Twins and Triplets and Quads, Oh My!

A review of MaterniT21 PLUS® assay results in multifetal pregnancies

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BACKGROUND

Since the 2011 introduction of noninvasive prenatal testing (NIPT), nearly half a million clinical samples have been analyzed. Over 18,000 (3.9%) were from high risk multifetal gestations. The increased risk for pregnancy complications with invasive testing in this population makes NIPT a valuable screening alternative. Here we describe our clinical assay findings in a multifetal cohort.

METHODS

Maternal plasma samples were subjected to DNA extraction and library preparation followed by massively parallel sequencing as described by Jensen et al. Sequencing data were analyzed to detect autosomal trisomies and other subchromosomal events as described by Zhao et al. Fetal fraction requirements were adjusted in proportion to fetal number (twice minimum for twins, three times minimum for triplets, etc). Outcome data were collected through provider solicitation.

RESULTS

The predominant indication for testing was maternal age, followed by abnormal ultrasound and serum screening. Overall positivity rates for trisomy 21 (1.6%), 18 (0.5%) and 13 (0.2%) were aligned with those found in our much larger singleton pregnancy cohort of over 400,000 patient samples (1.4%, 0.4% and 0.2%). Additionally, a total of 17 events associated with the Enhanced Sequencing Series (trisomy 16 and 22 and selected microdeletions) were reported in the multifetal cohort. Average turnaround time was ~5 calendar days. The non-reportable rate in this multifetal cohort was 7.6%, largely due to insufficient DNA, which is higher than our singleton cohort average of 1.6%.

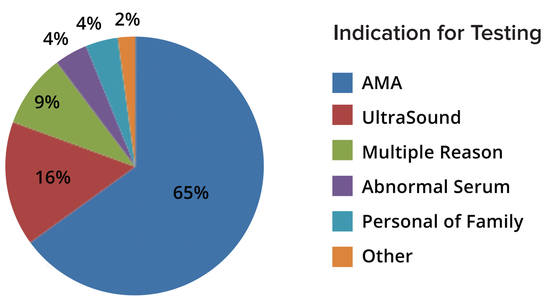

Figure 1. MaterniT21 PLUS test indication in multifetal pregnancies

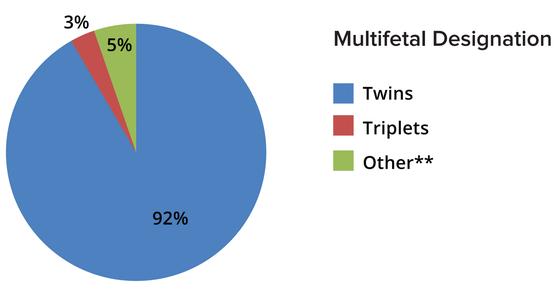

Figure 2. Multifetal designation as indicated on test requisition*

** Representative of 2014 data forward, as early requisitions did not specify fetal number beyond 'multifetal'. Previous samples are presumed to segregate similarly.*

***'Other' category has been historically used to indicate high order multiples, co-twin/triplet/quad demise, or selective reduction.*

RESULTS

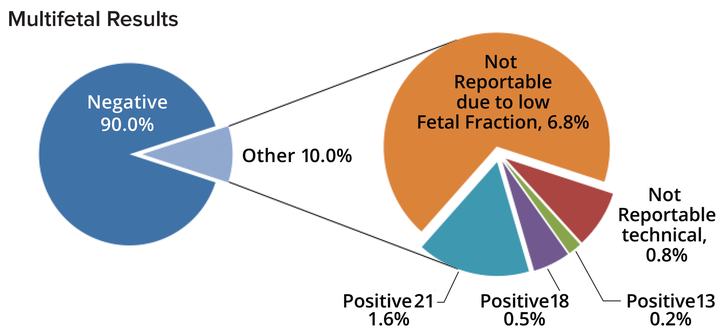

Figure 3.
Positivity rates: Multifetal gestations (updated through 8/31/2015, N = 18,651)

Demographics	
Average gestation age	14.0 weeks
Average turnaround time	6.2 calendar days / 4 business days
Median fetal fraction	11.7%
Non reportable (technical)	0.8%
Non reportable (QNS)	6.8%

Table 1.
MaterniT21 PLUS test in multifetal pregnancies

Chromosome	Number of MaterniT21 PLUS test cases reported as negative	Number of MaterniT21 PLUS test cases reported as positive	Number of discordant negatives communicated to Sequenom Laboratories	Number of discordant positives communicated to Sequenom Laboratories**	Relative Sensitivity	Relative Specificity	Relative positive predictive value
21	16,922	301	3	3	99.0%	>99.9%	99.0%
18*	17,077	99	3	4	96.9%	>99.9%	96.0%
13*	17,140	36	0	4	>99.9%	>99.9%	88.8%

Table 2.
Multifetal performance of core trisomies based on Ad Hoc feedback

Summary of clinical outcome feedback we have received from clinicians through 8/2015, and estimated analytical performance based on this feedback.

**T13 and T18 testing started in February 2012*

*** Majority of 'discordant positive' results reported with known co-twin demise, suggestion of placental mosaicism (abnormal biochemical screening, IUGR), or rescue due to co-segregation with translocation*

Condition	Total reported as positive	Confirmed True positive	Suspected due to clinical findings (co-twin demise, ultrasound findings)	No information	Confirmed discordant positive
Trisomy 16	9		7	1	1*
Trisomy 22	4		4		
5p deletion	0				
22q11 deletion	1	1			
1p36 deletion	1		1		
15q deletion	0				
4p deletion	0				
8q deletion	2		1		1
11q deletion	0				
TOTAL	17	1	13	1	2

Table 3.
MaterniT21 PLUS test: Enhanced Sequencing Series Performance in multifetal pregnancies

12,732 multifetal samples tested since Enhanced Sequencing Series launch

** IUGR noted on ultrasound*

DISCUSSION & CONCLUSIONS

MaterniT21 PLUS® testing in high-risk multifetal gestations has demonstrated positivity rates for trisomy 21, 18, and 13 mirroring those found in much larger cohort of singleton gestations, suggesting that the performance of the assay in multifetal gestations is comparable to that in singleton gestations, except for a higher non-reportable rate due to insufficient fetal DNA. Clinical performance concurs with expectations from the original validation studies.

A unique caveat of testing a multifetal population is enrichment of samples impacted by co-twin/triplet demise, selective fetal reduction, and IVF transfer of several embryos. These scenarios increase the likelihood of a 'discordant' NIPT result, in addition to hindering the clinician's ability to confirm the source of the abnormal clone. The assumption is made that the origin of 'discordant' NIPT results with such histories are likely from the non-viable fetal tissue being actively absorbed. Internal sequential studies on discrepant samples with a history of co-twin demise have shown the time lapse between loss and full absorption of the associated circulating cell free DNA to be extensive, commonly showing discernible signals at and beyond 10 weeks post fetal loss. Clinicians should be mindful of the impact such history has on NIPT data and of the implications for clinical interpretation.

REFERENCES

1. Jensen TJ1, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallelsequencing for fetal aneuploidy detection from maternal plasma. *PLoS One.*2013;**8**(3):e57381. doi: 10.1371/journal.pone.0057381. Epub 2013 Mar 6.
3. Zhao C, et al. Detection of Fetal Subchromosomal Abnormalities by Sequencing Circulating Cell-Free DNA from Maternal Plasma; *Clin Chem.* 2015 Feb 20.

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