NON-INVASIVE PRENATAL TESTING (NIPT) FOR FOETAL ANEUPLOIDY

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Non-Invasive Prenatal Testing (NIPT) for Foetal Aneuploidy DETECTS FOETAL WHOLE-CHROMOSOMAL ABNORMALITIES (TRISOMY AND MONOSOMY) FOR CHROMOSOMES 13, 18, 21, X, Y, AND TRIPLOIDY USING CELL-FREE DNA ISOLATED FROM MATERNAL PLASMA.

Test Highlights

- Non-invasive prenatal testing (NIPT) using a single-nucleotide polymorphism (SNP) and informatics-based approach to detect foetal copy number for the five chromosomes responsible for the majority of live-birth aneuploidies (chromosomes 13, 18, 21, X, Y and Triploidy).
- This method offers consistently high confidence across all five chromosomes, calculates specimen-specific accuracies, and has high sensitivity and specificity for all detected aneuploidies.

Clinical Background

- Traditional non-invasive screening methods that use biochemical markers in maternal serum, with or without foetal nuchal translucency, detect only 70% to 95% of foetal Down syndrome (trisomy 21) and 60% to 90% of foetal trisomy 18, at a screen positive rate that can be >20% in women over age 35.
- Invasive procedures, such as amniocentesis and chorionic villus sampling (CVS), carry a risk of pregnancy loss.
- High false-positive rates associated with traditional prenatal aneuploidy screening methods result in unnecessary diagnostic procedures, which can lead to procedure-related foetal loss of unaffected foetuses.
- NIPT, while still a screening test, combines very high sensitivity (>99%) with very high specificity (>99%) to provide a screening test that can detect >99% of the conditions listed below, at a greatly reduced false-positive rate.

Epidemiology

Incidence at birth for:

- Down syndrome (DS) is 1/600.
- Trisomy 18 (T18) is 1/3,000.
- Trisomy 13 (T13) is 1/5,000.
- Turner syndrome (TS) is 1/2,500 female births.

Indications for Ordering

- Primary screening of pregnant women at increased risk to have a foetus with specific whole-chromosomal abnormalities, including T13, T18, T21, and TS.
- Secondary/advanced screening of women who have had an abnormal test result by biochemical maternal serum screening and/or ultrasound abnormalities suggestive of any of the above disorders.

Contraindications for Ordering:

Alternative testing should be considered when either the patient or her partner is a known balanced carrier of a translocation or other chromosome rearrangement.

- This test should not be ordered under the following circumstances:
  * If the patient is pregnant with more than one foetus.
  * If the patient is NOT the genetic mother of the foetus (e.g., she used a donor egg or embryo, or if she is a surrogate).
  * If the patient ever had an allogeneic bone marrow transplant.
- These tests should not be used in place of routine ultrasound or diagnostic testing for chromosomal aneuploidies.

Please note that recommended treatment guidelines are for South Africa only. Please refer to the guidelines relevant to your country.

Additional Ordering Notes

- Each test order must be accompanied by a completed Lancet Laboratories “Patient History” form.
- It is recommended that a completed patient “Informed Consent” form be submitted.
- Collection kits containing the two required 10 mL Streck Cell-Free DNA (tan tiger-top) blood collection tubes and a buccal brush are available through Lancet Laboratories (please contact 011 358 0739 and order the Panorama Pack).
- **Specimen requirements:**
  - 20 mL maternal whole blood collected in two Streck Cell-Free DNA blood collection tubes (provided) shipped overnight at room temperature. Specimen **must** be collected in the correct tube.
  - A paternal specimen is requested, but not required. Use the provided brush to collect a paternal buccal specimen.
- The paternal buccal specimen, if provided, MUST accompany the maternal blood specimen. Please **do not** send the buccal brush home with the patient to collect the paternal specimen.
- **Unacceptable conditions:**
  - Clotted, refrigerated or frozen specimens.
  - Patient specimens drawn in any tube other that a Streck DNA tube.
  - Less than 16 mL maternal blood.
  - Paternal buccal specimen sent separately from the patient's (maternal) blood specimen.

Interpretation

- A written summary and an interpretation of the NIPT findings will be provided.
- Each risk assessment includes the age-related risk (pre-test risk) and the risk after NIPT.
- A “High Risk” result indicates that the test has detected a significantly increased risk (greater than/equal to 1/100) for the foetus to have either monosomy or trisomy for chromosomes 13, 18, or 21, or monosomy X.
- A “Low Risk” result means the test detected a very low chance (less than 1/100) for the foetus to have an abnormal number of chromosomes 13, 18, 21, X, or Y.
  - A foetal karyotype or other testing may still be appropriate if a foetus is found to have ultrasound anomalies, or if there are other concerns about the health of the foetus.
- A “Vanishing Twin/Triploidy” result means the test results indicated the presence of a possible vanishing twin pregnancy (a multiple gestation pregnancy in which one of the twins die in utero) or pregnancy with triploidy.
- A “No Call” test result* occurs relatively infrequently and describes the inability to confidently report a “High Risk” or a “Low Risk” test result using stringent quality-control guidelines.

This may happen when:

- Too little foetal DNA is present in the maternal specimen (low foetal fraction).
- Mosaicism for one of the targeted chromosomes is present in the foetus, the placenta, or the mother.
- An unrecognized multiple gestation pregnancy exists.
- The patient is not the genetic mother of the foetus.
- The patient has received an allogeneic bone marrow transplant in the past.
- The mother and the father of the foetus are related by blood (e.g., cousins).

▸ Under some circumstances, the laboratory may request a second patient specimen to clarify the test results.

- Patients with a high-risk NIPT result should be referred for genetic counselling and should be offered a foetal karyotype by chorionic villus sampling (CVS) or amniocentesis.

Limitations

- Will not detect all foetal chromosome abnormalities.
- Cannot be performed in women who are not carrying their biological offspring (i.e., pregnancies resulting from an egg donor, or if the patient is a surrogate).
- Cannot be performed in women who have received an allogeneic bone marrow transplant.
- Has not been tested in multiple gestations.
- Analytical sensitivity is >99%.
- Clinical sensitivity and specificity for all chromosomes examined are >99%.

Methodology

- Cell-free DNA is isolated from maternal plasma, maternal genomic DNA is isolated from the buffy coat, and (if a paternal specimen is provided) paternal genomic DNA is isolated from a buccal specimen. Targeted, polymorphic loci are amplified in 19,500-plex PCR assays and then sequenced using the Illumina HiSeq next-generation sequencing instrument. The results are then analyzed using the “Next-generation Aneuploidy Test Using SNPs” (NATUS) algorithm.
- The NATUS algorithm considers the parental genotypes and HapMap crossover frequency data to predict possible foetal copy number for each chromosome at various foetal fractions. Sequencing results from the cell-free DNA specimens are compared to the predicted foetal genotypes/foetal fractions. The software then sums the likelihoods of those results and identifies those predictions with the maximum likelihood as the foetal ploidy state and foetal fraction.

References

1. Lo YM, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997;;350(9076):485–487.
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