NATIVA

The new generation test for the analysis of fetal DNA

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NATIVA

THE NEW GENERATION TEST FOR THE ANALYSIS OF FETAL DNA

NATIVA is a new generation, non-invasive, prenatal test (NIPT - Non Invasive Prenatal Test). It is **a screening test** that provides answers **about the possible presence of fetal chromosomal abnormalities** such as Down Syndrome or other common anomalies.

It is fast and risk-free for the mother and child because it is not invasive. By analyzing fetal DNA fragments released from placental trophoblasts and circulating freely in maternal blood, it is possible to identify which subjects are at risk and for which a further investigation via invasive diagnostic techniques such as amniocentesis or chorionic villus sampling is recommended.

NATIVA is performed at BioRep laboratories located in San Raffaele Scientific Park in Milan, one of the reference institutions for Research and Clinical Diagnosis in Italy and in Europe.

NATIVA IS CE-IVD CERTIFIED (*In Vitro* Diagnostics) FOR TRISOMIES 21, 18 AND 13 AND SEX CHROMOSOME ANEUPLOIDIES.

NATIVA:
THE LATEST GENERATION NIPT

WHAT DOES IT ANALYZE?

NATIVA is the prenatal screening test able to detect **trisomies 21, 18 and 13, sex chromosome aneuploidies (variations in the number of X and Y chromosomes) and the sex of the unborn child.**

Some genetic diseases are hereditary, others, such as Down Syndrome, can occur in any pregnancy. The risk of chromosomal abnormalities is greater as gestational age increases, rising drastically after the age of 35.

NATIVA ascertains the possible risk of trisomies - namely, conditions that occur when a subject presents an extra chromosome as compared to the habitual chromosome pair - as in the case of Down Syndrome, Edwards Syndrome and Patau Syndrome.

- **Down Syndrome (trisomy 21):** this is the most frequent trisomy at birth and is caused by the presence of an extra copy of chromosome 21. It is associated with severe or moderate mental disabilities. It can also cause problems that affect the digestive and cardiovascular systems.
- **Edwards syndrome (trisomy 18)**: this is caused by the presence of an extra copy of chromosome 18 and is associated with severe malformations with a high risk of miscarriage and reduced life expectancy.
- **Patau Syndrome (trisomy 13)**: this is caused by the presence of an extra copy of chromosome 13 and is associated with a high risk of miscarriage. Children born with Patau Syndrome usually present severe congenital heart defects and other malformations and are unlikely to survive beyond the first year of life.

Frequency of the main trisomies in relation to maternal age

Maternal age	Down Syndrome Frequency	Edwards Syndrome Frequency	Patau Syndrome Frequency
15 - 19	1:1.400	1:17.000	1:33.000
20 - 24	1:1.250	1:14.000	1:25.000
25 - 29	1:1.100	1:11.000	1:20.000
30 - 34	1:700	1:7.100	1:14.000
35 - 39	1:200	1:2.400	1:4.800
40 - 44	1:60	1:700	1:1.600

EB. (1981) Rates of chromosomal abnormalities at different maternal ages, Obstetrics & Gynecology, 58(3):282-5

As for sexual aneuploidies, 1 in 400 men and 1 in 650 women suffer from sex chromosome aneuploidies. The consequences of these types of abnormalities are generally less severe than the previously listed trisomies.

- **Turner syndrome (45,X0): due to the presence of only one X chromosome in women, with a frequency of approximately 1 in 3,000.** Affected subjects have a female phenotype, but in 85-90% of cases, due to premature ovarian failure, they do not develop or only partially develop secondary sexual characteristics and are infertile or experience early menopause. Furthermore, these subjects may present with cardiac defects, renal abnormalities and short stature. Intelligence is usually normal but learning disorders, psychomotor retardation and behavioural disorders may be present.
- **Klinefelter Syndrome (47,XXY):** Klinefelter Syndrome is a condition characterized by the presence of an extra X sexual chromosome in male subjects. Affected subjects have low testosterone levels, and if not treated early during puberty, may suffer from hypogonadism and infertility. There is an increased risk of language delay and learning disability.
- **Chromosome X trisomy (47,XXX):** due to the presence of an extra X chromosome in females. This is the most common female chromosome abnormality, occurring in approximately 1 in 1,000 female births. It does not involve unusual phenotype characteristics. Proportionate tall stature is often reported. Pubertal development is normal, but in a third of cases reduced fertility, dysmenorrhoea and early menopause may be observed. In two thirds of cases a delay in psychomotor development may be observed.
- **Jacobs Syndrome or Y disomy (47, XYY):** Double Y syndrome has an incidence of approximately 1 in 1,000 male births. Most men are tall and show normal sexual development as well as usually preserved fertility. Intellectual development is usually normal, however, delays in language, learning disability and muscle hypotonia may be observed.

Characteristics	Klinefelter	XYY or Jacobs	Turner	Triple X
Karyotype	47,XXY	47,XYY	45,X0	47,XXX
Phenotypic sex	Male	Male	Female	Female
Gonads	Hypogonadism due to low testosterone	Normal	Ovarian insufficiency	Normal
Fertility	Infertility	Usually normal	Infertility	Usually normal
Intelligence	Normal	Normal	Usually normal	Normal
Behavioural problems	Sporadic	Sporadic	Sporadic	Sporadic
Other characteristics	Language disorder, learning disability	Language disorder, learning disability	Short stature, language disorder, learning disability, possible other abnormalities of heart, skeleton, kidneys	Language disorder, learning disability

From Harper PS: Practical Genetic Counseling, 6th ed. London, Arnolf Publisher, 2004, p.69.

WHO IS THIS TEST INTENDED FOR?

NATIVA is ideal for all pregnant women as it avoids the risks associated with invasive diagnostic tests. It can be performed from the 10th week of pregnancy onwards.

NATIVA can also be performed in the cases of:

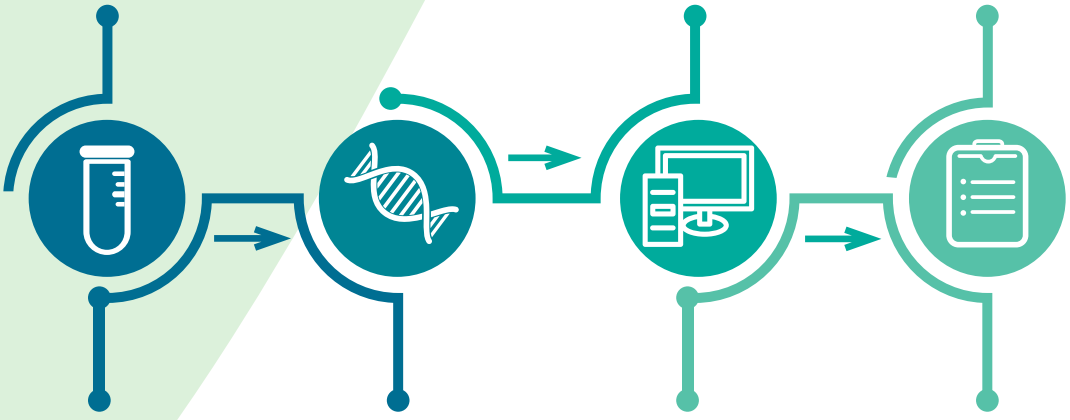
- **twin pregnancies;**
- **egg donation pregnancies;**
- **surrogate pregnancies.**

There are conditions for which a foetal DNA screening test is particularly recommended:

- **when the mother is over the age of 35;**
- **a POSITIVE result for the first or second quarter screening (Bi/Tri Test);**
- **pregnant women at risk of miscarrying;**
- **abnormalities in the child detected via ultrasound;**
- **family history pointing towards the risk of chromosomal abnormalities.**

HOW TO TAKE THE TEST

NATIVA analyzes the child's DNA fragments released by placental trophoblasts and circulating freely in maternal blood. The procedure is simple and the result is obtained in only 5/7 working days from receipt of the blood sample at the laboratory.
The test consists in taking a simple blood sample from the mother. It has no side effects, is not invasive and does not pose any risk to the mother or child. The blood sample is collected by express courier and delivered to BioRep laboratories.



MATERNAL BLOOD SAMPLE

LATEST GENERATION DNA EXTRACTION AND SEQUENCING

ANALYSIS OF FETAL DNA

REPORT PROCESSING

BioRep keeps offering you assistance after issuing the report: in case of positive results, our geneticists will be available for consultation and it will be possible to perform amniocentesis or chorionic villus sampling free of charge at affiliated centres.

NATIVA: THE MOST RELIABLE AND ACCURATE ANSWER

NATIVA is a non-invasive prenatal test validated on more than 3,000 pregnant women, that provides precise and timely information on trisomies 21, 18 and 13 and on sexual aneuploidies, and is able to determine the sex of the foetus with high reliability.

NATIVA, thanks to its increased sensitivity and specificity as compared to the combined test, can reduce the use of invasive diagnostic tests, thus reducing the ensuing risk of miscarriage.

The Specificity and Sensitivity values of **NATIVA** are very high and make the test extremely reliable.

SENSITIVITY

> 99.9%

IT IDENTIFIES THE PRESENCE OF ABNORMALITIES

IT HAS REDUCED POSSIBILITIES OF FALSE NEGATIVES

SPECIFICITY

> 99.9%

IT EXCLUDES THE PRESENCE OF ABNORMALITIES

IT HAS REDUCED POSSIBILITIES OF FALSE POSITIVES

CUTTING-EDGE TECHNOLOGY NEXT-GENERATION SEQUENCING (NGS)

NATIVA is based on Illumina's VeriSeq technology, the only automated solution that fully complies with CE-IVD guidelines for the screening of the most common fetal trisomies (trisomies 21, 18 and 13) and sex chromosome aneuploidies. The DNA of every living being comprises the repetition of only four molecules referred to as nitrogenous bases: Adenine, Thymine, Guanine and Cytosine. NGS technology makes it possible to know the sequence of human DNA, through the ordered reading of each single base making up the strands of DNA, and to carry out a chromosome count by using the maternal blood sample.

THE SEQUENCING PROCESS

Sample preparation

- **The maternal blood sample is collected in special EC-IVD tubes**, sent to BioRep laboratories and registered in the database, which guarantees traceability and eliminates risks in sample management.
- **The blood is centrifuged to separate the plasma, from which the circulating DNA** will then be extracted, maternal and fetal alike (ccfDNA, circulating cell free DNA).

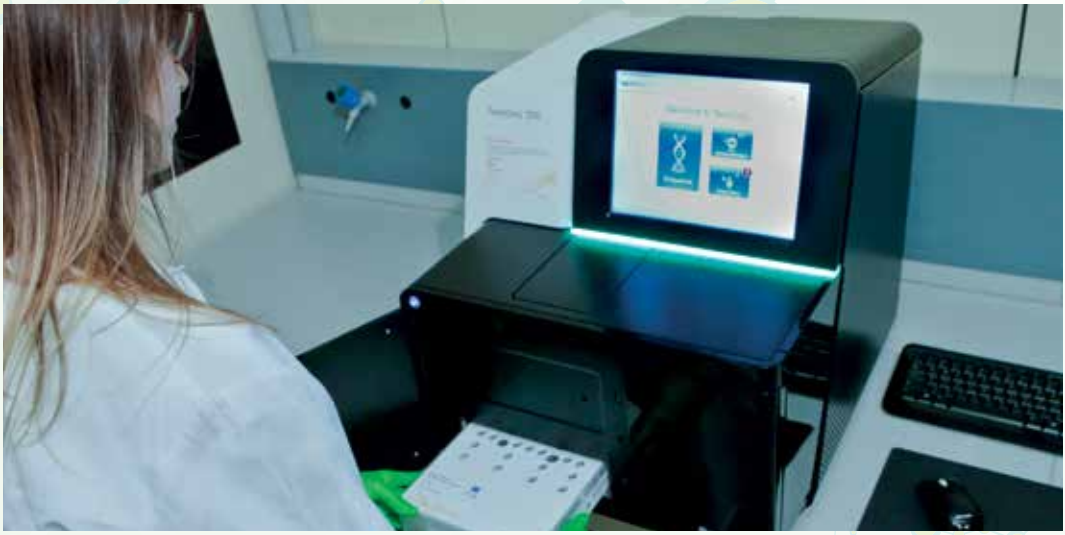
From DNA extraction to the nucleotide sequence

- **Circulating DNA is isolated via an automated extraction system** based on adsorption affinity of polymers. Up to 96 samples can be simultaneously processed through this technology without risk of errors because the process is fully automated.
- **The 96 samples are identified with a special molecular bar code** that will enable the identification of each DNA fragment from each sample. **Samples are prepared for sequencing (library) and grouped into a single "pool".**
- **The DNA pool is loaded into the sequencer that performs the sequencing** and quality control of the process.
- **The sequencer immobilises the DNA strands of the pool on a special support referred to as a "flow cell". Each fragment is amplified until a “cluster” is created,** namely a group of hundreds of millions of identical copies of the same fragment.

- Solutions containing the four bases forming the DNA strands (A, G, C, T) are sequentially injected into the flow cell. **Every time one of the bases is coupled to one of the filaments on the flow cell, a light emission is recorded with a colour specific to the incorporated base**. The sequencer recognizes the light emissions of billions of fragments at the same time and notes the sequence.

From the nucleotide sequence to the report

- Once the sequence of each fragment is obtained, the data are processed by a special **CE-IVD certified software** that differentiates foetal DNA from maternal DNA and counts and identifies fetal chromosomes.
- All the chromosomes are sequenced (Whole Genome Sequencing) but **abnormalities are analyzed only and exclusively for chromosomes 21, 18 and 13, X and Y (if present)**.
- **The new Illumina algorithm makes it possible to analyzed samples with a percentage of foetal fraction even lower than 4%** using a variable threshold analysis that modifies the sensitivity of the system based on the amount of fetal DNA detected. Furthermore, **NATIVA** uses the number of sequenced foetal DNA fragments as a test reliability parameter, making the limit of 4% foetal fraction obsolete.
- The 4% limit was historically fixed because previously used technologies (Array) made test results unreliable when the foetal fraction was below that threshold.
- **Analysis results are checked by the geneticist** who is available for consultation regarding the reports.



COMPARISON ACROSS PRENATAL SCREENING TECHNOLOGIES

NATIVA, due to the use of innovative methods and algorithms, is the only test based on CE-IVD certified NGS technology for each phase of the analysis process. By equencing the entire child genome, NATIVA is able to provide reliable results even in the case of a fetal fraction lower than 4%.

Type of test	Analysis methodology	Calculation of foetal fraction	Threshold foetal fraction	Rate of failures
NATIVA	Whole Genome Sequencing	Paired End Sequencing	<4%	0.68%*
Test based on array technology	Hybridisation on chip array	SNP	4%	3.00%**
Test based on sequencing limited only to chromosomes 13, 21, 18, X, Y	Target Sequencing	SNP	4-8%	6.40%***

* Illumina VeriSeq NIPT Solution Package Insert
** MCCullogh RM et al. PLoS ONE 2014
*** Dar et al. Am J Obstet Gynecol 2014

PAIRED END SEQUENCING

Free circulating DNA of both the child and the mother is present in maternal blood. It is therefore necessary to have an accurate detection system, which selects without ambiguity the free circulating DNA of the child on which to perform the aneuploidy analysis. The sequencing method known as Paired End Sequencing (PE), sequences each DNA fragment from both ends, rather than from a single end. In this way it is possible to know, in addition to the sequence, the exact size of each fragment analyzed. It has been proven that in a sample of maternal blood smaller fragments are usually of fetal origin whereas longer fragments tend to be of maternal origin. The introduction of the Paired End Sequencing method has increased the information generated by each sequencing process so that a more accurate analysis is obtained with one third the intensity of sequencing compared to other tests.

1 Lo YN, Chan KC, Sun H, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med. 2010;61(2):61-91.

CERTIFIED QUALITY

The complete NATIVA analysis flow is CE-IVD certified (*In-Vitro* Diagnostic) and is performed at the **SMeL (Servizio di medicina di Laboratorio) authorized** laboratories of BioRep, located in the San Raffaele Science Park in Milan.
CE-IVD certification, issued by the European Community, certifies that **NATIVA meets the essential requirements and expected performance levels for medical products**. Certification is issued only if the product complies with **Directive 98/79/EC** and is a guarantee of quality and safety.

A CLEAR REPORT, IN A SHORT TIME

The NATIVA report shows the percentage of foetal fraction analyzed and the presence or absence of abnormalities. If the report is positive it is necessary to undergo an invasive diagnosis.

The report is delivered within 5/7 working days of receipt of the sample and, if it is positive, you can contact our geneticist for advice.

COMPARISON ACROSS PRENATAL SCREENING PROCEDURES

Non-invasive standard first-trimester tests, such as the bi-test, do not identify more than 15%* of trisomy 21 cases.

Screening tests	Biological materials analyzed	Conditions analyzed	Detection rate for trisomy 21
Combined first trimester screening	Blood Tests PPAPP-A and hCG, plus an ultrasound	Trisomy 21 Trisomy 13 Trisomy 18	82% - 87%*
NATIVA	Blood analysis for free circulating foetal DNA	Trisomy 21 Trisomy 13 Trisomy 18 X0, XXX, XXY. XYY	99.9%

*Screening Tests for Birth Defects, American Congress of Obstetricians and Gynecologists, 2017.

NATIVA is a screening test proposed as an alternative to standard non-invasive tests. It is able to provide answers even if the foetal fraction is below 4%, decreasing the number of unnecessary invasive tests and reducing the ensuing risk of miscarriage.

WARNING

NATIVA is CE-IVD certified for the analysis of trisomies 21, 18 and 13, sex chromosome aneuploidies and foetal sex, as recommended by Ministerial Guidelines. If necessary, a doctor will be responsible for using the information obtained from the test to direct the patient towards additional genetic guidance or let her undergo further assessment via chorionic villus sampling or amniocentesis. The stated specificity and sensitivity do not exclude the presence of false positives or false negatives due to chromosomal placental modifications (mosaicism confined to the placenta) or chromosomal modifications of the mother or foetus (chromosomal mosaicism).

NATIVA does not exclude other abnormalities and malformations of the foetus, in that it exclusively analyzes the presence of trisomies 21, 18 and 13 and sex chromosome aneuploidies.

In case of twin pregnancies **NATIVA**:
- is able to detect trisomies 21, 18 and 13 but is unable to indicate which foetus is affected;
- it cannot detect sex chromosome aneuploidies;
- it can identify the presence of at least one male foetus or indicate if both foetuses are female.

NATIVA is not recommended in cases of:
- **Vanishing twin**
- **Multiple pregnancies with more than two foetuses**
- **Pregnant mother known to suffer from cancer**
- **Immunotherapy, radiotherapy or blood transfusion performed on the pregnant woman within the previous 3 months**
- **Organ allogeneic transplantation in the pregnant woman**
- **Chromosomal mosaicisms present in the mother involving the chromosomes under investigation**

WHY CHOOSE NATIVA

NATIVA is the prenatal screening test tested on over 3,000 pregnant women able to detect trisomies 21, 18 and 13, sexual aneuploidies and the sex of the foetus, providing a reliable result also in cases of a foetal fraction less than 4%.

FAST

The report is processed in a few working days from receipt of the sample at the laboratories.

PERFORMED IN ITALY

NATIVA is entirely performed at the BioRep laboratories located in the San Raffaele Scientific Park in Milan.

COMPLIANT

NATIVA complies with the provisions of the Ministry of Health guidelines for DNA-based non-invasive prenatal screening (JUL/3/2015).

CERTIFIED

NATIVA uses CE-IVD certified collection devices, laboratory processes and analyzes.

ACCURATE

Nativa uses an NGS platform that ensures the best performance in terms of accuracy and sensitivity, with the lowest rate of false positives and false negatives.

GENETIC CONSULTING

Our **Customer Care** and **Genetic Consulting Team** are available for a medical consultation before and after delivery of the report.

BioRep is a Sapio Group company operating in the field of biotechnology at an international level. Founded in 2003, BioRep is a **Biological Resources Centre** able to provide comprehensive solutions for the long-term preservation of biological material and laboratory services to public and private research institutes (hospitals, universities, clinics), biotech and pharmaceutical companies, ensuring the highest levels of quality and safety.

Thanks to the most advanced sequencing technologies with **Next-Generation Sequencing (NGS)** techniques, BioRep also offers **DNA** analysis services for diagnostic or research purposes. BioRep is UNI EN ISO 9001 certified and is equipped with one of the most advanced technological infrastructures on an global scale.

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