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8 years of testing and over one million patients screened: A statistical review of the latest MaterniT® 21 PLUS assay enhancements

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I. Introduction

MaterniT® 21 PLUS was the first to market cfDNA screening test for aneuploidy in October 2011. Since its introduction, over 1,000,000 patient samples have been run at Sequenom Laboratories®. Leveraging this experience has bolstered multiple assay enhancements in order to continue reporting timely and reliable screening results to patients and providers. We describe the laboratory experience and clinical performance of the most recent MaterniT®21 PLUS assay updates as compared to a 2018 review of over 600,000 samples.

II. Methods

Over 200,000 maternal blood samples have been submitted and run on the latest assay version. All samples were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.[1] Statistical analysis of this large patient cohort was performed. For all positive results, outcome data (e.g. cytogenetic/molecular results and/or birth outcomes) is largely dependent on feedback provided by the ordering provider. A statistical analysis was performed using a 2 sample, 2 sided T-test for cohort comparison.

III. Results

Enhancements to the most recent assay version resulted in an average turnaround time upon receipt at the laboratory of 2.8 calendar days, and a total non-reportable rate, including DNA quantity not sufficient (QNS) and low fetal fraction, of 1.06%. The turnaround time is an average 2.7 calendar days faster and the total non-reportable rate 0.42% lower when compared to the 2018 >600,000 sample cohort[2]. (**Table 1**) The average maternal age of the new assay cohort is 32.1 years, average gestational age is 13.4 weeks and the average fetal fraction 8.72%. (**Table 3**) More than 4,000 samples (2.00%) were multifetal gestations. Average risk is now the predominant indication for referral at 50.50% and advanced maternal age is the second most common indication for referral at 40.16%. This is a statistically significant difference (p-value <0.001) when comparing the 2018 >600,000 sample cohort where advanced maternal age was the most common indication for referral at 56.7% and average risk indication for referral was only 20.2%[2]. (**Table 2**) There were 2,301 samples which yielded a positive result for trisomy 21, trisomy 18 and trisomy 13; an overall positivity rate of 1.12%. (**Figure 1**) When comparing relative observed positive predictive values (PPV) between the 2018 >600,000 sample cohort to the new assay cohort respectively, overall PPV values improved: 99.2% vs. 99.7% (trisomy 21), 97.5% vs. 99.6% (trisomy 18), and 93.1% vs. 97.5% (trisomy 13).

Table 1: MaterniT® 21 PLUS laboratory experience comparing the latest assay cohort to the 2018 cohort of >600,000 samples

Averages	New assay cohort	>600k sample cohort	P-value	Interpretation
Turnaround time (calendar days)	2.8	5.5	<0.001	Statistically significant *decrease*
Gestational age (weeks)	13.4	14.2	<0.001	Statistically significant *decrease*
Non-reportable: Technical issues (%)	0.31%	0.53%	<0.001	Statistically significant *decrease*
Non-reportable: Low fetal faction (%)	0.75%	0.95%	<0.001	Statistically significant *decrease*

Table 2. MaterniT® 21 PLUS indication for referral comparing the latest assay cohort to the 2018 cohort of >600,000 samples

Referral Indication	New assay cohort	>600k sample cohort	P-value	Interpretation
Advanced maternal age	40.16%	56.7%	<0.001	Statistically significant *decrease*
Average-risk	50.50%	20.2%	<0.001	Statistically significant *increase*
Ultrasound findings	3.63%	9.5%	<0.001	Statistically significant *decrease*
Abnormal serum biochemical screening	2.15%	6.1%	<0.001	Statistically significant *decrease*

Table 3: MaterniT® 21 PLUS laboratory experience comparing the total sample cohort, singletons, and multifetal gestations run on the latest assay to the 2018 cohort of >600,000 singleton and multifetal gestations

Averages	Total sample cohort (n=205,812)	Singletons (n=201,696)	Multifetal gestations (n=4,116)	>600k sample cohort – singletons and multifetal gestations (n=654,710)
Turnaround time (calendar days)	2.8	2.8	3.0	5.5
Maternal age (years)	32.1	32.1	33.0	Not available
Gestational age (weeks)	13.4	13.4	13.6	14.2
Fetal fraction (%)	8.72%	8.68%	10.44%	10.2%

Figure 1. MaterniT® 21 PLUS indication per test requisition

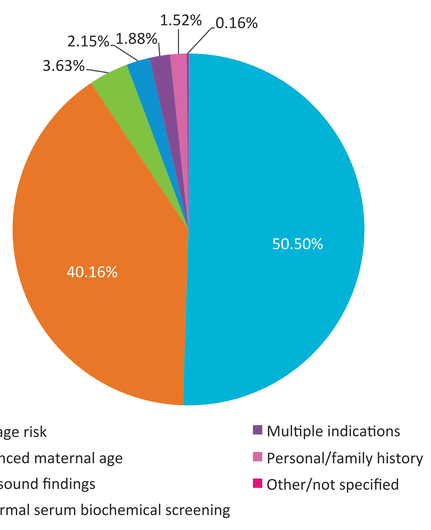

Figure 2. Positivity rates for MaterniT® 21 PLUS in total sample cohort

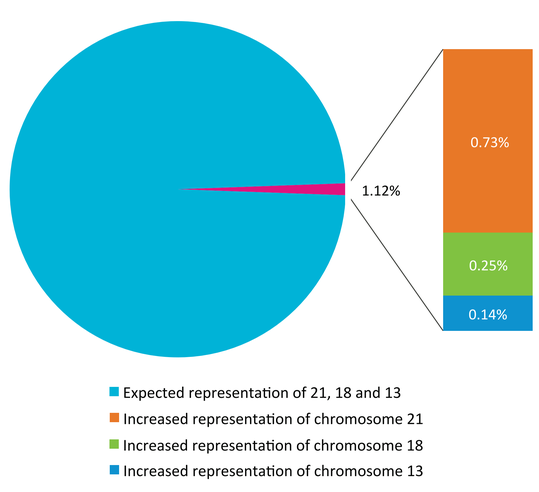

Table 4. Non-reportable rates for MaterniT® 21 PLUS comparing the total sample cohort, singletons, multifetal gestations, and 9.0-9.9 week samples run on the latest assay to the 2018 cohort of >600,000 singleton and multifetal gestations

	Total sample cohort (n=205,812)	Singletons (n=201,696)	Multifetal gestations (n=4,116)	9.0-9.9 week cohort (n=6,697)	>600k sample cohort – singletons and multifetal gestations (n=654,710)
Low fetal fraction	0.75%	0.68%	4.00%	0.97%	0.95%
Technical issues	0.31%	0.31%	0.10%	0.21%	0.53%
Total non-reportable rate	**1.06%**	**0.99%**	**4.10%**	**1.18%**	**1.48%**

Table 5: MaterniT® 21 PLUS laboratory experience comparing the total sample, positive, and 9.0-9.9 week cohorts to non-reportable samples, all run on the latest assay

Averages	Total sample cohort (n=205,812)	Positive cohort (n=2,301)	9.0-9.9 week cohort (n=6,697)	Non-reportable: Technical (n=630)	Non-reportable: Quantity not sufficient (n=1,537)
Turnaround time (calendar days)	2.8	3.3	2.8	6.0	7.1
Maternal age (years)	32.1	35.4	32.3	33.0	33.6
Gestational age (weeks)	13.4	14.0	9.0	13.2	12.1
Fetal fraction (%)	8.72%	8.84%	7.69%	7.47%	2.57%
Multifetal gestations (%)	2.00%	2.26%	1.70%	0.63%	10.74%

Table 6. Combined singleton and multifetal gestation ad hoc feedback and performance

Chromosome	Number of MaterniT®21 PLUS cases reported as negative	Number of MaterniT®21 PLUS cases reported as positive	Number of false negatives communicated to Sequenom Laboratories®	Number of false positives communicated to Sequenom Laboratories®
21	202,145	1,500	10	4
18	203,128	517	7	2
13	203,361	284	3	7

Chromosome	Relative observed sensitivity	Relative observed specificity	Relative observed PPV
21	99.3%	>99.9%	99.7%
18	98.7%	>99.9%	99.6%
13	98.9%	>99.9%	97.5%

IV. Conclusions

While the landscape of aneuploidy screening continues to evolve with the increased adoption of cfDNA screening for both average and high risk pregnancies, MaterniT® 21 PLUS continues to be a reliable screening tool for fetal aneuploidy.[3] A comparison between the new assay version cohort and the 2018 >600,000 sample cohort reveals that providers are ordering cfDNA screening more routinely in average-risk patients and earlier in gestation. The reduction in referrals due to abnormal serum biochemical screening or abnormal ultrasound findings suggests that cfDNA has gained ground as a first-tier screening test for common aneuploidies, instead of as a secondary screening test after an abnormal first trimester screen or abnormal ultrasound finding. MaterniT® 21 PLUS recent assay enhancements aid in the delivery of quicker results to patients while maintaining a low non-reportable rate, even in 9 week samples. The assay enhancements also result in clinical performance that meets, or exceeds the original validation study and continues to improve upon previous assay enhancements made between 2011-2017 as shown by the improved relative observed PPVs.[2]

Key points:

- Recent assay enhancements result in an average turnaround time in the laboratory of 2.8 calendar days.
- The non-reportable rate has shown a statistically significant reduction from 1.48% for samples submitted between 2011-1017, to 1.05%.
- 9.0-9.9 week samples continue to have a high success rate, 98.82%, with the recent assay enhancements.
- Providers are continuing to order MaterniT® 21 PLUS as a first-tier aneuploidy screening test for both average and high risk women.

V. References

1. Gregg AR, Skotko BG, Benkendorf JL, et al., Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genet Med* 2016; 18:1056-1065.
2. Chibuk J et al., Over a half million noninvasive prenatal tests: a clinical laboratory experience. Poster presented at: *ISPD 22nd International Conference on Prenatal Diagnosis and Therapy*; 2018 Jul 8-11; Antwerp, Belgium.
3. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. *PLoS One* 2013; 8(3):e57381. doi:10.1371/journal.pone.0057381. Epub 2013 Mar 6.

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