B-278

Third trimester cell free DNA screening: A clinical laboratory experience

Kimberly Fanelli MS, CGC, Brittany Dyr MS, CGC, Samantha Caldwell MS, CGC, Eyad Almasri, MS
Sequenom Inc., a wholly owned subsidiary of Laboratory Corporation of America® Holdings, San Diego, California

Integrated GENETICS
LabCorp Specialty Testing Group
sequenom

I. Introduction

Cell free DNA (cfDNA) is a well-established test for screening high risk, and more recently average risk pregnancies for common aneuploidies.[1] Aside from fetal ultrasound, cfDNA is the only aneuploidy screening option available to women after 21 weeks gestation. Although no samples ≥27 weeks were part of the initial validation study cohort, late gestational age was not expected to influence test results based on known methodology and performance characteristics.[2] We describe the laboratory experience and clinical performance of MaterniT®21 PLUS for samples submitted in the third trimester.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT®21 PLUS were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.[2] Over 10,000 samples were submitted at ≥27 weeks gestation. Statistical analysis of this patient cohort was performed. For all positive results, outcome data (e.g. cytogenetic/molecular results and/or birth outcomes) is dependent on feedback provided by the ordering provider.

III. Results

Analysis of 10,063 samples received from August 2014 – August 2018 at ≥27 weeks gestation yielded 242 results positive for trisomy 21, trisomy 18 and trisomy 13; an overall positivity rate of 2.42% among reported samples. Of those 242 positive results, 2 false positives for trisomy 13 were reported to the laboratory and 1 false negative each for trisomy 21 and trisomy 18. Only 66 samples (0.66%) did not yield a result due to 1) low fetal fraction (0.07%) or 2) technical issues (0.59%); thus 99.34% of samples received positive or negative test results. The average fetal fraction was 17.42% and the average gestational age was 30 weeks and 5 days. Indications for referral included ultrasound abnormalities (45.59%), advanced maternal age (23.03%), average risk (11.60%), multiple indications (6.63%), positive serum screen (5.90%), other/not specified (3.74%), and personal/family history (3.51%). Multifetal gestations accounted for 250 (2.48%) of samples in this cohort.

Table 1. Third trimester ad hoc feedback and performance

Clinical outcomes and analytical performance: August 2014 – August 2018

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	9,827	170	1	0
18	9,956	41	1	0
13	9,966	31	0	2

Chromosome	Relative observed sensitivity	Relative observed specificity	Relative observed positive predictive value
21	99.42%	>99.99%	>99.99%
18	97.62%	>99.99%	>99.99%
13	>99.99%	99.98%	93.55%

Table 2. MaterniT®21 PLUS test in the third trimester: Laboratory experience

Demographics	
Number of tests submitted in the third trimester	10,063
Average gestational age	30 weeks, 5 days
Multifetal pregnancy	2.48%
Average fetal fraction	17.42%
Not reportable (technical)	0.59%
Non-reportable (QNS)	0.07%
Success rate	99.34%
Average turnaround time (calendar days)	5 days

Figure 1. Positivity rates for MaterniT®21 PLUS samples in the third trimester

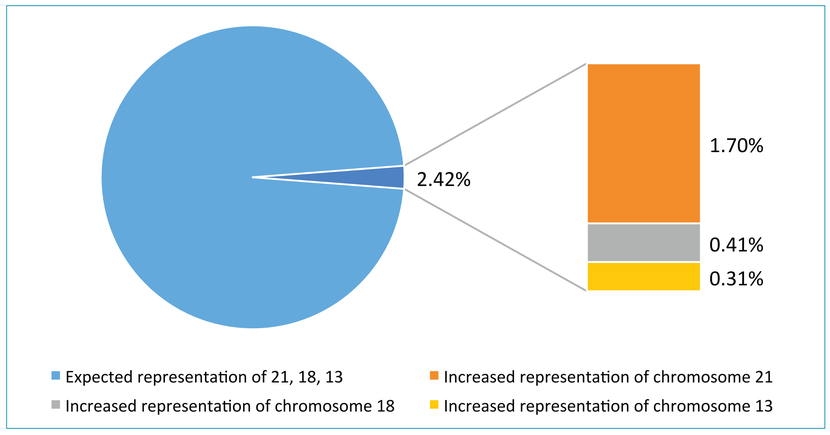
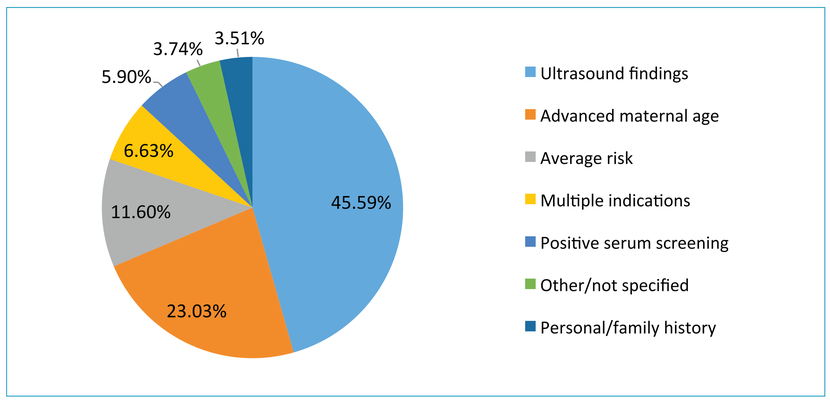


Figure 2. Test indications for late gestational age samples per test requisition



IV. Conclusion

MaterniT®21 PLUS offers patients reliable screening for fetal aneuploidy at late gestational age, when screening options are limited. Aneuploidy screening in the first or second trimester allows for earlier pregnancy management options. However, MaterniT®21 PLUS can be considered for patients in the third trimester for indications including but not limited to late to care, reconsideration of aneuploidy screening, or ultrasound findings discovered in the third trimester. The higher positivity rate in the third trimester (2.42%) compared to 2018 data analyzing the first 600,000 samples (1.81% in singletons) is likely due to the increased number of referrals for ultrasound abnormalities (9.5% vs. 45.59%), which is 4-times higher in this sample cohort.[3] One notable performance metric is the relatively high success rate of cfDNA screening in the third trimester (99.34%), likely due to the higher average fetal fraction in this cohort. Analytical performance and laboratory experience in late gestational age is comparable to 2018 data analyzing >600,000 samples.[3]

Key Points:

- MaterniT®21 PLUS is a reliable screening tool in the third trimester with comparable performance characteristics to cfDNA drawn in the first and second trimester.
- The higher average fetal fraction in the third trimester contributes to a 99.34% success rate for results.
- Third trimester cfDNA can be considered for patients with indications including but not limited to: late to care, ultrasound findings in the third trimester, or reconsideration of aneuploidy screening.

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. 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.
3. 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.

LabCorp

Poster presented at the *NSGC Annual Conference*; 2018 November 14-17; Atlanta, GA.