SISTEMAS GENÓMICOS
A MEMBER OF SYNLAB

INCLUYE ASESORAMIENTO GENÉTICO AL ESPECIALISTA

NON-INVASIVE PRENATAL TESTING (NIPT)

SG BABYTEST *Plus / Advanced*

This **non-invasive prenatal testing (NIPT)** enables genetic testing in the unborn baby using a sample of maternal blood containing circulating cell-free foetal DNA. It determines at an early stage in the pregnancy the risk of the foetus carrying an aneuploidy (abnormal number of chromosomes) or partial aneuploidy (CNVs) associated with 10 known genetic syndromes.

Non-invasive prenatal testing: does not involves risk to either the foetus or the mother.

Avoids the anxiety of undergoing an invasive procedure to obtain the foetal sample.

Significantly reduced rate of false positives compared to other prenatal screening tests.

SG BabyTest can be performed on any pregnant woman, regardless of her genetic condition or family history. It can also be performed in pregnancies achieved using assisted reproductive techniques, with egg donation and in consanguineous couples. **SG BabyTest** is performed in the first trimester of the pregnancy from the ninth week of gestation.

It is particularly indicated in:

- Pregnancy with an increased risk of chromosomal abnormalities due to:
  - Advanced maternal age.
  - History of previous pregnancies with chromosomal aneuploidy.
  - Family history of chromosomal abnormalities.
  - High or intermediate risk determined by the biochemical screening performed in the first trimester.
- Foetuses with certain ultrasound abnormalities.

It is also indicated in the general population of pregnant women.

CE-IVD marking of the bioinformatic algorithm for the evaluation of aneuploidies of chromosomes 13, 18, 21, X and Y and for the detection of CNVs>6Mb associated with microdeletion syndromes.

ISO 15189 accreditation for the screening for foetal aneuploidies (chromosomes 13, 18, 21, X and Y) and determination of foetal sex in maternal blood.

We actively take part in external quality controls such as European Molecular Genetics Quality Network (EMQN)quality controls.

SG BabyTest analyses the risk of aneuploidy in all chromosomes

SG BABYTEST *Plus*

Risk of autosomal chromosomes aneuploidy:

- Trisomy 21, associated with Down syndrome.
- Trisomy 18, associated with Edwards syndrome.
- Trisomy 13, associated with Patau syndrome.
- Trisomies 16 and 22, associated with higher rates of miscarriage, as well as other less common aneuploidies, such as chromosome 9 and chromosome 15 aneuploidies.

Risk of sex chromosomes aneuploidy:

- Turner syndrome (45,X0).
- Klinefelter syndrome (47,XXY).
- Jacobs syndrome (47,XYY).
- Triple X syndrome (47,XXX).
- Polysomy X

Determination of sex and foetal fraction

SG BabyTest also offers:

- Determination of foetal Rh(D) genotype in Rh(–) women.

SG BABYTEST *Advanced*

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Risk of sex chromosomes aneuploidy:

- Turner syndrome (45,X0).
- Klinefelter syndrome (47,XXY).
- Jacobs syndrome (47,XYY).
- Triple X syndrome (47,XXX).
- Polysomy X

Determination of sex and foetal fraction

Risk of partial aneuploidies (CNVs):

- Limitation: CNV size > 6 Mb
- Detection of 10 microdeletions associated with genetic syndromes.

SYNDROME*	CHROMOSOME REGION SIZE	INCIDENCE
Angelman syndrome (15q11)	5-7 Mb	1/12,000-20,000
Prader-Willi syndrome (15q11)	4-6 Mb	1/10,000-30,000
1p36 deletion syndrome	1.5-10 Mb	1/5,000-10,000
Cri-du-chat syndrome (5p-)	0.560-40 Mb	1/15,000-50,000
Wolf-Hirschhorn syndrome (4p16.3)	0.5-30 Mb	1/50,000
Jacobsen syndrome (11q23)	5-20 Mb	1/100,000
Langer-Giedion syndrome (8q24.1)	2.8-14 Mb	0.2-1/1,000,000
DiGeorge II syndrome (10p14-13)	4.19-12.072 Mb	1/200,000
16p11.2-p12.2 deletion syndrome	7.1-8.7 Mb	-
Phelan-McDermid syndrome (22q13.3)	0.1-9 Mb	-

* https://decipher.sanger.ac.uk/disorders#syndromes/overview

TECHNOLOGICAL BASIS

During pregnancy, small fragments of foetal DNA from the cytotrophoblast are released into the bloodstream and circulate alongside other fragments of maternal DNA in the mother's blood plasma. The sample of maternal blood is collected in tubes specially designed to prevent degradation of circulating cell-free DNA, thus reducing the risk of rupture of blood cells that would contaminate the sample. After plasma separation and DNA purification, the sample is analysed by means of low-coverage whole-genome sequencing using the latest next-generation sequencing (NGS) technology.

Next, a bioinformatics algorithm we developed (GeneSystems ©-G-NIPT) is used to estimate the number of sequences obtained for each chromosome and they are compared with reference samples to detect whether there is an increase or decrease in the number of readings associated with each of the chromosomes, or any chromosomal fragment in the ***Advanced*** version. The change in the number of readings indicates that there may be a gain or a loss in the chromosome identified.

WORKING PROTOCOL

Collection of the maternal blood sample in the tube recommended and dispatch to our laboratories. All measures should be taken to ensure that the sample is received at the laboratory within 72-96 hours of collection. The sample is kept at room temperature (6°C-35°C) at all times.

Isolation of circulating cell-free DNA from plasma obtained by centrifugation.

Generation of libraries from circulating cell-free DNA fragments and low-coverage whole-genome sequencing using NGS.

Bioinformatics analysis with our own algorithms to identify the deviations in the number of foetal DNA readings. Determination of foetal fraction.

Report with simple, easy-to-interpret graphical display of results.*

*Time elapsed between receiving the sample and issuing the report: 4-6 calendar days (see informed consent).

DETERMINATION OF FOETAL FRACTION

For an accurate result, **SG BabyTest** requires at least 3.5% foetal DNA in the sample to detect the presence or absence of aneuploidies and/or microdeletions.

SG BabyTest determines the foetal fraction using a bioinformatics algorithm we developed based on foetal-specific nucleosome profiles.

As increased body mass reduces the foetal fraction, pregnant women with obesity (BMI >30) **are advised to undergo a prior molecular test that determines the percentage of foetal DNA and also reports the baby's sex.**

Sensitivity and specificity greater than 99.9% for chromosomes 13, 18 and 21

Sensitivity greater than 99% and specificity greater than 99.9% for sex chromosomes

Accuracy greater than 99% for determination of foetal sex

ADVANTAGES

Non-invasive test.

Analysis of all chromosomes, **greater reliability** in detecting aneuploidies associated with specific chromosomes and greater efficiency in identifying these aneuploidies.

Significantly reduced rate of false positives, resulting in a **reduced number of subsequent invasive procedures**.

RESULTS	RECOMMENDATIONS
Low risk: Very low likelihood of foetal chromosomal aneuploidy in any of the chromosomes analysed or a microdeletion (in the ***Advanced*** version).	**Routine patient information.**
High risk: High likelihood of foetal chromosomal aneuploidy in the chromosome indicated or a microdeletion (in the ***Advanced*** version).	**Genetic counselling and free confirmation of the result by means of a diagnostic test.***
Undetermined risk or non-informative result: The risk of aneuploidy in the chromosome indicated or microdeletion cannot be determined by the values obtained from the test, because although the result may not be normal, it is not sufficiently abnormal to be high risk.	**Review medication, treatments and clinical data (abnormalities, ultrasounds, etc.)****
Inconclusive result: Either the sample or the result obtained does not pass the quality controls established for the test (high degree of haemolysis, low foetal fraction or results with a high dispersion value).	**Review medication, treatments and clinical data (abnormalities, ultrasounds, etc.)****

* If an aneuploidy or microdeletion is suspected, the result should be confirmed by performing a definitive diagnostic test. Ascires Sistemas Genónicos offers invasive prenatal genetic diagnosis on chorionic villus or amniotic fluid samples (QF-PCR, FISH, Array or Karyotype) free of charge.

** In cases of undetermined or inconclusive results, it may be necessary to repeat the test with a new sample, aiming to eliminate factors (if known) that may have affected the first non-valid sample.

THE REPORT INCLUDES

- Details of the sample and the request
- Method
- Results (percentage of cell-free foetal DNA, risk for whole-chromosome aneuploidies, microdeletions in the ***Advanced*** version and foetal sex)
- Interpretation of the results
- Recommendations on how to proceed depending on the result
- Graphical report of chromosomes

GENETIC COUNSELLING

Our experts in prenatal genetic diagnosis and in the NIPT test will answer any questions that might arise during the process.

SG BABYTEST

FEATURES	SPECIFICITY	SENSITIVITY
Trisomy 21	>99.9%	>99.9%
Trisomy 18	>99.9%	>99.9%
Trisomy 13	>99.9%	>99.9%
Trisomies 9, 16 and 22	>99.9%	-
Sex chromosome aneuploidies (XO, XXY, XXX and XYY)	>99.9%	>99%
False positives (sex chromosome aneuploidies detected)	<0.1	
Foetal fraction	Bioinformatics algorithm	
Method	Bidirectional NGS	
% of non-informative results	0.6%	
Valid from which week of pregnancy	Ninth	
Valid for twins	Yes*	
Valid for egg donation	Yes	
Turnaround time	4-6** calendar days	
Confirmation with an invasive prenatal test (chorionic villus sampling or amniocentesis)	3 working days (QF-PCR and FISH)*** 5 working days (Array)	

* Consult informed consent.
** Consult our experts for further information.
*** Sistemas Genómicos has fine-tuned the technology to perform the QF-PCR, FISH, Array and Karyotype techniques. An invasive procedure for confirmation or ultrasound follow-up may be recommended depending on the chromosomal abnormality detected.

PRE-TEST CONDITIONS THAT CAN AFFECT THE SG BABYTEST NON-INVASIVE PRENATAL TEST

- **Low concentration of total circulating cell-free DNA in plasma:** the yield of the extraction of circulating cell-free DNA from maternal plasma is insufficient to perform the test.
- **Haemolysed sample:** the blood sample has undergone haemolysis (cell rupture) which has contaminated the plasma with maternal genomic DNA.
- **Low foetal fraction:** the percentage of circulating cell-free foetal DNA versus the total obtained is less than 3.5%, the minimum required to be able to perform the test.
- **Problems associated with biological conditions or other factors** related to therapeutic actions (taking medication, transfusions, transplants, etc.):* for example, treatment with low-molecular-weight heparin prior to collection of a blood sample can affect the test; therefore, it is recommended that such biological conditions or other factors be reported in advance so that the best conditions under which blood collection should be performed may be determined.

All medical treatments should be reported for assessment and confirmation that the test is viable: compatibility with treatments with Adiro®, progesterone and vitamins.

LIMITATIONS

- The NIPT technique may yield inconclusive results in:
    - Gestational age less than 9 weeks.
    - Mosaicism or partial chromosome abnormalities in the foetus or in the mother.
    - Cases of genetically non-identical twins, since the circulating cell-free foetal DNA load in the bloodstream of one of them may be below the limit of detection.
    - Multiple pregnancies (3 or more foetuses).
    - Pregnant women with obesity (BMI >30), who are advised to undergo determination of foetal fraction prior to the test.

- **SG BabyTest** is not a diagnostic test but a screening test. A low risk result does not completely rule out the possibility of foetal chromosome abnormality.

- The test does not rule out the presence of other genetic abnormalities not analysed, the existence of polyploidies (triploidy or tetraploidy) or the possibility of the foetus carrying congenital defects not caused by any of the abnormalities detected by the test.

- **SG BabyTest** requires a foetal fraction of at least 3.5%.

- **SG BabyTest *Advanced*** has a limit of detection of microdeletions corresponding to a size greater than 6 Mb.

- Some syndromes associated with microdeletions in the ***Advanced*** version may be due to changes other than microdeletions (e.g. mutations) that would not be detected in this test.

SAMPLE SHIPMENT

- Collection of maternal blood in a tube with a specific preservative for plasma cell-free DNA.
  Depending on the test ordered, 1 or 2 tubes will be required.

- Receipt of the **unrefrigerated** sample within 72-96 hours of collection.

Test fully developed and produced in Spain

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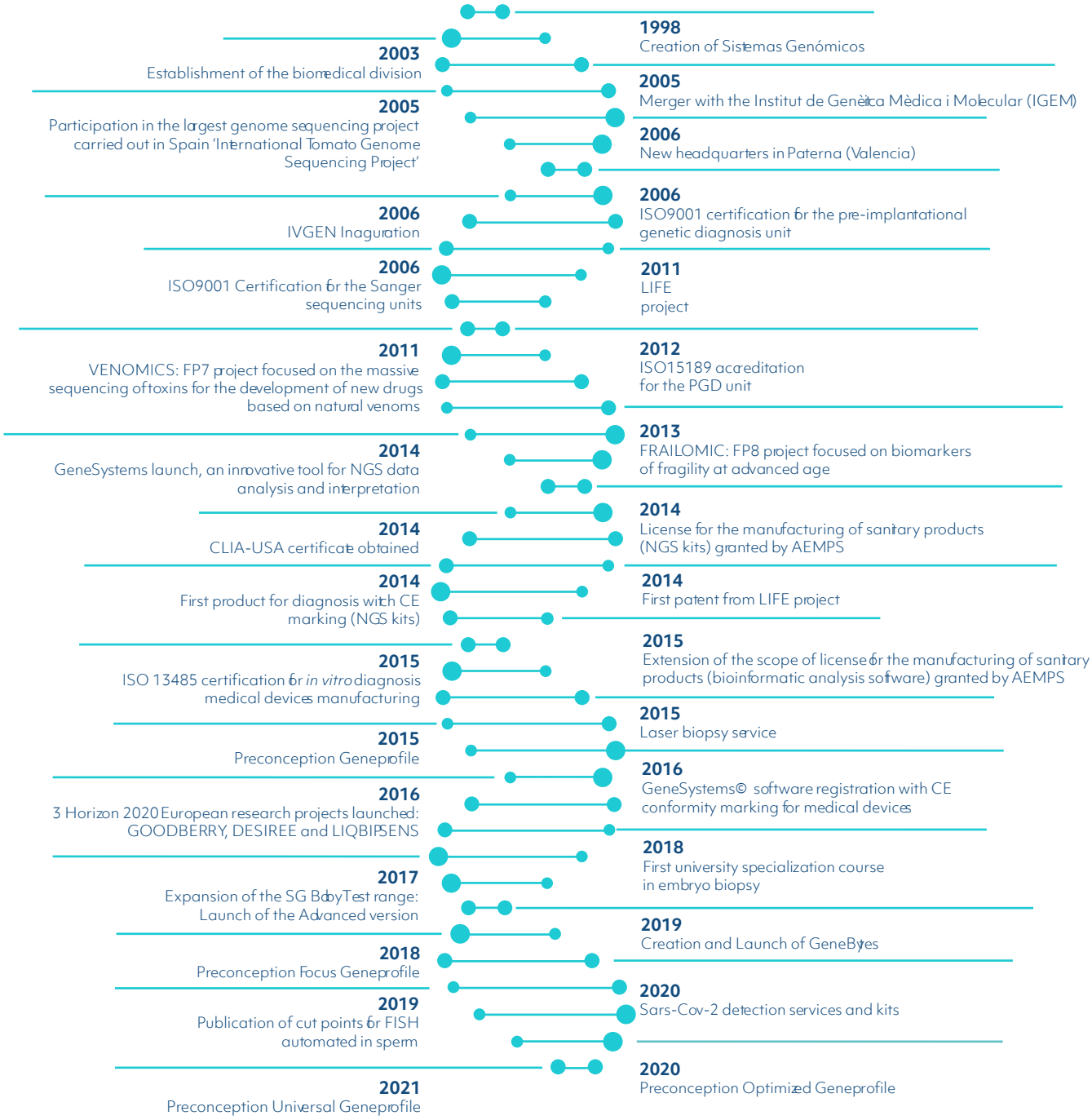
At **Sistemas Genómicos** we are **committed to improving the quality of life** of patients and their families. To achieve this, we design high-quality diagnostic tools, combining the most advanced technology with the latest scientific research.

We are **pioneers in applying next-generation sequencing (NGS) technology to genetic diagnosis**. We are also **the organisation with the best track record in Spain and internationally** in research and development for genetic diagnosis services **based on our extensive experience in the study of genomes and human genetics.**



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