Twins and Triplets and Quads, Oh My!
A review of MaterniT21 PLUS® assay results in multifetal pregnancies

Theresa Boomer1, Eyad Almasri1, Jenna Wardrop1, Nilesh Dharajiya1, Thomas Monroe2, William B. Paxton1, Daniel H. Farkas3, Sidra Boshes1, Ron McCullough1

1Sequenom® Laboratories, San Diego, CA; 2Sequenom® Laboratories, Morrisville, NC; 3Sequenom® Laboratories, Grand Rapids, MI

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%.

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 origin of 'discordant' NIPT results with such histories are likely from the non-viable fetal tissue being actively absorbed. 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