

# Cell-free DNA: a new era in aneuploidy testing

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Cell free DNA testing (cfDNA), or non-invasive prenatal testing (NIPT), has been welcomed into the offices of fetal medicine specialists worldwide and is increasingly sought by the general obstetrician-gynecologist. And no wonder: it has superior performance over traditional screening methods for the risk assessment for trisomies 13, 18 and 21.

This shift in the prenatal screening protocol is significant. Maternal serum biochemistry has been the source for risk assessment for over 40 years since decreased levels of alpha-fetoprotein (AFP) were associated with an increased risk for Down syndrome. Different iterations on maternal serum screening evolved over time by adding new informative analytes (AFP shifted to a triple screen, which then became the quad screen). Tests were built upon the same premise of measuring maternal serum biochemistry, calculating the variance from normal of these analytes, and assigning a risk to the pregnancy.

Eventually, first trimester sonographic assessment of the fetal nuchal translucency (NT) was included. This measurement of the fluid pocket in the back of the fetal neck must be assessed between 11 and 13.9 weeks of gestation and requires a skilled technician. As an isolated marker, an increased NT is associated with increased risks for fetal aneuploidy, heart defects, and poor outcome. When NT measurement is combined with maternal serum analytes, aneuploidy screening becomes more sensitive. For example, NT along with maternal serum collected in both the first and second trimesters has a detection rate for Down syndrome of approximately 95%. However the screen positive rate for all of these screening tests is high and ranges from 3–5%.

In 2011, the first commercially available cfDNA test launched and the term non-invasive prenatal testing (NIPT) was adopted. NIPT evaluates fragments of DNA from the fetus and placenta that are present in maternal circulation. The amount of cfDNA from the relevant chromosomes is evaluated and more than the expected amount from any particular chromosome suggests aneuploidy. Detection rates are greater than 99% with false positive rates of less than 0.1%. With these statistics, it's easy to see why NIPT is so popular.

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## Benefits of non-invasive prenatal testing

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NIPT is simple. All it requires is a single blood draw from a patient as early as 10 weeks gestation with results available within about 10 days. There is no need for skilled technologists, as is necessary for NT measurements. Not only can NIPT be performed earlier in pregnancy than traditional screening, it can also be performed at any point in pregnancy (even in the third trimester). This provides an opportunity for screening for those patients who have limited access to providers or who present late to prenatal care.

Because NIPT provides lower screen positive rates, fewer patients require invasive diagnostic testing. Previously, 5% of patients having serum screening would receive a result suggesting an increased risk for Down syndrome or trisomy 18. These patients would then seek counsel regarding this result, often traveling quite a distance to meet with a specialist. Diagnostic testing via CVS and amniocentesis required to confirm a diagnosis is accompanied by risk to the fetus. Patients would be counseled to consider the risk to the fetus for aneuploidy, the risk to the pregnancy for miscarriage due to the diagnostic test, along with their own beliefs and values. These diagnostic testing options are restricted to certain times in pregnancy and typically only available at high risk centers, contributing to the decision making process. This entire experience creates significant anxiety for patients and their families.

## Implementation of non-invasive prenatal testing in clinical practice

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Determining the best way to utilize NIPT in your practice depends on several factors, including frequency of prenatal visits, access to ultrasound, and patient volume. It should be included in a manner that most enhances your approach to prenatal care. Regardless of when NIPT is offered to patients, it is critical to include patient counseling both before and after testing.

Pre-test counseling should include a description of the technology and its ability to provide risk assessment for trisomies 13, 18, and 21. Analysis of the X and Y chromosomes may be optional, and should be reviewed with the patients. The types of results should be discussed, as should the manner in which results are disclosed.

Pre-test counseling should also include a discussion about the patient's desire to have testing and that testing is optional. Personal attitudes towards risk ought to be reviewed. Patients should consider the potential outcomes and be aware of the consequences of each. This counseling should be non-directive and aim to encourage patient decision making.

Post-test counseling will vary based on the results. The majority of results fall into two categories: high risk or low risk. It is important to remember that NIPT is not a diagnostic, but rather a very accurate screening test. Results should always be considered in the context of other screening tests (i.e. ultrasound) and discussed with the patient.

In general, a patient with a low risk result and no other risk factors would simply continue with routine obstetrical care. She should be informed that these results do not eliminate the risk for an abnormal outcome, but rather the risk for these specific chromosomal conditions is extremely low. It is important to note that NIPT cannot identify all risks to the pregnancy and that other screening tests, particularly ultrasound, may be advised for further risk assessment.

If a patient has had negative/low risk NIPT results in the context of a fetal anomaly identified on ultrasound, diagnostic testing with fetal microarray should be considered as the risk for other chromosomal anomalies may be significant.

A patient with a high-risk result should be counseled that the pregnancy may be affected, but diagnostic testing is required for diagnosis. All patients with a high-risk result should be offered diagnostic testing, with the appropriate counseling. Historically, counseling for an abnormal screening test (i.e. Quad screen) has been generally reassuring, as most women with abnormal screening have a normal baby. Post-test counseling for patients with high risk NIPT results should still include some optimism, though the chance for a normal outcome is much lower. Because CVS samples the placenta it may be more appropriate to confirm a high-risk NIPT result with an amniocentesis, because that test samples cells from the fetus itself.

## Fetal fraction

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Only non-invasive prenatal tests that measure and report the amount of fetal cfDNA present in the sample (the “Fetal fraction”) should be considered for use in patient care because of the risk of inaccurate results when the fetal fraction is not measured accurately. Because the chromosomes of interest compose a small percentage of the entire genome, and because aneuploidy results in a 50% increase in the amount of that chromosome, there is a statistical threshold for accuracy (generally accepted at 4%). The average fetal fraction approximates 11% but varies within the population. Fetal fraction increases during pregnancy; slowly until the 22<sup>nd</sup> week of pregnancy and then at an increased rate. Fetal fraction is also inversely proportional to maternal weight. This may be clinically relevant in helping determine the appropriate timing for sample collection in a particular patient population.

## Contraindications

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While NIPT is largely appropriate for universal application in obstetrical care, there are several indications that preclude test accuracy. At this time, testing on multiple gestations is limited to twins. Validation studies on higher order multiples have not been completed and accuracy is uncertain.

Twin pregnancies in which a demise of one twin has occurred should also not have NIPT. The contribution of cell free DNA from the demised fetus is uncertain and could potentially overshadow the remaining pregnancy, thus

increasing both the false positive and false negative rate. Finally, women who have had a bone marrow transplant or an organ transplant should not have NIPT given the presence of another source of DNA in circulation.

## Conclusion

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Cell-free DNA testing is gaining in popularity with patients and providers. Its ease of use and high accuracy are obvious benefits. Implementation within obstetrical practice requires an emphasis on pre- and post-test counseling and careful attention to result interpretation.