# Doubling down: fetal fraction change and success on cfDNA redraw in twin pregnancies

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## 1. Introduction

Fetal fraction (FF) is one important metric in evaluating cell free DNA (cfDNA) samples. The minimum FF for a reportable result is dependent on methodology, platform, and number of fetuses and is approximately 3-4% in singleton pregnancies and roughly double, or 6-8%, for twin pregnancies. However, it is also known that in reality, FF is not directly proportional to the number of fetuses in a single pregnancy. As such, FF is not necessarily doubled in a twin pregnancy as compared to a singleton pregnancy at the same gestational age (GA).[1] Here we describe FF in 7,132 consecutive MaterniT®21 PLUS twin samples including a subset of samples from the same pregnancy to assess change in FF over time and redraw success rate.

## 2. Methods

Maternal blood samples submitted for MaterniT®21 PLUS testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.[2] Sequencing data were analyzed to detect autosomal trisomies and other subchromosomal events as described by Zhao et al.[3] Analysis of FF data was performed on samples with reportable results or nonreportable results due to low FF. Samples that were nonreportable due to technical or sample related issues were excluded from the FF analysis as this measurement is not reliable when other quality metrics are not met in the sample. Study data was statistically described using counts, rates, and measures of central tendency. Statistical analysis and generation of plots and figures was performed using R version 4.0.5 and the dplyr, ggplot2, and ggpubr packages.[4-7] When comparing the groups with a reported result on redraw versus the group with a second non-reportable result, normality of gestational age data was tested using a Shapiro-Wilk test and compared using a Mann-Whitney U test.

## 3. Results

In the initial cfDNA analysis of 7,132 consecutive twin pregnancies at one laboratory 97.2% of samples received a reportable result, 2.5% were nonreportable due to low FF, and 0.2% were nonreportable due to technical failures. These 16 technical failures were excluded from FF analysis as the data is unreliable. The majority of samples (55%) were submitted during the first trimester of pregnancy between 9 and 12 weeks gestation and more than 75% were submitted prior to 16 weeks gestation. The average FF for reportable samples was 10.4% (range 3.7-39.6%), with an average gestational age of 13.7 weeks. The average FF in nonreportable samples due to low FF was 3.6% (range 0-6.4%), with an average gestational age of 12.4 weeks. We received redraws for 41% of samples that failed due to low FF, and the success rate for a reportable result on redraw was 79%. The average time between the initial draw and the redraw was just under three weeks. When comparing the change in FF in the same pregnancy, between the initial draw to the redraw the average change in FF was a gain of 1.1% per week. Sixteen samples received a repeat nonreportable result due to low FF, the average change in FF in this small subset of samples was 0.35% per week.

## 4. Conclusions

More than 97% of twin pregnancies that underwent cfDNA screening had sufficient FF on the initial draw, despite the roughly double FF requirement imposed over that of singleton pregnancies. Taking into account a 79% rate of success on redraw the overall reportable rate for twins is greater than 98%. Furthermore, three quarters were submitted prior to 16 weeks gestation. This highlights cfDNA in twin pregnancies as a successful screening tool in the first trimester or early pregnancy. When comparing this twin cohort to more than 200,000 singleton pregnancies8 screened on the same assay, the average total FF was higher in twins (10.2%) but clearly not doubled over that of the singleton cohort (8.7%). Additionally, the average change in FF per week as measured on samples submitting redraw after a failed first attempt due to low fetal fraction is similar to change in FF per week in singleton pregnancies, ~1%.9 Additional evidence that two fetuses do not equate to double the FF, which contributes to slightly increased low FF nonreportable rates in twin pregnancies. It is important to note that many factors impact a sample's FF, such as maternal weight, gestational age, and potential impact of maternal medication interaction. This data provides additional understanding of FF change per week in twin pregnancies but should be considered with these other factors in mind. This data may aid providers in counseling patients with a twin pregnancy about the risk for a low FF nonreportable result and when/if to redraw.

## Tables + Figures

**Figure 1. Breakdown of sample results and redraw results**

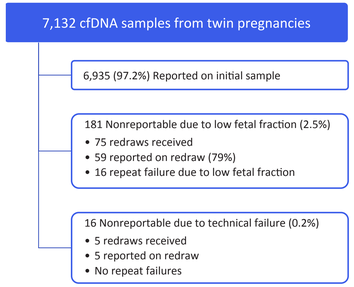



**Figure 2. Measured fetal fraction across draws from the same pregnancy (initial draw in green and redraw in blue). 93% of redraws showed an increase in fetal fraction compared to the initial sample.**

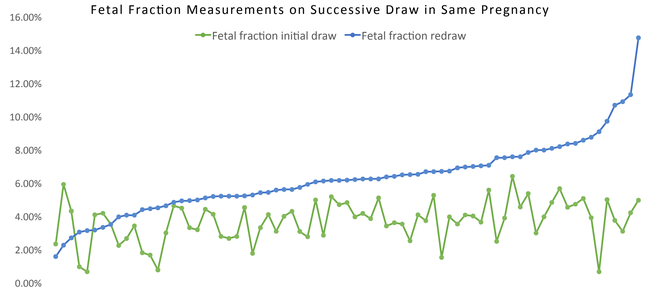



**Figure 3. Comparing the fetal fraction change between initial and redraw samples in patients with a low FF nonreportable. The FF change was significantly higher in samples that were successfully reported on redraw p=0.01005.**

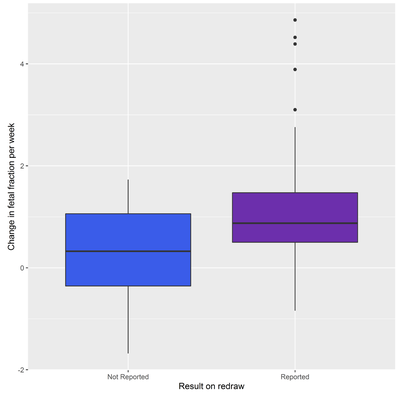
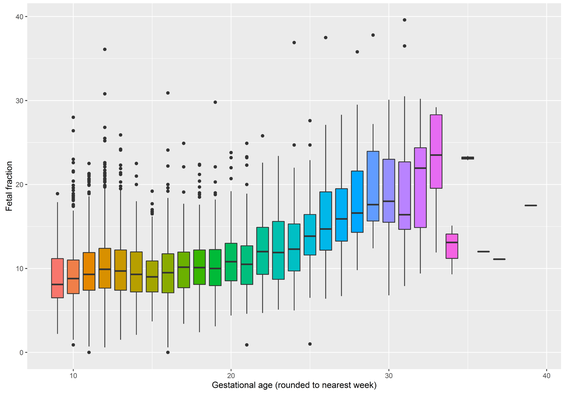



**Figure 4. Fetal fraction by gestational age for total cohort**

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