DNA extracted from maternal blood samples. Total cell-free DNA isolated ranged from 388.5 to 1658.5 GE/ml plasma collected (median 701.7 GE/ml).



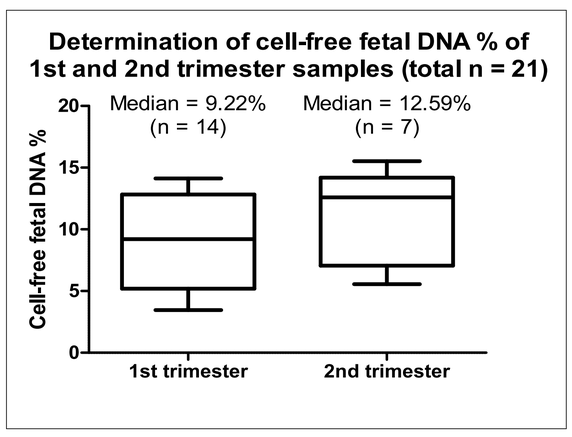


Figure 3a (left). Determination of cell-free fetal DNA (cff-DNA) extracted from maternal blood samples (21 Y-positive samples). Fetal DNA isolated ranged from 31.63 – 116.21 GE/ml (median = 67.95 GE/ml). Figure 3b (right). Cell-free fetal DNA % was studied by trimester. For first trimester samples (8 -11 weeks, n = 14), fetal DNA content ranged from 3.45 % to 14.13% (median = 9.22%, 67.43 GE/ml). For second trimester samples (14-22 weeks, n=7), fetal DNA content ranged from 5.56 to 15.53% (median = 12.59%, 95.65 GE/ml).

	Median fetal DNA concentration (%)				
Gestation age	This Study (Real-time)	Conventional Method (Real-time)	Study A [2] (Real-time)	Study B [4] (Real-time)	Study B [4] (Digital PCR)
First Trimester	9.22	4.52	6.8	4.8	9.7
Second Trimester	12.59	7.82	N/A	4.1	9.0

Table 1. Comparison of fetal DNA concentration with other studies. Fetal DNA isolated from both first and second trimester samples in this study were higher than conventional method (unpublished data) and other studies [2, 4]. In comparison with Study B, extraction yield of this study was comparable with digital PCR detection method.

Conclusion

This workflow enables cffDNA to be preserved and collected in higher yield than conventional collection method (K3EDTA tube collection)[5]. Fetal to total DNA ratio of this study was about 2 folds of the conventional method (i.e. median ranged from 9.22 and 12.59% compared to 4.52% and 7.82% for first and second trimester respectively, unpublished data). Furthermore, the collected blood sample can be preserved up to 7 days with no significant change (unpublished data which was comparable to other study [2]) . Therefore, this workflow greatly opens up the possibility of performing downstream application for non-invasive fetal diagnosis for patient.

References

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