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INFORMATION ON THE SCREENING TEST  
FOR ANEUPLOIDY OF CHROMOSOMES 21, 18, 13 AND SEX CHROMOSOMES BY  
MEANS OF SEQUENCING CELL-FREE DNA IN THE MATERNAL PLASMA  
(NIPT, Non Invasive Prenatal Testing - singleton pregnancies and twin pregnancies)

This information is intended to illustrate the characteristics and limitations of the non-invasive prenatal test NIPT, which has high sensitivity and specificity in determining, by sequencing the free-cell DNA circulating in maternal plasma, the risk of certain specific aneuploidies in the foetus (presence of an abnormal number of chromosomes in a cell):

- aneuploidies of chromosomes 13, 18, 21 and sex chromosomes in singleton pregnancies;
- aneuploidies of chromosomes 13, 18, 21 in twin pregnancies.

The test is validated exclusively for singleton and twin pregnancies.

Each cell contains 46 chromosomes (23 pairs), divided into 22 pairs of autosomes plus two sex chromosomes (XX in females, XY in males). **Aneuploidies** are abnormal numbers in an individual's set of chromosomes (karyotype). When an additional chromosome is present for one of the pairs, it is called **trisomy** (e.g. three chromosomes 21, trisomy 21 which causes Down syndrome). The term **monosomy** indicates the lack of a chromosome in one of the pairs (e.g. lack of one of the sex chromosomes, monosomy X, which causes 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. The most common monosomy is found in sex chromosomes


**NIPT:**

- a) is a screening test and is therefore not a substitute for diagnostic tests (foetal karyotype test on chorionic villus and amniotic fluid) and is not designed to give a diagnosis;
- b) assesses the risk of trisomy/monosomy of chromosomes 13, 18 and 21 in singleton and twin pregnancies;
- c) in singleton pregnancies, it identifies the foetal genetic sex and the risk of monosomies/trisomies of sex chromosomes;
- d) in twin pregnancies, it identifies the possible presence of the Y chromosome but is not able to define the genetic sex of the foetuses, nor the risk of aneuploidies of the sex chromosomes;
- e) does not provide information on genetic diseases other than those involving the number of the chromosomes involved in the test (for example, this test is unable to identify monogenic diseases due to alterations of a single gene);
- f) is performed on a maternal venous blood sample (about 10-15 ml) from the 10th week of pregnancy as estimated by ultrasound;
- g) is performed using the CE-IVD VeriSeq™ NIPT Solution version 2 at the SOD Diagnostica Genetica of the Careggi University Hospital (AOU Careggi).

From a technical point of view, NIPT is performed by sequencing the cell-free DNA in the maternal plasma. In a pregnant woman, cell-free DNA is a mixture of maternal and foetal DNA derived from the part of the placenta that generally represents the genetic characteristics of the foetus (cytotrophoblast). The ratio of foetal DNA to total cell-free DNA is the foetal fraction, FF. The FF value is calculated as an integral part of the test and is a very important parameter: the reliability of the result is strictly dependent on the size of the FF. The FF considered adequate for estimating the risk of aneuploidies is  $\geq 4\%$  for single pregnancies and  $\geq 8\%$  for twins.

**Test sensitivity and specificity**

The following are the manufacturer's stated test performance characteristics for the VeriSeq™ NIPT Solution version 2.

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### ***Single pregnancies***

For trisomies 21, 18 and 13 the test has a sensitivity (ability to identify affected fetuses) and a specificity (ability to identify unaffected fetuses) of over 99%.

For the analysis of sex chromosomes, the test has a sensitivity and specificity of over 99% for the attribution of foetal genetic sex and for trisomies XXX and XXY, 91% for trisomy XYY and 90% for monosomy X.

### ***Twin pregnancies***

The test has a sensitivity (ability to identify affected fetuses) of 99% for the Y chromosome, 96.4% for trisomy 21, 95.7% for trisomy 18 and 93.6% for trisomy 13; it is not possible to attribute the identified result to the individual foetus.

The test has a specificity (ability to identify unaffected fetuses) of over 99% for trisomies 21, 18 and 13 and for the identification of the Y chromosome.

### **Test risks and limitations**

The test does not involve any risks for the foetus or the mother.

The test does not provide information on possible polyploidy (for example triploidy, i.e. the presence of three chromosomes of each type).

In a small percentage of cases (<1%), the test may fail or provide an inconclusive result; in these cases, the estimated risk of aneuploidy cannot be calculated.

A FF lower than 4% increases the risk of a false negative result and the rate of non-informativeness of the test (for further information: Ministerial Guidelines.

[https://www.salute.gov.it/portale/documentazione/p6\\_2\\_2\\_1.jsp?lingua=italiano&id=2381](https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2381) and Ministerial Recommendations [https://www.salute.gov.it/portale/documentazione/p6\\_2\\_2\\_1.jsp?lingua=italiano&id=3097](https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=3097)).

The value of FF in maternal plasma depends on certain characteristics of the pregnancy and is influenced by a series of both maternal and foetal factors (e.g. it increases with gestational age; decreases in case of obesity, certain autoimmune diseases or taking certain drugs, or with increased levels of certain endogenous substances such as triglycerides or haemoglobin).


In the case of twin pregnancies, the test used is unable to attribute the FF to an individual foetus; therefore, to ensure a sufficient contribution of the DNA of each twin, the FF in dichorionic pregnancies must be  $\geq 8\%$ .

In the case of FF lower than 4% for single pregnancies, 8% for twins, or failure, a repeat sampling will be proposed. However, taking a second sample does not guarantee that a result will be obtained: the data shows that, in such cases, 50% fail to get a result from the second sample.

In single pregnancies, in case of  $FF \geq 3\%$  at the second sampling, a report will be issued with the estimate of the risk of chromosomal aneuploidies, which will have lower sensitivity and specificity than the estimates described above.

The implications of the above results should be explained during post-test genetic consultation to assess their significance in relation to the overall obstetric and anamnestic picture.

The test performed is a screening test, therefore:

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- any high-risk results require confirmation by karyotyping on foetal tissue (amniocentesis/chorionic villus sampling);
- it is not free from false positive results (i.e., cases that appear high risk according to NIPT, but are not confirmed by invasive prenatal diagnosis);
- it is not free from false negative results (i.e., cases that appear low risk according to NIPT, but for which an aneuploidy is identified at invasive prenatal diagnosis or at birth);
- is not free from results that lead to discrepancies between foetal genetic sex and ultrasound sex.

The reliability of the test could be influenced by certain factors, both maternal and foetal, including:

- with a different chromosomal makeup from the foetus (fetoplacental mosaicism);
- cell lines with two or more different karyotypes in the pregnant woman (maternal mosaicism);
- cancer (even undiagnosed), blood transfusions, organ transplants, surgical interventions, immunotherapy or stem cell therapies in the pregnant woman;
- pregnancy that began with twins with the early loss of one of the foetuses (vanishing twin).

**It is important to understand that:**

- 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 could accidentally reveal unexpected information that is not related to the purpose for which it was performed;
- the test does not provide a diagnosis: possible chromosome anomalies in the prenatal period can only be confirmed or excluded by undergoing chorionic villus sampling or amniocentesis, through the reconstruction of the foetal karyotype;
- certain cases require the evaluation of the chromosome structure in the mother and/or the father, or other blood tests or specialist examinations.

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 undergo any further testing in relation to the chromosomal makeup of the foetus. However, you may decide to undertake invasive diagnostic assessment, taking into account the regional access criteria.

**Statement of having received, read and understood the information**

First Name and Last Name of the patient or other authorised person

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Signature of the patient or other authorised person.....

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Signature of the Health Care Professional

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Date.....

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References:

- 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 Testing', Ed. February 2014.
- Ministry of Health, Supreme Health Council, Section I: 'Non-invasive fetal DNA screening (NIPT) in public health', recommendations drawn up by the working group, 9 March 2021.
- <https://www.regione.toscana.it/-/test-per-il-rischio-di-gravidanza-con-anomalie-cromosomiche> (forms and informational video for pregnant women)