Finger Stick Self-Collection of Maternal Blood for Non-Invasive Prenatal Testing

H. Milot, C. Jacob
Gateway Genomics, San Diego, CA

Abstract

Noninvasive prenatal testing (NIPT) is currently limited to maternal venous blood collection by trained phlebotomists, which can restrict accessibility to prenatal screening. We conducted the first evaluation of self-collected capillary blood samples compared with phlebotomist-collected venous blood samples for the determination of fetal sex. Pregnant women (11-37 weeks gestation) self-collected capillary blood samples by lancet finger stick. Venipuncture samples were collected from another set of pregnant women to allow for comparison of test results with those obtained from capillary samples. Plasma was separated from whole blood by centrifugation and cell-free DNA was isolated using a commercial DNA extraction kit. Real-time quantitative PCR was performed to detect fetal DNA using a multi-copy sequence on the Y chromosome. An autosomal control gene was used to measure total cell-free DNA (maternal and fetal). Test results for capillary blood samples were compared to venipuncture blood sample results. Cell-free DNA was detected in all capillary and venous blood samples. Y-chromosome specific sequences were detected in capillary and venous blood samples in all pregnancies confirmed to have a male fetus. All gender results were in concordance with known fetal sex, without false-positive or false-negative results. For fetal sex, self-collected capillary blood samples and venous blood samples from pregnant women produced a sensitivity of 100%, specificity of 100% and accuracy of 100%. Prenatal diagnosis of fetal gender from self-collected capillary blood is simple, accurate, and reliable. The results of this study demonstrate that fetal DNA analysis using self-collected capillary blood is highly feasible and easily adaptable for population screening. This method simplifies blood collection of maternal blood and should increase the accessibility of noninvasive prenatal testing.

Introduction

The potential of utilizing maternal plasma for noninvasive prenatal diagnosis was first described in 1997 by Dennis Lo.[1] Lo was the first investigator to demonstrate the presence of cell-free fetal DNA in maternal plasma.[1] The maternal plasma samples in Lo's study were obtained from pregnant women via venous blood draw.

Almost twenty years later, noninvasive prenatal testing still utilizes venous blood sampling for the detection and analysis of cell-free fetal DNA. Venous blood sampling requires a licensed phlebotomist and a centralized laboratory which can limit access to NIPT. A simpler and more convenient method for collecting maternal blood for noninvasive prenatal testing was investigated in this study.

We demonstrated the feasibility of a new method for obtaining cell-free fetal DNA, capillary blood via lancet finger stick, that can be used for noninvasive prenatal diagnosis. This new collection method has the potential to increase the accessibility of noninvasive prenatal testing.

In addition, we conducted a validation study to show that the performance of a fetal sex determination assay (SneakPeek®) was similar between venous and self-collected capillary blood samples.

Materials and Methods

After obtaining informed consent, capillary blood samples were obtained from 18 pregnant women (11-37 weeks gestation). For the validation study, venous blood samples were collected from 18 pregnant women (9-17 weeks gestation). Venous blood was collected via standard venipuncture and capillary blood was self-collected via finger stick. Plasma was separated from whole blood via centrifugation. Cell-free DNA was extracted and purified using a commercial DNA extraction kit.

Following isolation of cell-free DNA, real-time quantitative PCR was performed to detect the presence of male cell-free fetal DNA using a multi-copy target sequence on the Y chromosome. In addition to the Y-target, an automsomal control gene was used to measure the amount of total cell-free DNA (maternal and fetal) present in the sample.

Gender calls were made using the SneakPeek® algorithm. The algorithm incorporates C_T values of the Y-target sequence and autosomal control gene.

All fetal sex test results were confirmed via ultrasound by a licensed sonographer.

Results

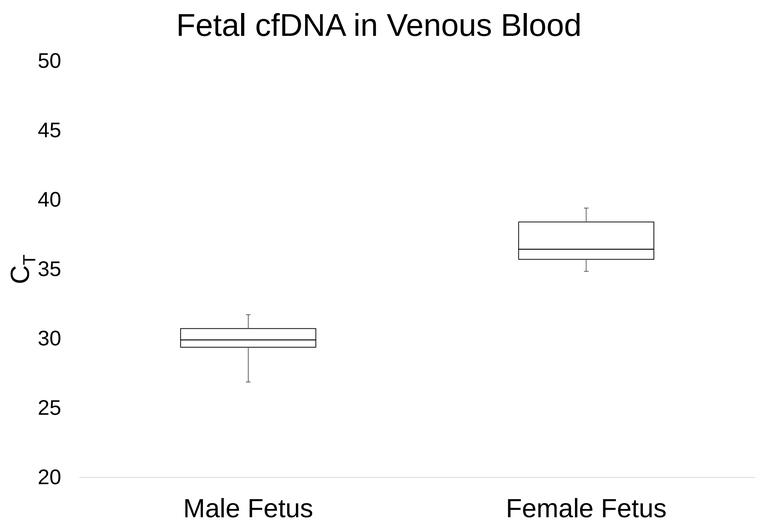

Figure 1.Cycle threshold results for Y-target qPCR

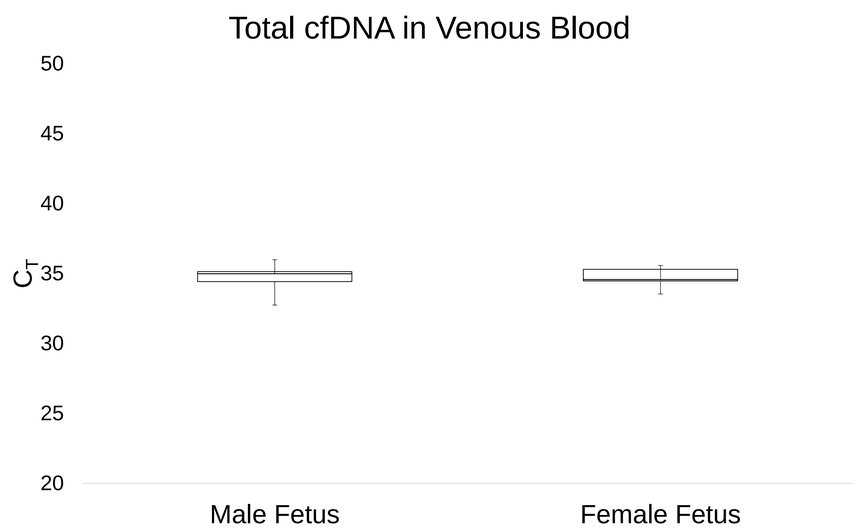

Figure 2. Cycle threshold results for autosomal target qPCR

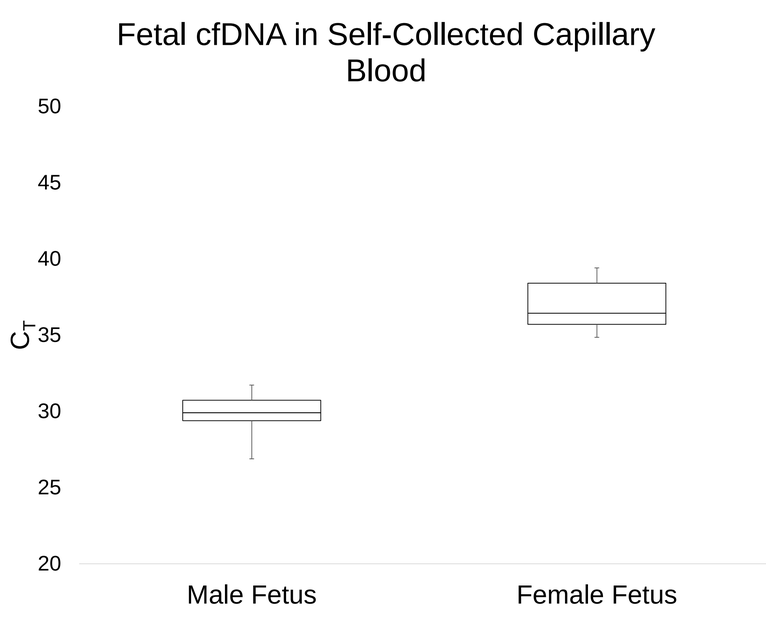

Figure 3. Cycle threshold results for Y-target qPCR

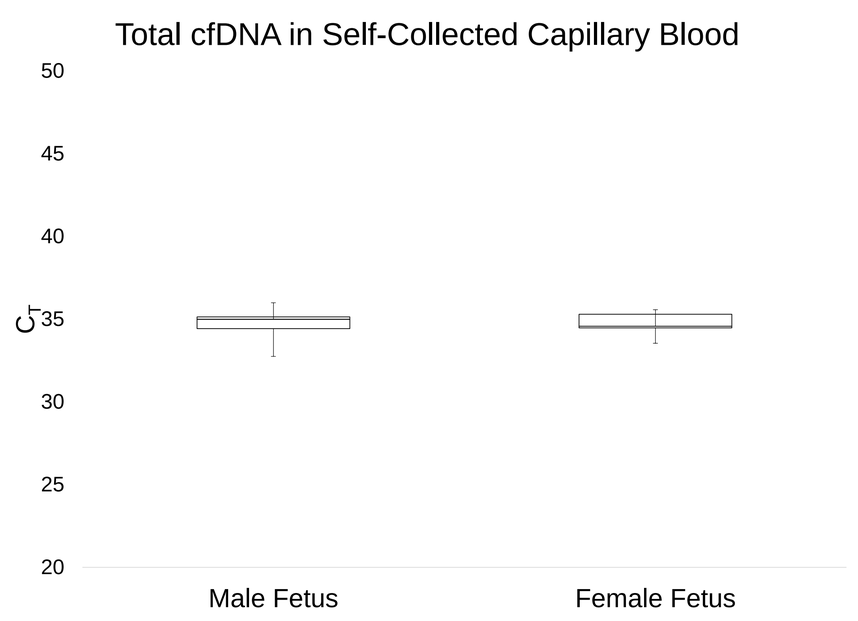

Figure 4. Cycle threshold results for autosomal target qPCR

Table 1. Statistical Parameters of the Self-Collection Study	
Samples Analyzed	18
Female Fetuses	11
Male Fetuses	7
False Positives	0
False Negatives	0
Sensitivity	100%
Specificity	100%
Accuracy	100%

Conclusion

Cell-free DNA (cfDNA) was detected in plasma samples obtained via venipuncture and self-collected finger stick. Total cfDNA levels were similar between venous and capillary plasma samples. Y-chromosome cell-free fetal DNA was successfully detected in 100% of samples from confirmed male bearing pregnancies (both venous and capillary blood). Fetal cfDNA levels were similar between venous and capillary plasma samples demonstrating that self-collection of capillary blood via finger stick is a viable method of obtaining maternal plasma for prenatal diagnosis.

Amplification of Y-chromosome DNA from maternal capillary plasma enabled highly accurate fetal sex determination. This new method of using self-collected maternal capillary blood via finger stick for prenatal diagnosis of fetal sex is simple, accurate, and reliable.

The results of this study demonstrate that self-collected maternal capillary blood is highly accurate and yields comparable results to samples collected via venipuncture. Finger stick collected maternal blood has the potential for being adapted to broad population screening.

This study showed that the SneakPeek® test was 100% accurate for determining fetal sex as early as 9 weeks gestation. This new method of using maternal blood for prenatal diagnosis of fetal gender is simple, accurate, and reliable. This study shows that genetic testing with self-collected maternal blood is feasible for fetal sex determination and has potential for various applications for noninvasive prenatal testing.

The ability to use self-collected maternal blood has the potential to increase the accessibility of noninvasive prenatal testing for broad population screenings. For example, the option to collect patient samples by finger stick could facilitate a more convenient collection process especially in regions where phlebotomy services are not readily available.

Reference

[1] Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485–487.