NON-INVASIVE PRENATAL TESTING: THE NEW PANORAMA™ TEST

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INTRODUCTION

In Q1 of 2013, Natera Inc. began offering its non-invasive prenatal aneuploidy screening test, Panorama™, for detection of fetal Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), Monosomy X (Turner syndrome), and, if requested, fetal sex.[1,2] The test analyzes maternal and fetal cell-free DNA (cfDNA) from maternal plasma and can be performed as early as 9 weeks of gestation. The test employs unique molecular biology and bioinformatics approaches that are significant advances over traditional non-invasive screening and other cfDNA-based methods, and which allow for unparalleled sensitivity and specificity.

TRADITIONAL SCREENING METHODS

Trisomy 21, Trisomy 18, and Trisomy 13 are the three most common aneuploidies and together occur in approximately 1 in 450 live births; of these, Trisomy 21 is the most common.[3,4] Until recently, the primary screening modality involved serial detection of maternal serum biochemical markers in the first and second trimesters, in combination with first trimester ultrasound to measure nuchal translucency (NT). Although designed to detect Trisomy 21, this integrated screening approach has also detected Trisomies 13 and 18, and increased NT (as well as cystic hygroma and other indicators) is associated with certain sex chromosome aneuploidies. However, large studies reported detection rates for Trisomy 21 ranging from only 79% to 90% and included a false positive rate of 5%.[5] Positive screen results also require an invasive follow-up procedure, such as chorionic villus sampling or amniocentesis, to confirm the results. However, these procedures have up to a 1 in 300 rate of procedure-induced pregnancy loss,[6] and the low detection rates mean that a proportion of affected pregnancies go undetected until birth. Furthermore, these methods are not specifically designed to detect sex chromosome aneuploidies, which are thus not reliably detected.

FETAL CELL-FREE DNA IN MATERNAL BLOOD

The identification of fetal cell-free DNA (cfDNA) circulating in maternal plasma permits a new method of screening for fetal aneuploidies: non-invasive prenatal testing (NIPT). Because fetal cfDNA traverses the blood-placental barrier and enters maternal circulation, a simple maternal blood draw allows for detection of fetal chromosomal copy number while avoiding the risks of invasive procedures. Fetal cfDNA is heavily diluted with maternal cfDNA, and on average rep-

resents approximately 10% of the total cfDNA.[7] This "fetal fraction" is positively correlated with gestational age and preliminary evidence suggests that it is negatively correlated with maternal weight. Accurate determination of fetal chromosomal copy number using cfDNA isolated from maternal plasma requires cfDNA amplification and subsequent bioinformatics analysis. To date, there are two major bioinformatics approaches: the first-generation "quantitative" or counting approach used by most cfDNA-based tests, and the second-generation approach that incorporates genotypic information used by Natera.

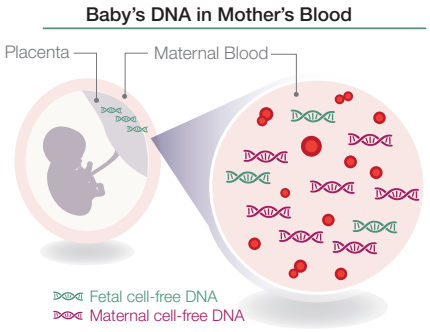

FIGURE 1: CELL-FREE FETAL DNA IN MATERNAL CIRCULATION

CELL-FREE DNA-BASED METHODS FOR DETECTING FETAL CHROMOSOMAL COPY NUMBER

The Panorama test from Natera is the only method that specifically amplifies and sequences single-nucleotide polymorphisms (SNPs) and thus can differentiate between maternal and fetal genotypes. Panorama uses a proprietary, patented algorithm called **Next Generation Aneuploidy Test Using SNPs** (NATUS). Panorama is the only NIPT that utilizes the mother's white blood cells to isolate and identify her DNA, then uses this information to "subtract out" the maternal genotype, resulting in a more robust fetal genotype and thus higher accuracy even at fetal fractions as low as 4% (Figure 2). This is the only method that does not require a reference chromosome, and as such is uniquely able to detect triploidy, a condition which is estimated to occur in approximately 1:1,500 pregnancies at 10-14 weeks.[8] Additionally, the method's unique bioinformatics approach results in greater quality control capabilities. Indeed, in validation studies Panorama reported sensitivities of >99% and specificities of >99% when detecting the autosomal trisomies and fetal sex, and 91.7% sensitivity with >99% specificity when detecting Monosomy X (Turner Syndrome).[2] Significantly, Panorama is the only screening test that always reports high or low risk for Monosomy X, an aneuploidy that is present at mid-trimester more frequently than the autosomal trisomies combined.[9] Finally, in research approximately 5% of cases failed to meet Natera's stringent quality control metrics. In clinical practice, in most instances this results in a request for a second blood draw, and preliminary clinical data shows that over 90% of these "redraws" then pass quality control, returning a confident result; this results in an overall no-call rate of <1%.[10]

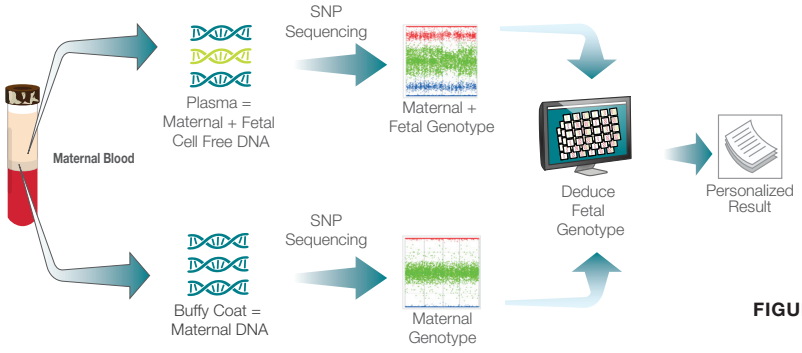

FIGURE 2: HOW PANORAMA WORKS

Panorama is not the only non-invasive prenatal test available on the market today. First-generation NIPT technologies use a purely quantitative "counting" method to determine fetal chromosomal copy number, and do not differentiate between maternal and fetal genotypes in the cfDNA. These methods compare the relative number of sequence reads from a chromosome-of-interest, like chromosome 21 (where trisomy results in Down syndrome), to a reference chromosome that is presumed to be euploid. While this approach is more effective at detecting Trisomy 21 than biochemical and ultrasound-based testing methods, it is not as effective for Trisomy 18, Trisomy 13, or sex chromosome aneuploidies.[11,13,14] Additionally, accuracy suffers at low fetal fractions; below 8% fetal fraction the detection rates from quantitative tests can fall to only 75% (Table 1).[12] Research has demonstrated that approximately one quarter of pregnant women between 9 and 14 weeks gestation have fetal cfDNA fractions between 4% and 8%.[7] In addition, the reported sensitivities when detecting Trisomy 13 range from only 80% to 91.7%, and Monosomy X generally is only reported when detected. Each of these tests also resulted in false positive rates ranging from 0.1% to 1.1%, resulting in poor positive predictive values.[11-14] Panorama does not suffer from these challenges.

%Fetal Fraction of cell-free DNA	Counting Down Syndrome Sensitivity[12]	Panorama Down Syndrome Sensitivity[2]
≥8%	>99%	>99%
4-8%	75%	>99%

TABLE 1: TRISOMY 21 SENSITIVITY

UTILIZATION OF THE PANORAMA TEST

Panorama can be performed as early as nine weeks gestation – earlier than any other test – and consists of a simple blood draw with an optional buccal swab from the father. The father's sample is helpful in 1-2% of cases to minimize the chances of failure, but is not required as it does not impact the accuracy of results.

For sample collection, special blood tubes are used which protect the cfDNA, and these are sent at room temperature to Natera's CLIA-certified laboratory in San Carlos, California. After samples are processed, a report is generated that contains personalized risk scores for each of the chromosomes evaluated. Fetal sex is reported if requested. For patients identified as high-risk for a fetal chromosomal abnormality, follow-up testing is recommended. Genetic counseling is recommended for all patients.

CONCLUSIONS

The Panorama test offers superior overall performance when detecting Trisomy 21, Trisomy 18, Trisomy 13, Monosomy X, and fetal sex. The low false-positive rate will reduce the number of unnecessary invasive diagnostic procedures when compared to biochemical and ultrasound screening methods, and offers early reassurance. Similarly, the low false-negative rate means that more affected pregnancies will be detected earlier, allowing mothers/parents and clinicians more time to prepare.

The rapid adoption of cfDNA-based testing suggests a paradigm shift in prenatal screening. Indeed, professional organizations such as the American College of Obstetricians and Gynecologists and the International Society of Prenatal Diagnosis have already recommended cfDNA-based testing for non-invasively screening for fetal aneuploidies in high-risk women. Because Panorama test interrogates SNPs, it is also uniquely poised to efficiently detect sub-chromosomal copy number variations, such as microdeletions, thus expanding the clinical scope and broadening the clinical value for patients.

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