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MaterniT® 21 PLUS Performance in Lower Fetal Fraction Samples

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I. Introduction

Fetal fraction (FF) is a cornerstone of cfDNA quality metrics, as insufficient FF can lead to non-reportable results. The minimum acceptable FF for each laboratory is dependent on their methodology and platform. The MaterniT® 21 PLUS fetal fraction for a reportable result was initially set at 4%, but with enhancements over time, that firm boundary has been replaced in favor of a more relevant metric: signal-to-noise ratio (SNR).[1] Here we describe the laboratory and clinical performance of >5,000 MaterniT® 21 PLUS reportable samples with <4% FF (mean FF=3.1%) between April 2016 – March 2018.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories 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] Fetal fraction calculation is described in Kim et al.[3] As with all samples, samples reported with <4% fetal fraction were required to meet signal-to-noise ratio (SNR) cut offs. SNR allows confident reporting of high quality data with minimal noise at lower fetal fractions.[1] Follow up information and pregnancy outcomes were primarily dependent on feedback from clinicians or elicited as part of routine, ongoing laboratory protocol for positive cases. A 2 sided, 2 sample proportional Z test was used to calculate significance.

III. Results

Table 1. Outcome of reportable samples with <4% FF based on ad hoc feedback (autosomal aneuploidies)

Chromosome (n=5,390)	MaterniT® 21 PLUS cases reported as negative	MaterniT® 21 PLUS cases reported as positive	Reported False Negatives	Reported False Positives
21	5,341	49	0	1
18	5,351	39	0	0
13	5,366	24	0	1

Table 2. Outcome of reportable samples with <4% FF based on ad hoc feedback (sex chromosome aneuploidies)

Sex chromosome aneuploidy (SCA) (n=3,552)*	MaterniT® 21 PLUS cases reported as positive	Reported False Negatives	Reported False Positives
XO	14	1	1
XXX	7	0	0
XXY	3	0	0
XYY	0	0	0

*includes samples which opted in for SCA analysis

Table 3. Performance of <4% FF samples vs. total MaterniT® 21 PLUS samples based on ad hoc feedback

Chromosome	Relative Observed Sensitivity (<4% Cohort)	Relative Observed Sensitivity (600k Cohort)[7]	Relative Observed Specificity (<4% Cohort)	Relative Observed Specificity (600k Cohort)[7]	Relative Observed PPV (<4% Cohort)
21	>99.9%	99.4%	>99.9%	>99.9%	97.96%
18	>99.9%	98.6%	>99.9%	>99.9%	>99.9%
13	>99.9%	99.3%	>99.9%	>99.9%	95.83%
XO	92.86%	94.4%*	>99.9%	99.7%*	92.86%

Note: insufficient number of samples to calculate performance for the other sex chromosome aneuploidies, PPV=Positive Predictive Value, *sens/spec from Mazloom et al.[6]

Figure 1. Positivity rate in <4% FF cohort

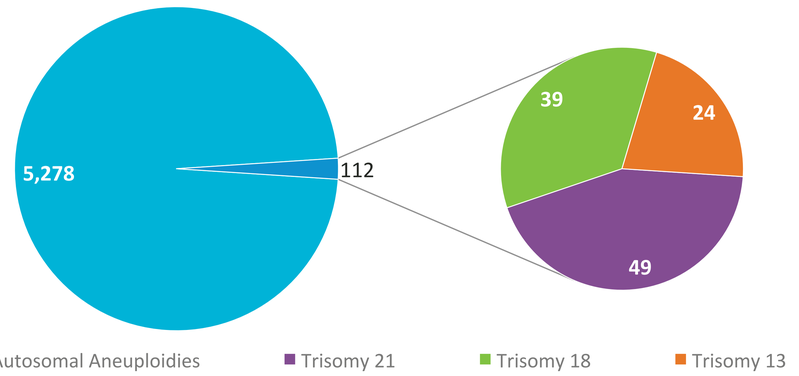

Figure 2. Chromosome representation between positive and negative samples in this cohort shows evident elevation of positive samples, allowing for clear reporting calls

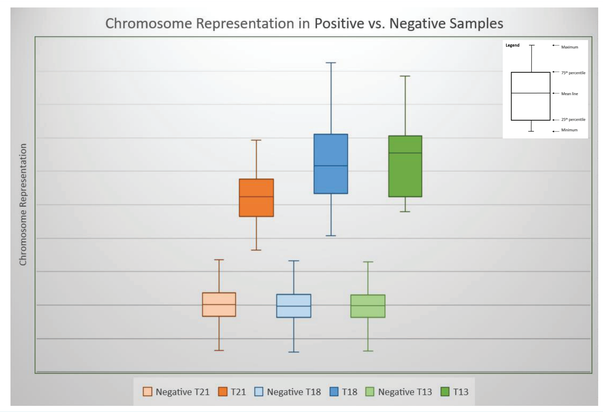

Table 4. Average chromosome Z scores in <4% FF cohort

	Positive T21 (n=49)	Negative T21 (n=5,341)	Positive T18 (n=39)	Negative T18 (n=5,351)	Positive T13 (n=24)	Negative T13 (n=5,366)
Average Z Scores	6.5	0.01	8.4	-0.07	8.9	-0.07

IV. Discussion

Fetal fraction (FF) is associated with maternal BMI, gestational age, placental physiology, and in some cases, aneuploidy. Confidently being able to report results at lower fractions while maintaining high specificity and sensitivity ultimately captures a broader patient population –namely early GA testers and patients with significant BMIs. These patients may have a harder time achieving robust fetal fractions and are often suboptimal candidates for invasive procedures and ultrasound screening.

Sensitivity and specificity for common aneuploidies are significantly similar with that reported in validation studies and clinical experience (all p values >0.05).[4-7] While this cohort included only reportable MaterniT® 21 PLUS results, aneuploidy enrichment was not observed in samples reported with <4% FF. In fact, positive autosomal aneuploidy rate in this cohort (2.07%) is statistically similar to the overall MaterniT® 21 PLUS positivity rate (1.8%), (p=0.12).[7] While fetal fraction lower limits and related QC metrics are unique to each platform, achieving reliable results at reduced fetal fractions may alleviate anxiety surrounding invasive testing decisions, as is currently part of societal recommendations after receiving a non-reportable cfDNA screen.[8]

There was also concern that minimal FF samples would be at risk for false negatives, which was not observed in this cohort. The only reported false negative was for a monosomy X case. Additionally, there were three false positives (one trisomy 21, trisomy 13 and a monosomy X). Distribution of trisomic samples is markedly elevated over negative samples in this cohort (figure 2 & table 4), allowing for clear reporting calls. Overall, this data shows the ability to obtain a reliable result at <4% fetal fraction using SNR.

Key Points:

- Sensitivity, specificity, and positivity rate in reportable <4% fetal fraction samples are statistically consistent with overall MaterniT® 21 PLUS performance.
- SNR bioinformatics enhancements enable reliable cfDNA results at <4% fetal fraction, which may be particularly important for early GA testers and patients with high BMIs.
- Positive samples in this cohort show distinct and elevated Z scores as compared to their negative counterparts, allowing for clear reporting.

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V. References

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8. Gregg A, Skotko B, Bendendorf J, et al., (2016) ACMG statement on noninvasive prenatal screening for fetal aneuploidy, 2016 update. *Genet Med* 18:1056–1065.

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