**INTRODUCTION**

Panorama™ is a non-invasive prenatal screening test for fetal chromosomal anomalies. The screening test analyzes fetal cell-free DNA (cfDNA) isolated from maternal plasma and can be performed as early as 9 weeks of gestation with high accuracy. Panorama was originally designed to screen for Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), Monosomy X (Turner syndrome), sex chromosome trisomies, triploidy and, if requested, fetal sex (1-5). Recently, the clinical scope of Panorama was expanded to include screening for five microdeletion syndromes. This Panorama Extended Panel now screens for the 22q11.2 deletion (DiGeorge), 1p36 deletion, Cri-du-chat, Prader-Willi, and Angelman deletions. Validation studies have demonstrated sensitivities of greater than 93% and specificities of greater than 99% for each microdeletion condition.

**MICRODELETION PREVALENCE AND TRADITIONAL MICRODELETION DETECTION**

Clinically relevant microdeletions and microduplications are more common than previously thought, occurring in up to 1 in 60 pregnancies, and can occur in pregnancies lacking ultrasound anomalies (6). The combined at-birth incidence of the 5 microdeletion syndromes covered by this screening test is approximately 1 in 1,000 (7-11), approaching the overall rate observed for Down syndrome (12). The most common microdeletion, 22q11.2 deletion, is more common than Edwards syndrome and Patau syndrome combined, and is more common than cystic fibrosis (Figure 1)(10,12,13). Further, the risk for microdeletions is independent of maternal age, unlike whole chromosome aneuploidies like Trisomy 21 (Down syndrome) that are more prevalent in women of advanced maternal age. For pregnant women under the age of 29, this means they are more likely to have a fetal microdeletion than fetal Down syndrome (Figure 2). Most concerning is that these microdeletion syndromes are severe and can result in serious physical and/or intellectual impairment.

Until now, prenatal diagnosis of microdeletion syndromes required an invasive procedure, like amniocentesis or chorionic villus sampling (CVS), followed by fluorescence in situ hybridization (FISH) or chromosomal microarray analysis. Recently, the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) released joint guidelines...
supporting the use of microarray testing for pregnant women who are undergoing invasive testing, regardless of maternal age (14). However, due to the risk of pregnancy loss associated with invasive procedures (15, 16), most women do not undergo invasive testing without another high-risk indication.

Traditional non-invasive tests such as serum screening and ultrasound have very low detection rates for these microdeletions. This means that many babies with microdeletions go undiagnosed until after birth, oftentimes well into adulthood. Thus, there is a clear need for a prenatal screening approach that is both accurate and non-invasive.

**NON-INVASIVE DETECTION OF MICRODELETIONS USING CELL-FREE DNA**

Prenatal screening for fetal aneuploidies has recently been revolutionized by non-invasive prenatal tests (NIPT) that analyze fetal cfDNA present in maternal plasma. This cfDNA is a mixture of maternal and fetal cfDNA, with the fetal percentage referred to as the “fetal fraction” (17). Among cfDNA-based screening tests, Panorama is the only one that incorporates genotypic information from the mother’s white blood cells and thus can differentiate between maternal and fetal genotypes in the plasma. Panorama targets 19,488 SNPs covering chromosomes 21, 18, 13, X, and Y, and it uses a patented algorithm called Next-Generation Aneuploidy Test Using SNPs (NATUS) to screen for whole chromosomal fetal aneuploidy and fetal sex. In clinical trials, this approach delivered a more robust fetal signal and greater quality control capabilities than first-generation quantitative “counting” methods that do not incorporate genotypic information (2, 5, 18-21).

Panorama uses similar methodology for detecting microdeletions. By specifically targeting SNPs within the microdeletion regions-of-interest, Panorama determines whether the full region is present or absent, and at the same time avoids inadvertent identification of “variants of unknown significance” (VUS), or abnormalities without clear clinical manifestations. Also, because Panorama analyzes maternal white blood cells separately from the plasma, it may also identify a previously undetected microdeletion in the mother, which would be reported.
VALIDATION OF PANORAMA FOR MICRODELETIONS

The validation study for Panorama Extended Panel reported sensitivities of greater than 93% and specificities of greater than 99% for each microdeletion condition (22). These sensitivities reflect the presence or absence of the entire targeted region. The study examined 358 pregnant women, including 6 women carrying fetuses affected with the 22q11.2, Cri-du-Chat, and 1p36 microdeletions.

In addition to the 358 pregnancy plasmas, Natera’s research team also developed a novel scientific method called “plasmART” to validate assay performance in detecting rare disorders. PlasmART samples are mixtures of fragmented genomic DNA collected post-natally from a mother and her affected child. They are designed to replicate the cfDNA profile in maternal plasma at numerous fetal fractions. This approach is important for two reasons. First, some microdeletions are exceedingly rare, such that collecting a high number of affected pregnancy samples would take many years. Second, the pregnancy samples available for research are generally collected later in pregnancy, after diagnosis by an invasive procedure. Because fetal fraction increases with gestational age (23), these research samples have higher fetal fractions than clinical samples collected from women undergoing NIPT in the first trimester.

To address this problem, Natera’s plasmART method can mimic fetal fraction profiles expected during the first trimester of pregnancy (24), which allows for rapid validation that more closely represents clinical performance. In the future, this method may be used to validate new assays for screening other disorders that generate circulating cfDNA, such as cancer.

Furthermore, the Panorama Extended Panel achieves high sensitivity with a limited increase in sequencing depth. By contrast, “counting”-based screening tests require much greater sequencing power (and expense) to detect a microdeletion, especially for cases with low fetal fraction. The counting methods do not differentiate between maternal and fetal genotypes; instead they compare the number of sequence reads from a region-of-interest, such as chromosome 22q11.2, to the number of sequence reads from a reference chromosome that is presumed to be euploid (normal copy number). A low ratio indicates a deletion. The challenge, however, is that microdeletion regions are much smaller than whole chromosomes, so the only way to get enough data is to sequence more – a lot more. Indeed, published studies have shown that the counting method required 20 to 50 times greater sequencing power to detect microdeletions when compared to the number
of reads required for detecting Trisomy 21 (25, 26). The Panorama Extended Panel is the only available method that targets SNPs and does not use a counting method. This difference means that Panorama is a more efficient approach to microdeletion screening, which should allow for broader clinical adoption.

**CLINICAL UTILIZATION**

Since microdeletions occur independently of maternal age and other traditional risk factors, conventional notions of “high-risk” and “low-risk” pregnancies may be revised. As a result, the Panorama Extended Panel is already being discussed by many physicians as a test for all pregnant women, not just those considered high-risk. Panorama can be ordered as early as nine weeks gestation and consists of a simple blood draw with an optional buccal swab from the father. The father’s sample maximizes the chances that a sample will return a result, but is not required. For sample collection, special blood tubes are used which protect the cfDNA. Samples are sent at room temperature to Natera’s CLIA-certified laboratory in San Carlos, California. For pregnancies identified as high-risk by the Panorama Extended Panel, follow-up invasive testing with SNP microarray analysis is recommended. Genetic counseling is recommended for all patients.

**CONCLUSIONS**

Panorama, already established as a highly-accurate non-invasive screen for fetal whole-chromosome aneuploidies, has now been scientifically validated as a screening tool for the most common and severe microdeletions. As the incidence of microdeletion syndromes is not related to maternal age, it may be important to screen all pregnancies, not only those women of advanced maternal age. The initial panel of microdeletions includes 22q11.2 deletion (DiGeorge), 1p36, Cri-du-chat, Prader-Willi, and Angelman syndromes. This panel may expand in the future.

While NIPT cannot currently screen for all of the conditions identified by amniocentesis with microarray analysis, the clinical scope of the Panorama Extended Panel offers expectant parents accurate results regarding determining the health of their unborn child through a safe, non-invasive screening test which can be performed during routine first-trimester screening.
REFERENCES


22. Natera Internal Data (www.panoramatest.com).


