DNA-Based Non-Invasive Prenatal Testing (NIPT) for Fetal Aneuploidy

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In the decade following the description of increased maternal serum alpha fetoprotein (MSAFP) levels in women carrying fetuses affected by open neural tube defects (NTDs), an association was reported between low levels of MSAFP and fetal aneuploidy, most notably Down syndrome (trisomy 21). Since then, additional maternal serum biochemical markers, including human chorionic gonadotropin (HCG), unconjugated estriol (uE3), dimeric inhibin A (DIA), and pregnancy-associated plasma protein-A (PAPP-A) have expanded prenatal screening from second trimester “triple” and “quad” screening (AFP, uE3, HCG, DIA) to first trimester screening (HCG and PAPP-A), to sequential and integrated first and second trimester screening. Biochemical and NT screening for fetal aneuploidy are cost effective, but have limits on detection rates and relatively low predictive value.

Fetal nuchal translucency (NT) measurement by certified ultrasonographers is also used in first trimester, sequential, and full integrated screens. Regardless of the screening panel used, the objective is to derive likelihood ratios by which to adjust the age-associated risk of carrying an aneuploidy pregnancy.

Maternal serum biochemical and NT screening is a useful and cost-effective method for calculating age-adjusted risk for the presence of NTD and fetal aneuploidy, and as an aid in determining which women should undertake the risk of undergoing definitive diagnostic procedures such as chorionic...
villus sampling or amniocentesis. However, the best performing biochemical / NT-based prenatal screens still have detection rates only in the 90 – 95% range, false positive rates in the 1 to 2% range, and positive predictive values of around 5%.

Late 2011 saw the first commercial release of maternal serum prenatal aneuploidy screening methods based on analysis of maternal serum free DNA, rather than assessment of biochemical analytes. Currently there are four companies offering DNA-based maternal plasma prenatal screening (often referred to as “non-invasive prenatal testing” or NIPT). Each is in the form of a proprietary laboratory developed test (LDT); each established, marketed, and performed by the test’s manufacturer.

Two of the tests currently on the market (MaterniT21™ by Sequenom and Verifi™ by Verinata) use massive parallel (“next generation”) sequencing of random DNA fragments in maternal serum, and the other two tests (Harmony™ by Ariosa and Panorama™ by Natera) involve more targeted DNA amplification methods. Three of the current tests are based on counting methods that compare expected quantities of genetic material from a given chromosome to observed quantities in maternal serum, and then calculate the likelihood that deviations from expected quantitative ratios are due to fetal aneuploidy. The fourth method (Panorama™, Natera), uses genotyping by assessment of single nucleotide polymorphisms (SNPs) in maternal and fetal DNA in maternal circulation. Currently, all marketed DNA-based prenatal screening tests are proprietary, available through the tests’ manufacturers, although some manufacturers are entering into marketing agreements with certain reference laboratories.

In March 2013, Warde Medical Laboratory entered into an agreement with Natera to begin offering the non-invasive prenatal test Panorama™ (test code: PANOR), based on SNP array technology as noted above. Published data and claims from the manufacturer indicate detection rates of greater than 99% for trisomy 21, trisomy 18, and trisomy 13 for maternal blood samples that contain an adequate fetal DNA fraction, and false positive rates of less than 0.1%. For monosomy X there is not as much data, but detection rates to date exceed 90% per the manufacturer. The Panorama™ test requires a special sample collection kit that is available to clients via Warde Laboratory; please call our client services line for information on obtaining these kits.
Biochemical vs DNA-Based Serum Screening

With the high level of performance of DNA-based testing, why continue with biochemical methods for screening? There are several factors for physicians and patients to consider when selecting whether and how to pursue prenatal serum screening.

First, biochemical approaches remain substantially less expensive than DNA-based approaches (less than $100 per test versus $900 to over $2700 per test for DNA-based testing), although out-of-pocket expenses may be capped for high-risk patients with insurance coverage.

Second, the American College of Obstetricians and Gynecologists (ACOG) currently recommends that biochemical screening be offered as an option to all pregnant women, whereas ACOG currently recommends that DNA-based serum testing be offered only to patients at increased risk for aneuploidy as defined by the criteria listed in Table 1. The National Society of Genetic Counselors (NSGC) also “...does not currently support NIPT as a routine, first-tier aneuploidy screening test in low-risk populations: To date, these technologies have been validated primarily in pregnancies considered to be at an increased risk for fetal aneuploidy, based on maternal age, family history, or positive serum and/or sonographic screening tests.” The NSGC position also states that “…NIPT results should not be considered diagnostic, and any abnormal results should be confirmed through a conventional prenatal diagnostic procedure, such as chorionic villus sampling or amniocentesis.” ACOG adds that “cell free fetal DNA testing should not be offered to low-risk women or women with multiple gestations because it has not been sufficiently evaluated in these groups.” ACOG cites detection rates across all methodologies of around 98% with false-positive rates of less than 0.5%.

Table 1. Indications for Considering the Use of Maternal Free Plasma DNA-Based Non-Invasive Pre-Natal Screening (reproduced from ACOG 2012).

<table>
<thead>
<tr>
<th>Indications</th>
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<tbody>
<tr>
<td>Maternal Age 35 years or older at delivery</td>
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<tr>
<td>Fetal ultrasonographic findings indicating an increased risk of aneuploidy</td>
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<tr>
<td>History of a prior pregnancy with a trisomy</td>
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<tr>
<td>Positive (serum / biochemical) test result for aneuploidy, including first trimester, sequential, or integrated screen, or a second trimester quadruple (“quad”) screen</td>
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<tr>
<td>Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21</td>
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Third, DNA based testing is limited to the detection of genetic abnormalities such as aneuploidy (and in the future, possibly other specific inborn genetic disorders), and cannot be used for screening for open NTDs. Currently, biochemical MSAFP assessment remains the standard screen for open NTDs.

Fourth, expected turnaround times are longer for any DNA-based NIPT than for biochemical screening. Currently, the Panorama™ test has a stated turnaround time of 15 business days, with the goal of achieving 10 business day turnaround within the next two months.

**The Method**

The Natera Panorama™ prenatal test may be performed as early as 9 weeks gestation, as compared to the minimum 10 weeks for the initial draw of an integrated (first and second trimester) screen, 11 weeks gestation for a first trimester biochemical / NT screen, and the minimum 15 weeks gestation for a second trimester (quad) screen. The test is currently designed for the assessment of risk for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and monosomy X (Turner syndrome). This test can also supply information on the likely gender of the fetus, but gender information is provided only when requested on the requisition, and patients may opt out of this portion of the report.

In contrast to the three other marketed technologies that rely on some form of counting of expected quantities of genetic material in maternal circulation, the Panorama™ test uses a genotyping method based on targeted amplification of highly heterogeneous single nucleotide polymorphisms (SNPs). The manufacturer refers to this as NATUS (Next-generation Aneuploidy Testing Using SNPs). The SNP pattern of DNA of entirely maternal origin (cellular DNA from the buffy coat of a whole blood sample) is compared to the SNP pattern of free DNA from maternal plasma, which contains a mixture of maternal and fetal DNA. An informatic algorithm assesses possible SNP combinations, including those of monosomic, disomic, and trisomic fetal genotypes. The relative likelihood of each combination is calculated based on an individual patient’s data. Likelihoods are summed for a given level of fetal DNA fraction and a given pattern of aneuploidy, and a risk score is generated. If the father is also available at the time of the mother’s blood draw, a cheek swab may be obtained from the father for testing, thereby decreasing the probability of an uninformative result (see information below about “No Result”).

The SNP-based methodology allows for meaningful results with lower fetal DNA fraction than is generally required of counting-based methods. (Table 2) Also, because of the particular genomic regions targeted for SNP analysis,
the Natera methodology is less susceptible than counting-based methods to bias that may be introduced by differential amplification of DNA segments based on relative content of guanine-cytosine (G-C) versus adenine-thymine (A-T) base pairs.

Several conditions may lead to the issuing of a “No Result” report (currently at a rate of approximately 5 to 6% of cases). First, the Panorama™ test requires a minimum threshold of fetal DNA fraction in maternal plasma in order to be interpretable (although, in general, this threshold is lower than that of counting-based DNA methods). Samples with fetal fractions below interpretable range, or with low DNA quality after extraction will be reported as “No Result,” but such cases will be re-tested if a repeat sample is submitted. Conversely, there are some conditions in which a “No Result” will be issued without opportunity for a repeat sample. These include samples from pregnant women who conceived using an egg donor or who are acting as surrogates, samples from women who have received hematopoietic stem cell transplants, or samples for which the SNP data between mother and fetus is not considered informative. Also, the Natera Panorama™ test has not been validated in non-singleton pregnancies (twins, triplets, etc).

**Table 2: Detection Rates at Low Fetal DNA Fractions**

<table>
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<tr>
<th>%Fetal DNA Fraction</th>
<th>Down Syndrome Detection Rate By Counting Methods</th>
<th>Down Syndrome Detection Rate By SNP-based method (Natera)</th>
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<tbody>
<tr>
<td>&gt;10%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>8 – 10%</td>
<td>91%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>6 – 8%</td>
<td>75%</td>
<td>&gt;99%</td>
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**Barriers to routine use include cost and a lack of broad experience in multiple gestations and low-risk settings.**

The field of maternal plasma cell-free DNA analysis for prenatal aneuploidy detection has advanced significantly in just the past year or two, and holds substantial promise for increasing the sensitivity, specificity, and predictive value of noninvasive screening compared to current non-DNA-based methods. Barriers to routine use of this technology for prenatal screening include cost, and a lack of broad experience in multiple gestations and low-risk settings. Currently this testing is still considered a high level screen rather than a diagnostic test, and national organizations are...
recommending that it be offered only to women at increased risk for aneuploid pregnancies based on age, prior serum biochemical screening, ultrasonographic data, or medical history. As the field continues to advance, Warde Medical Laboratory is pleased to offer NIPT to co-tenant and client laboratories through the Natera Panorama™ test. The special collection kits required for this test may be obtained by calling Warde client services.

References


9. American College of Obstetricians and Gynecologists Committee on Genetics: Noninvasive prenatal testing for fetal aneuploidy. ACOG Committee Opinion 2012; number 545.

Occasionally I receive calls regarding unexpected laboratory results. A typical query might involve an extensive workup for a potential infectious disease in which one of many agent-specific antibodies (usually IgM antibodies) ordered on a patient returns a positive result that is discordant with clinical findings. I am generally asked whether this could represent a false positive. Invariably, a thorough review of processes and procedures yields no evidence that a given test is operating outside expected performance characteristics. The process from there becomes an exercise in educated guessing: Is the result near the positive threshold or is it well beyond the threshold? Were the reference values for the day unusually low or high? Were there an unusual number of positive results for that particular run? Each of the questions might be potentially useful, but ultimately there is no magic formula for determining whether an individual positive result is true or not. What may be most useful is a thorough evaluation of the clinical scenario in which the test was ordered, and the statistical and epidemiologic issues surrounding the meaning of any given laboratory result.

The meaning of a “positive” test of any kind is dependent on a number of pre-test variables, including the clinical probability that a patient has the condition the test is being used to detect. This tenet is met with varying skepticism throughout the health care community, but remains an important principle in laboratory medicine. The most astute medical practitioners treat laboratory results not as yes-or-no diagnostic switches, but as ways to either increase or decrease the probability of a given disease relative to the probability that existed before the results were received.

These ideas may seem abstract or ill-defined (or they may seem like ways for laboratory directors to dodge accountability), but they are actually embedded principles, quantifiable by inferential statistics. Let’s take, for example, a test for which 1% of the general population tests positive regardless of clinical status (for instance from known rates of analytic interference of serum or plasma elements, or known rates of cross-reacting antibodies). If a patient’s clinical signs and symptoms place them in a group for which the prevalence of the disease is 20%, then the predictive value of a positive test (assuming, for the sake of argument, 100% sensitivity) is 96%. That is, 96 of every 100 patients in that group that test positive for disease will actually have that disease; this is likely to be a clinically useful result. Conversely, if a patient’s clinical signs and symptoms place them in a group for which the prevalence of disease is 1%, then the predictive value of a positive test is only about 50%. That is, only about half of the patients with a positive result
Pre-Test Probability Matters: A Comment Regarding “Shotgun” Testing

will have the disease—a much less clinically useful result. This discordance is due to the fact that, given a consistent rate of false positive results for a given assay, as true positives decrease in the population, the probability of any given positive result being false increases. An extreme example would be a disease for which populational prevalence is zero. In that case, all positives are by definition false positives, and the predictive value of a positive result is zero.

There may be some utility in assessing for certain disorders even in low-probability settings, since the negative predictive value of a negative result is often quite high for very sensitive assays. However, uncertainty as to the meaning of a positive result in these settings might abrogate the usefulness of the negative result. Again, an informed approach from an astute clinician as to how the laboratory information will be used is essential.

There is a culture in medicine that resists the principle that a laboratory test (whether a routine chemistry panel, a next-generation molecular test, or a traditional surgical pathology evaluation) is a means by which to hone pre-test probability, rather than the magic answer to a given medical question. To our credit, the field of pathology and laboratory medicine has established such a high level of reliability for most tests that each result is treated as that magic answer, and deviations from perfection are attributed to some ill-defined “lab error” instead of to the natural performance characteristics of a given test within a given patient population.

Many laboratory tests enjoy “gold standard” status for the answering of many medical questions. Every test, however, is influenced by the populational statistics that underlie the disease for which testing is done. Consequently, every patient is best served by medical ordering practices that take into account the pre-test probability of a given medical condition. “Shotgun” testing doesn’t just drive up the cost of health care; it also measurably decreases the value of medical information.