REGIONE TOSCANA

INFORMATION ON THE SCREENING TEST FOR ANEUPLOIDY OF CHROMOSOMES 21, 18, 13 AND SEX CHROMOSOMES THROUGH SEQUENCING THE CELL-FREE FOETAL DNA IN MATERNAL PLASMA (NIPT)

The purpose of this information is to illustrate the reasons for screening for aneuploidy (alterations in the normal number of chromosomes) of chromosomes 21, 18, 13, and of sex chromosomes, on the basis of the cell-free foetal DNA (Non-Invasive Prenatal Testing - NIPT) and the characteristics and limits of the test performed.

NIPT is highly sensitive and precise testing used to define the risk of Trisomy 21 (Down Syndrome), Trisomy 18 (Edwards Syndrome), Trisomy 13 (Patau Syndrome), trisomies of sex chromosomes (XXY or Klinefelter Syndrome, XXX or Triple X Syndrome, XXY or Jacobsen Syndrome), monosomy of the X chromosome (Turner Syndrome) and analysis of foetal sex. The term *trisomy* refers to a chromosomal abnormality that involves the presence of three instead of two pairs of a chromosome (e.g. Trisomy 21 that causes Down Syndrome). The term *monosomy* refers to the presence of one instead of two pairs of a chromosome (e.g. X Monosomy or Turner Syndrome). The most common trisomies are those involving chromosomes 21, 18, and 13 that make up between 50 and 70% of all chromosome disorders, their frequency varying according to maternal age. The most common monosomy is found in sex chromosomes.

NIPT:

1 it is ascreening test and is therefore not a substitute for diagnostic tests (foetal karyotype test on chorionic villi and amniotic fluid) and is not designed to give an definitive diagnosis;

2 it assesses the risk of trisomy of chromosomes 21, 18, 13, and the sex chromosomes (XXX, XXY, and XYY) and of monosomy of the X chromosome;

3 it identifies the foetal sex;

4 it does not provide any information about other genetic diseases and/or chromosome disorders that the foetus may be suffering;

5 it is performed by taking a maternal venous blood sample (about 10-15 ml) from the 10th week of pregnancy as estimated by ultrasound;

6 the test is not performed in the case of multiple pregnancies or pregnancies that began with multiple foetuses;

7 the test is performed using the CE-IVD VeriSeq™ NIPT Solution system at the SOD Diagnostica Genetica (Genetic Diagnostics Departmental Organizational Unit) at Careggi University Hospital.

The NIPT is performed by reading the DNA in the maternal plasma (massive parallel sequencing of cell-free DNA). The cell-free DNA is part maternal and part foetal (Foetal Fraction, FF), which comes from a section of the placenta (the cytotrophoblast), that usually displays all the genetic foetal characteristics. The FF in the maternal plasma increases with the advancement of the pregnancy, and is also influenced by a series of maternal factors (for example, it can be reduced by obesity, by certain maternal autoimmune diseases, by drugs,...). According to the Ministerial guidelines, an FF of less than 4% is unable to give a result.

Test sensitivity and specificity

The test sensitivity (capacity to identify affected foetuses) of:

- 99.9% for Trisomy 21
- 93.3% for Trisomy 18
- 99.9% for Trisomy 13
- 80% for Trisomy X (XXX)
- 99.9% for the other aneuploidies (Monosomy X, XXY and XYY)
- 97% foetal sex

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The test specificity (capacity to identify non-affected foetuses) is 99%.

Test risks and limitations

- The test does not does not involve any risks for the foetus or the mother.
- The literature indicates that the test has a failure rate (no result/inconclusive result and lack of response) of less than 1%. The failure rate is linked in most cases to the characteristics of the sample under examination (low FF, DNA foetal fraction), or the characteristics of the method used.
- Around 2% of the samples taken at the end of the first trimester have an FF rating of less than 4%. In the case of FF being less than 4%, it is not possible to provide a risk assessment since the test is not reliable. The test has to be repeated on a new blood sample, taken at least 7 days from the first. In half of these cases, the FF level is still too low. At this point a genetic and/or prenatal consultation is recommended, in order to assess any maternal or foetal factors relating to the low FF, and the subsequent diagnostic path.

False positive results are, in most cases, due to:

- the placenta and foetus having different genetic characteristics (fetoplacental mosaicism);
- the presence of cells with different genetic characteristics in the mother (constitutional mosaicism);
- certain maternal illnesses (known and unknown);
- a pregnancy that began as a multiple pregnancy, with the loss of one of the foetuses ("vanishing twin").

It is important to understand that**:**

- Certain cases require the evaluation of the chromosome structure in the mother and/or the father, or other blood-tests or specialist examinations.
- A low risk result does not ensure that there is no abnormality.
- In the case of a high risk result, it is recommended to seek advice from a specialist in genetics or gynaecology and a obstetrician expert in prenatal diagnostics.
- The test does not provide diagnostic information: only performing diagnostic tests such as chorionic villus sampling or amniocentesis can actually confirm or exclude with certainty during pregnancy the existence of a chromosome abnormality in the foetus.
- If you judge the risk assessment provided to be exhaustive and choose not to perform the NIPT offered as part of the regional antenatal pathway, you can decide not to carry out further examination in relation to the chromosome structure of the foetus. However, you may decide to undertake invasive diagnostic assessment, taking into account the regional access criteria.

References: - The Ministry of Health, Supreme Health Council, Section I: "Guidelines for Non-Invasive Prenatal Testing (NIPT), May 2015; - Italian Human Genetics Society, SIGU: "Guidelines on the use of Non-Invasive Prenatal Ttesting", Ed. February 2014. https://support.illumina.com/documento numero 1000000031192 v02ITA