A-277

Cell free DNA screening at 9 weeks: 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] Cell free DNA screening provides patients the earliest information regarding aneuploidy risk during pregnancy, allowing for early prenatal diagnosis to be considered. We describe the laboratory experience and clinical performance of MaterniT®21 PLUS for samples submitted at 9.0-9.9 weeks gestation.

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 8,000 samples were submitted between 9.0-9.9 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 8,805 samples received from August 2014 – August 2018 at 9.0 to 9.9 weeks yielded 119 results positive for trisomy 21, trisomy 18 and trisomy 13; an overall positivity rate of 1.39% among reported samples. Of those 119 positive results, there was 1 false positive and 1 false negative for trisomy 21 and 1 false positive for trisomy 18 reported to the laboratory. There were no known false positives or negatives for trisomy 13. Only 183 samples (2.08%) did not yield a result due to 1) low fetal fraction (1.77%) or 2) technical issues (0.31%); thus 97.92% of samples received positive or negative test results. The average fetal fraction for all samples was 6.8%. Indications for referral included advanced maternal age (59.10%), average risk (26.88%), other/not specified (7.97%), personal/family history (3.92%), multiple indications (1.45%), and ultrasound abnormalities (0.68%). Multifetal gestations accounted for 270 (3.1%) of samples in this cohort.

Figure 1.
Positivity rates for MaterniT®21 PLUS samples between 9.0-9.9 weeks

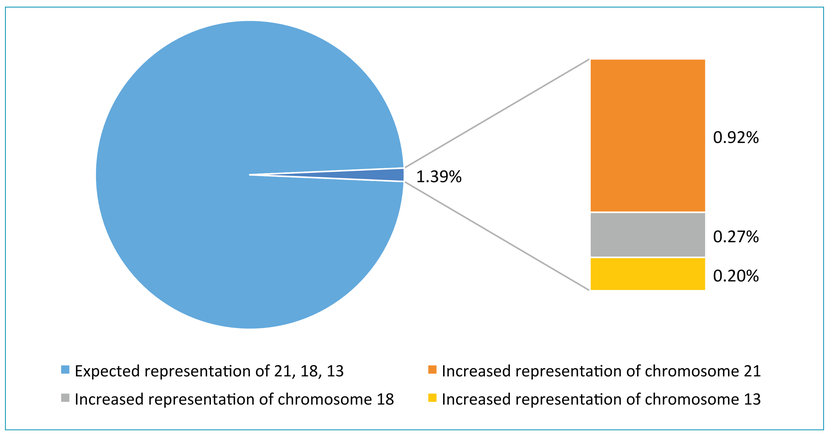

Figure 2.
Test indications for 9.0-9.9 week samples per test requisition

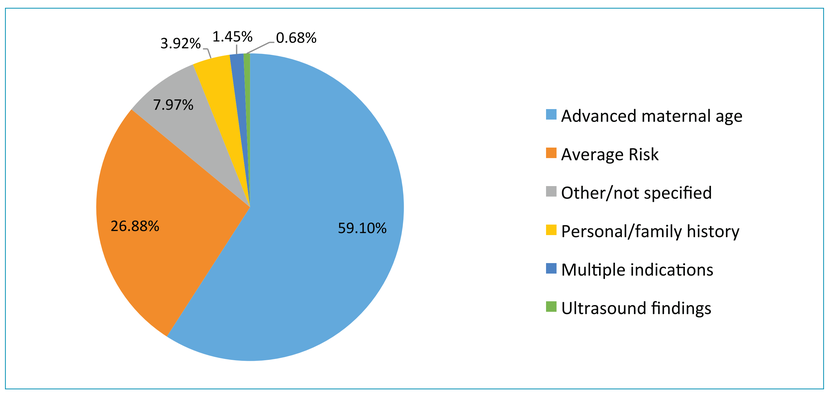

Table 1: 9.0-9.9 week ad hoc feedback and performance

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	8,543	79	1	1
18	8,599	23	0	1
13	8,605	17	0	0

Chromosome	Relative observed sensitivity	Relative observed specificity	Relative observed positive predictive value
21	98.73%	99.99%	98.73%
18	>99.99%	99.99%	95.65%
13	>99.99%	>99.99%	>99.99%

Table 2. MaterniT®21 PLUS test at 9.0-9.9 weeks: Laboratory experience

Demographics	
Number of tests submitted between 9.0-9.9 weeks	8,805
Multifetal pregnancy	3.1%
Average sample fetal fraction	6.8%
Average singleton fetal fraction	6.76%
Average multifetal gestation fetal fraction	9.59%
Overall success rate	97.92%
Average turnaround time (calendar days)	4 days

Table 3: Non-reportable rates for MaterniT®21 PLUS at 9.0-9.9 weeks compared to 2018 data analyzing >600,000 samples at all gestational ages

	Total sample population	Singletons	Multifetal gestations	2018 data (singletons and multifetal gestations)
Low fetal fraction	1.77%	1.46%	11.48%	0.95%
Technical issues	0.31%	0.30%	0.74%	0.53%
Total non-reportable rate	**2.08%**	**1.76%**	**12.22%**	**1.48%**

IV. Conclusion

MaterniT®21 PLUS offers patients reliable screening for fetal aneuploidy, even at 9 weeks gestation. Earlier access to cfDNA results allows patients the opportunity to discuss prenatal diagnosis options with their healthcare provider. The non-reportable rate for multifetal gestations from low fetal fraction is likely due to the increased fetal fraction requirements for multiples (approximately double that of singletons) and the direct relationship between placental size and gestational age. This is similar to findings analyzing >30,000 multifetal gestations which also showed a modest increase in non-reportable rates.[4] As expected, the vast majority of samples submitted in this cohort had an indication of advanced maternal age or average-risk, as generally there is a paucity of ultrasound information at 9 weeks gestation. Over 7% of samples did not have an indication for referral on the test requisition, though upon manual review some samples were referred due to abnormal biochemical screening in a prior pregnancy, and likely parental anxiety. Analytical performance and laboratory experience between 9.0-9.9 weeks are similar when compared to 2018 data analyzing >600,000 samples.[3]

Key Points:

- The overall success rate for samples received between 9.0-9.9 weeks is 97.92%.
- The non-reportable rate is higher in multifetal gestations as compared to singleton pregnancies due to increased fetal fraction requirements to report a sample.
- Analytical sensitivity and specificity in this cohort is similar to overall MaterniT®21 PLUS performance.

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.
3. Chibuk J. A new era in aneuploidy screening for multifetal gestations: clinical laboratory experience screening >30,000 cell-free DNA samples. Oral presentation at: *ISPD 22nd International Conference on Prenatal Diagnosis and Therapy*; 2018 July 10; Antwerp, Belgium.

LabCorp

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