SISTEMAS GENÓMICOS
ΔSCIRES

GENETIC COUNSELLING TO SPECIALISTS

NON-INVASIVE PRENATAL TEST (NIPT)

SG BABYTEST *Plus* / *Advanced*

SG BabyTest range is designed to **assess the risk of aneuploidy** (abnormal number of chromosomes) **in the fetus early in pregnancy:**

- Trisomy 21 associated with Down syndrome.
- Trisomy 18 associated with Edwards syndrome.
- Trisomy 13 associated with Patau syndrome.
- Trisomies for chromosomes 16 and 22, most often associated with spontaneous abortion, as well as other less common types of aneuploidy such as those of chromosomes 9 and 15.
- Sex chromosome aneuploidies:
    - o Turner syndrome (presence of a single X sex chromosome).
    - o Klinefelter syndrome (XXY).
    - o Triple X syndrome.
    - o Polysomy X.

SG BabyTest *Advanced*, the amplified version of SG BabyTest identifies partial aneuploidies (CNVs) related to **10 known microdeletion syndromes*.**

SYNDROME	CHROMOSOMAL REGION SIZE	INCIDENCE
Angelman Syndrome (15q11)	5 - 7 Mb	1/12,000 - 20,000
Prader Willy Syndrome (15q11)	4 - 6 Mb	1/10,000 - 30,000
1p36 deletion Syndrome	1.5 - 10 Mb	1/5,000 - 10,000
Crit-du-chat Syndrome (5p)	0.560 - 40 Mb	1/15,000 - 50,000
Wolf-Hirschhorn (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
Di George II Syndrome (10p14-p13)	4.19 - 12.072 Mb	1/200,000
16p11.2-p12.2 deletion	7.1 - 8.7 Mb	-
Phelan-McDermind Syndrome (Deletion 22q13.3)	0.1 - 9 Mb	-

* Some of these syndromes may be due to other alterations (i.e. mutations) that would not be detected by this test.

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

SG BABYTEST

This non-invasive prenatal test (NIPT) makes fetal genetic analysis possible using **a maternal blood sample that contains cell-free fetal DNA.**

- **Non-invasive test:** there is no increased risk for either the fetus or the mother.
- **Avoids the anxiety** of undergoing an invasive procedure to obtain the fetal sample.
- Reduction of false positives.

SG BabyTest can be used for **all pregnant women**, including **pregnancies achieved through assisted reproductive techniques and oocyte donation**, who want to rule out chromosomal aneuploidies regardless of their genetic condition or family history.

SG BabyTest should be performed in the first trimester of pregnancy, from week 9.

It is particularly recommended in:

- **Women with high risk** of having a fetus carrying chromosomal abnormalities:
  - o Advanced maternal age.
  - o History of pregnancies with chromosomal aneuploidy.
  - o High or intermediate risk established by the biochemical screening performed in the first trimester
- Fetuses with certain **ultrasound abnormalities**.

TECHNOLOGY BEHIND SG BABYTEST

During pregnancy, small fragments of fetal DNA coming from the trophoblast are released into the bloodstream and circulate alongside other fragments of maternal DNA in the mother's blood plasma.

Maternal blood sample is extracted in a suitable Streck® tube developed for optimal preservation of cffDNA from degradation and reduces the risk of blood cell lysis. **The plasma is then isolated** and the DNA extracted and analysed using bidirectional, whole-genome **next-generation sequencing**.

An **in-house bioinformatics algorithm** is applied to the estimation of the number of sequences aligned to each chromosome and they are compared with reference samples to **detect whether there is an increase or decreased in number of reads associated to each chromosome or CNVs in *Advanced* test**. Changes in the number of reads indicate that a gain or loss of can be present in the reported chromosome.

PROTOCOL

1. Extraction of the maternal blood sample in the recommended tube and dispatch to our laboratories.
   All measures should be taken to ensure that the sample arrives at the laboratory within no more than 72-96 hours after it was taken.
   The sample should be kept at room temperature at all times (6-35°C).
2. Isolation of cell-free fetal DNA from plasma obtained by centrifugation.
3. Determination* of the fetal fraction.
4. Generation of libraries from cell-free fetal DNA fragments and low-coverage whole-genome sequencing using NGS.
5. Bioinformatics analysis with our own algorithms to identify the deviations in the number of fetal DNA readings.
6. Report** with simple, easy-to-interpret graphical display of results.

*Determination by bioinformatics algorithm or molecular techniques (ddPCR) according to BMI of the pregnant woman

**Time from sampling reception to issue of report: 6-7 working days, consult informed consent

DETERMINING THE FETAL FRACTION

For optimal results, SG BabyTest requires at least 3.5% fetal DNA in the sample to detect the presence or absence of aneuploidies or CNVs.

SG BabyTest determines fetal fraction by an own bioinformatics algorithm associated to the massive sequencing process of the distribution frequencies of cell-free fetal DNA sizes, which are smaller in the case of fetal DNA. It is also determined regarding to the presence and quantification of Y chromosome, in addition to the algorithm, in case of male fetus.

As body mass increase reduces fetal fraction, in the case of pregnant women with obesity **(BMI>30), a previous molecular test is recommended to determine the percentage of fetal DNA and, it also reports on the gender of the fetus.**

Over 99% sensitivity and specificity for chromosomes 21, 18 and 13 and 97% for sex chromosomes

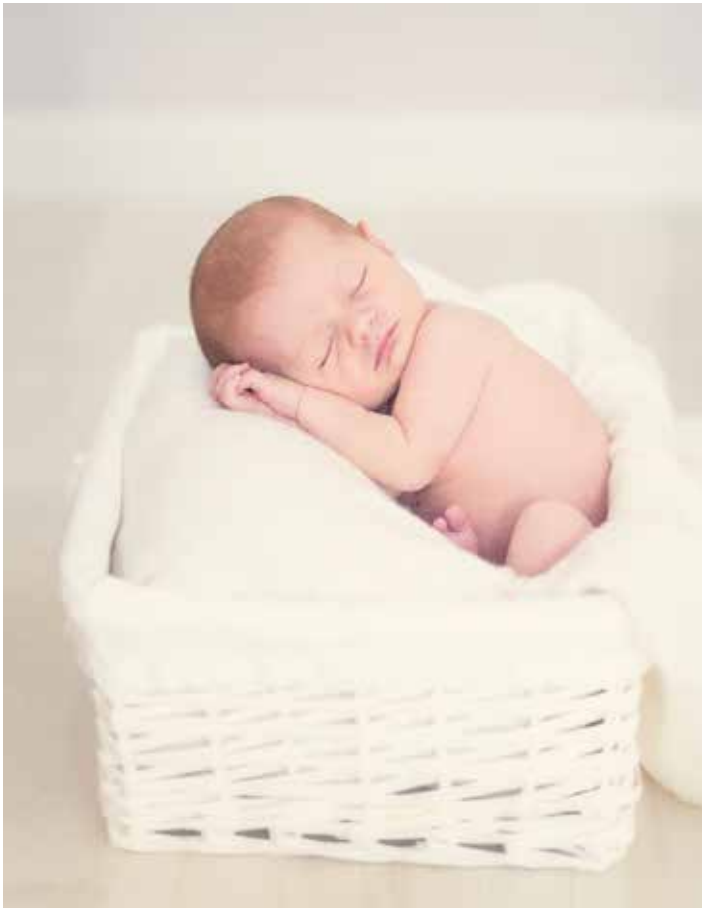
Accuracy greater than 98% for determination of the fetal gender

NIPT COMPARED TO TRIPLE SCREENING

- As well as triple screening, it is a non-invasive test.
- Increased reliability in the detection of aneuploidies associated with specific chromosomes.
- Higher efficiency in the identification of aneuploidies.
- Reduction in the number of subsequent invasive procedures thanks to the minimal number of false positives.

RESULTS

POSSIBLE OUTCOMES



Low risk:

Very low probability of fetal chromosomal aneuploidy in any of the analysed chromosomes, and microdeletion in ***Advanced*** test.

High risk:

High probability of fetal chromosomal aneuploidy in the indicated chromosome, or microdeletion in ***Advanced*** test. The result should be confirmed by performing a definitive diagnostic test such as invasive prenatal chorionic villus sampling or amniotic fluid testing.

Undetermined risk or non-informative result:

Obtained values do not allow to determine risk of aneuploidy in the indicated chromosome or microdeletion, the result may not be normal but it is not sufficiently abnormal to be high risk.

Result inconclusive:

Either the sample or the result obtained does not pass the quality controls established for the test (high degree of haemolysis, low fetal fraction, results with high dispersion value, etc.).

In cases of inconclusive results, it may be necessary to repeat the study with a new sample, aiming to eliminate factors that may have affected the first non-valid sample.

STRUCTURE OF THE REPORT

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

Our experts in prenatal diagnosis and in the NIPT test will resolve any queries that might arise during the process.

In case of chromosome suspected chromosome abnormality, Sistemas Genómicos offers free invasive procedure (QF-PCR, FISH or Array)

SG BABYTEST

FEATURES	Specificity	Sensitivity
Trisomy 21	> 99.9%	> 99%
Trisomy 18	> 99.9%	> 99%
Trisomy 13	> 99.9%	> 99%
Trisomy 9,16,22	> 99.9%	-
Sex chromosome aneuploidies (XO,XXY,XXX,XYY)	97%	-
Overall detection rate (CR.21,18,13)	> 99%	> 99%
False positives (sex chromosome aneuploidies detected)	2.6%	
Fetal Fraction	Bioinformatics algorithm	
Method	Bidirectional NGS	
% of non-informative results	2.5%	
Valid from which week of gestation	9	
Valid for twins	Yes*	
Valid for oocyte donation	Yes	
Turnaround time	6-7** working days	
Confirmation with invasive prenatal test (chorionic villus sampling or amniotic fluid)	3 working days (QF-PCR & FISH)*** 5 working days (Array)	

* Consult informed consent
** Consult our experts for more information
*** Sistemas Genómicos has 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 chromosome abnormality detected.

PRE-ANALYTICAL CONDITIONS THAT CAN AFFECT SG BABYTEST

• Haemolysed sample: the blood sample has undergone haemolysis (cell rupture), contaminating the plasma with maternal genomic DNA.

• Low concentration of cffDNA in plasma: the yield of the DNA extraction from maternal plasma was so low that it is insufficient to perform the test.

• Low fetal fraction: the fetal DNA content in the sample is very small compared to the DNA of the mother (less than 3.5% detected by the bioinformatics algorithm) and so, if there is an aneuploidy, it may not be detected.

Problems associated with biological conditions or other factors related to therapeutic actions (taking medication, transplants, etc.)*: for example, treatment with low molecular weight heparin prior to sampling can affect the test, so we recommend notifying in advance to determine the optimum conditions under which blood collection should be performed.

*Any medical treatment should be reported in order to be assessed and to confirm that the test is viable: compatibility with Adiro®, treatments, progesterone and vitamins.

EXPERIENCE IN PRENATAL STUDIES

Specificity	Object	Result	Year
European CEQAS/EMQN Quality Programme	Fetal sex determination	Highest score	2015
European CEQAS/EMQN Quality Pilot Programme	Fetal aneuploidies	Highest score	2016-2017
External Validation	21, 18, 13, 9, 16, 22 Fetal sex	100% correlation	2016
European CEQAS/EMQN Quality Pilot Programme	Fetal sex and fetal CNVs determination	Highest score	2017

SAMPLE SHIPMENT

- Extraction of maternal blood into one or two 10 ml STRECK BCT tubes (specific preservative for free plasma DNA), regarding to the choosen test.
- Sample shipment without refrigeration in less than 96 hours.

LIMITATIONS

- The NIPT technique may give inconclusive results in:
    - o Gestational age less than 9 weeks.
    - o Mosaicism or partial chromosome abnormalities in the fetus or in the mother.
    - o Twin or multiple pregnancies, if the twins are not genetically identical, since the fetal DNA load in the bloodstream of one or other of them may be below the limit of detection.
    - o In morbidly obese pregnant women, as the fetal fraction has been shown to be lower.
- SG BabyTest is not a diagnostic test but a screening test. As it is a screening test, a low risk result does not totally exclude the possibility of fetal chromosome abnormality.
- The test does not exclude the presence of other genetic abnormalities which are not analysed, nor the existence of polyploidy (triploidy or tetraploidy) or that the fetus may have congenital defects of which the cause is not any of the alterations detected by the test.
- SG BabyTest requires a minimum of 3.5 % of fetal fraction, in adition, the Advanced version has a minimum detection size of 6 Mb for microdeletions.

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Sistemas Genómicos is the largest Spanish company specialized in genetic sequencing with nearly two decades of experience in the field of molecular biology and genetics. Internationally, it is the only firm that is able to provide genetic analysis and diagnostic services in all stages of the human life cycle. Pioneer in mass sequencing and the implementation of this technology, the company participates in different R&D projects, its mission is to place advances in genomics and genetics at the service of society and the medical and research community. Its most important asset is a dedicated interdisciplinary (molecular biology, bioinformatics, medicine and genetics) team of more than 120 professionals.

Sistemas Genómicos is the genomics and genetics division of ASCIRES Biomedical Group which focuses its activity on diagnosis and treatment, reinvesting on average an average of 20% of the R & D & I benefits, allowing us to be at the forefront of incorporation of technology and the latest scientific advances. All this with a team of 700 people working in personalized medicine that share identity signs: vocation for the patient, passion for technological innovation and humanization of treatment.

1998
Creation of Sistemas Genómicos

2003
Establishment of the biomedical division

2005
Participation in the largest genome sequencing project carried out in Spain 'International Tomato Genome Sequencing Project'

2006
New headquarters in Paterna (Valencia)

2006
ISO9001 certification for the pre-implantational genetic diagnosis unit

2006
IVGEN Inagurations

2006
ISO9001 Certification for the Sanger sequencing units

2011
LIFE project

2011
VENOMICS: FP7 project focused on the massive sequencing of toxins for the development of new drugs based on natural venoms

2012
ISO15189 accreditation for the PGD unit

2013
FRAILOMIC: FP8 project focused on biomarkers of fragility at advanced age

2014
GeneSystems launch, an innovative tool for NGS data analysis and interpretation

2014
License for the manufacturing of sanitary products (NGS kits) granted by AEMPS

2014
CLIA-USA certificate obtained

2014
First patent from LIFE project

2014
First product for diagnosis witch CE marking (NGS kits)

2015
ISO 13485 certification for *in vitro* diagnosis medical devices manufacturing

2015
Extension of the scope of license for the manufacturing of sanitary products (bioinformatic analysis software) granted by AEMPS

2016
3 Horizon 2020 European research projects launched: GOODBERRY, DESIREE and LIQBIPSENS

2016
Ampliation of SG Baby Test Range launched of Advanced version