Information sheet GeneSAFE®

What is GeneSAFE®

GeneSAFE® is a **non-invasive screening** test aimed at assessing the risk that the fetus is affected by certain **monogenic disorders**. The test is **not diagnostic**: it does not provide absolute certainty but indicates the possibility that the fetus may present specific genetic conditions.

The test is performed from the **10th week of pregnancy onwards** through a **maternal blood sample**, from which small fragments of circulating cell-free DNA (cfDNA), derived from the placenta and originating from the fetus through the placenta, are analyzed. The test does not pose any direct risk to either the mother or the fetus.

In some cases, in order to ensure correct interpretation of the result, **additional investigations** on a **paternal** biological sample may be required. It is therefore **recommended** that such a sample be **made available at the time of the maternal blood draw** for GeneSAFE® analysis. In the case of pregnancies achieved through donor conception, it may also be necessary to obtain **documentation relating** to any genetic testing performed on the **donors**; such documentation may be requested at the time of the maternal sample collection or at a later stage.

Who GeneSAFE® is intended for and when to perform it

GeneSAFE® can be performed from the 10th week of pregnancy in:

- singleton pregnancies, both spontaneous and obtained through medically assisted reproduction (MAR);
- twin pregnancies, both dichorionic and monochorionic.

The test is **not validated** for pregnancies in which the presence of **more than two placentas** has been identified by ultrasound.

GeneSAFE® is a screening test and should be proposed in pregnancies at **low risk for genetic disease**; however, it may also be considered as a first non-invasive approach when:

- there is a known family history of genetic diseases or previously identified variants in relatives;
- clinical or ultrasound findings during pregnancy raise suspicion of one of the investigated conditions;
- the couple prefers a non-invasive first-line approach, being aware that a “high-risk” result requires confirmation.

Which conditions GeneSAFE® can identify

GeneSAFE® can identify different types of **fetal monogenic disorders**, that is, genetic conditions caused by alterations in a single gene.

These include, on one hand, certain **inherited autosomal recessive disorders**, in which the risk for the fetus arises when **two pathogenic variants in the same gene** are inherited, one from each parent. In these cases, the parents may be **healthy carriers**, meaning that they do not show clinical manifestations despite carrying a single variant.

On the other hand, GeneSAFE® can identify certain **de novo genetic disorders**, caused by variants that may arise for the **first time in the fetus at conception** and are therefore not necessarily present in the parents. These conditions may be associated, for example, with **skeletal dysplasias, congenital heart defects, multiple congenital anomalies, and/or neurodevelopmental or cognitive impairment**. Many of these conditions cannot be identified through preconception carrier screening performed on the parents, and some may not be detectable by ultrasound in the first trimester. In addition, some of these conditions may be associated with **advanced paternal age.**

Available GeneSAFE® testing levels

GeneSAFE® is available in **three** different testing **levels**, which differ based on the type of genetic conditions investigated:

- **GeneSAFE® Inherited**: investigates selected autosomal recessive inherited genetic disorders that are relatively frequent in the general population.
- **GeneSAFE® De Novo**: focuses on selected non-inherited genetic disorders, caused by variants that arise de novo in the fetus.
- **GeneSAFE® Complete**: includes both the Inherited and the De Novo panels, thus providing the most comprehensive assessment among the three testing levels.

For each testing level, the genes analyzed and their associated conditions are reported in the following tables.

*Table 1 Genes and conditions included in **GeneSAFE®** Inherited*

GeneSAFE® Inherited	
GENE	**Associated disorder(s)**
Gene	**Patologie associate**
CFTR	Cystic Fibrosis
HBB	Beta-thalassemia
	Sickle cell anemia
GJB2 (CX26)	GJB2-Related Autosomal Recessive Nonsyndromic Hearing Loss (type 1A)
GJB6 (CX30)	GJB6-Related Autosomal Recessive Nonsyndromic Hearing Loss (type 1B)

Table 2 Genes and conditions analyzed by GeneSAFE® De Novo- The conditions included in the GeneSAFE® De Novo panel can be grouped into major clinical categories. The following tables report the analyzed genes and their main associated disorders.

GeneSAFE® De Novo	
GENE	**Associated disorder(s)**
Noonan Spectrum Disorders	
BRAF	Cardiofaciocutaneous Syndrome, type 1

CBL	Noonan Syndrome-like disorder with or without juvenile myelomonocytic leukemia (NSLL)
KRAS	Noonan Syndrome, type 3
MAP2K1	Cardiofaciocutaneous Syndrome 3
MAP2K2	Cardiofaciocutaneous Syndrome 4
NRAS	Noonan Syndrome, type 6
PTPN11	Noonan Syndrome, type 1
	LEOPARD Syndrome 1
RAF1	Noonan syndrome, type 5
	LEOPARD Syndrome 2
RIT1	Noonan syndrome, type 8
SHOC2	Noonan syndrome-like disorder with loose anagen hair
SOS1	Noonan syndrome, type 4
Craniosynostosis	
FGFR2	Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis
	Apert Syndrome
	Crouzon Syndrome
	Jackson-Weiss Syndrome
	Pfeiffer Syndrome, type 1, 2, 3
	Pfeiffer Syndrome, type 2
FGFR3	Pfeiffer Syndrome, type 3
Skeletal Disorders	
COL2A1	Achondrogenesis, type II or hypochondrogenesis
FGFR3	Achondroplasia
	CATSHL Syndrome
	Crouzon syndrome with acanthosis nigricans
	Hypochondroplasia
COL1A1	Ehlers-Danlos syndrome, classic
	Ehlers-Danlos syndrome, type VIIA
	Osteogenesis imperfecta, type I, II, III, IV
COL1A2	Ehlers-Danlos Syndrome, cardiac valvular form
	Ehlers-Danlos, type VIIB Syndrome
	Osteogenesis imperfecta, type II, III, IV
Syndromic Disorders	
JAG1	Alagille Syndrome
CHD7	CHARGE Syndrome
NIPBL	Cornelia de Lange Syndrome, type 1
SMC1A	Cornelia de Lange Syndrome, type 2
SMC3	Cornelia de Lange Syndrome, type 3
RAD21	Cornelia de Lange Syndrome, type 4
HDAC8	Cornelia de Lange Syndrome, type 5
MECP2	Rett Syndrome
SETBP1	Schinzel-Giedion Syndrome
SIX3	Holoprosencephaly

Tests that can be performed in combination with GeneSAFE®

In some cases, GeneSAFE® may be integrated with additional tests in order to expand the range of information obtainable during pregnancy.

PrenatalSAFE® is a non-invasive prenatal test that assesses the risk of certain fetal chromosomal abnormalities, including the most common trisomies, sex chromosome aneuploidies and, in its more extensive levels, also rare chromosomal abnormalities, structural alterations and microdeletions. The combination of GeneSAFE® with PrenatalSAFE® therefore allows the assessment of both the risk of fetal monogenic disorders and the risk of fetal chromosomal abnormalities.

GeneScreen® is a test performed on the parents, aimed at identifying carrier status for certain inherited genetic disorders. It is therefore not a fetal test, but rather an additional assessment useful to better define the reproductive risk of the couple.

RhSafe® is a non-invasive prenatal test that determines the fetal Rh(D) status through the analysis of circulating DNA in maternal blood. It is an optional test, performed only in pregnancies where the mother is Rh(D) negative and the partner is Rh(D) positive, and it is useful in the management of pregnancies at risk of maternal–fetal Rh incompatibility.

For further details on these additional tests, please refer to the dedicated information leaflets.

Table 3 Possible integrations of GeneSAFE® with other available tests

	Included investigations					Special considerations	
Test	**Karyo**	**Karyo Plus**	**GeneSAFE® Complete**	**GeneScreen® Focus**	**RhSafe***	**Dichorionic twin pregnancy**	**donor conception**
PrenatalSAFE® Complete	✓	X	✓	X	Optional	✓	✓
PrenatalSAFE®Complete Plus	X	✓	✓	X	Optional	X	✓
PrenatalSAFE® Full Risk	X	✓	✓	✓	Optional	✓#	X

* RhSafe® can be performed **only in cases where the pregnant woman is Rh(D) negative,** and **the partner is Rh(D) positive**. In twin pregnancies, a positive result does not allow determination of whether one or both fetuses are RhD positive.
# In dichorionic twin pregnancies, the PRENATALSAFE® FULL RISK pathway includes PrenatalSAFE® Karyo analysis and not PrenatalSAFE® Karyo Plus analysis.
The **Complete** and **Complete Plus** combinations are defined in the standard documentation as the association of **PrenatalSAFE® Karyo + GeneSAFE® Complete** and **PrenatalSAFE® Karyo Plus + GeneSAFE® Complete**, respectively.
The **Full Risk** package includes **PrenatalSAFE® Karyo Plus + GeneSAFE® Complete + GeneScreen® Focus** (female partner) + **GeneScreen® Focus** (male partner).

Possible results of GeneSAFE®

GeneSAFE® may yield one of the following results:

Low risk: A low-risk result indicates that no pathogenic variants have been identified in the analyzed genes. A low risk result significantly reduces, but does not completely exclude, the presence of variants in the analyzed genes or the expression of conditions associated with those genes.

High risk of being AFFECTED: A variant in an autosomal dominant gene or two or more variants in the investigated autosomal recessive genes have been identified, indicating an increased risk that a condition related to the specific gene may manifest. In other words, this result indicates a **high probability that the fetus is affected** by the condition identified by the test. A high-risk result must be followed by a diagnostic test. During pregnancy, this is generally performed through **invasive procedures** carried out at different gestational stages: chorionic villus sampling (CVS, 11th–13th week) or amniocentesis (16th–18th week). After birth, diagnostic testing is typically performed on a blood sample or another biological sample (e.g., buccal swab) from the newborn. The **most appropriate diagnostic pathway** should always be discussed during **genetic counseling** and/or with a prenatal diagnosis specialist, taking into account the GeneSAFE® result.

High probability of being a CARRIER: A variant in an autosomal recessive gene has been identified. This result indicates that the fetus has a high probability of being **a carrier of an autosomal recessive condition** and does not imply an increased risk of expressing the condition. In such cases, the result should be discussed during genetic counseling in order to evaluate the appropriate follow-up and future reproductive risk (including potential carrier screening in the biological parents).

Inconclusive: The test did not provide an interpretable result. The most common causes may include insufficient fetal fraction, inadequate sequencing coverage, or the presence of artifacts/noise in the analyzed regions.

- If the inconclusive result occurs at the **first blood draw**, a **repeat analysis** on a second sample is generally requested.
- If the result remains inconclusive after the **second sample**, the test is not repeated further. In such cases, **genetic counseling** is recommended to discuss the most appropriate prenatal diagnostic pathway and possible options for invasive prenatal diagnosis.

Partially inconclusive: In rare cases, the test may yield a partially inconclusive result, meaning that **no interpretable result** was obtained for **one or more specific regions** among those analyzed. The remaining analyzed regions will have produced one of the results described above.

If **additional investigations** on a **paternal biological** sample are required for the interpretation of the result, these will be performed by the laboratory **exclusively** as **support** for the GeneSAFE® analysis and **do not constitute an independent diagnostic service**. No separate report will be issued, and the results obtained will be used solely for the purpose of generating the GeneSAFE® report.

Methodology and performance of GeneSAFE®

GeneSAFE® is a cfDNA-based test performed using high-depth Next-Generation Sequencing on the Ion S5™ platform with a targeted high-depth panel. Depending on the selected testing level, the panel is designed to analyze the genes listed in Tables 1 and 2, limited to specific genomic regions available upon request. The average sequencing coverage is high (≥2000×), with approximately 90% of the target regions covered at ≥500×. Only pathogenic or likely pathogenic variants (Class IV and V), according to ACMG/AMP standards, are reported.

GeneSAFE® has demonstrated an analytical performance >99% in detecting clinically relevant variants in the investigated genes, with false-positive and false-negative rates <1%. As with all cfDNA-based tests, results are probabilistic and must be interpreted within the clinical context: in case of a high-risk result, diagnostic confirmation (amniocentesis or chorionic villus sampling) is always recommended before any clinical decision is made.

Limiti di GeneSAFE®

GeneSAFE® is a **screening test** and **not** a **diagnostic** test: results indicate a risk, not absolute certainty. A **Low risk** result significantly **reduces the probability** that the fetus is affected by one of the investigated genetic conditions but **does not completely exclude it**. Similarly, a **High risk of being AFFECTED** or **High probability of being a CARRIER** result does not constitute a diagnosis and must always be interpreted in the clinical context and, when appropriate, confirmed by diagnostic testing.

GeneSAFE® is intended to provide information **exclusively** on the **genes included in the selected testing level**, **limited to the analyzed regions** and **to the conditions** explicitly **reported** in the tables 1 and 2. The test does not detect genetic diseases other than those included in the selected panel and cannot identify all possible genetic alterations within the analyzed genes. In particular, it does not detect variants located in intronic regions beyond ±5 nucleotides from exon–intron boundaries, nor deletions, inversions, or duplications larger than 20 bp, nor mosaicisms. In addition, some genomic regions may not be analyzable or may have insufficient coverage to ensure reliable interpretation.

The ability of the test to detect a variant also depends on technical and biological factors, including fetal fraction and DNA quality. In a small percentage of cases, the test may yield an **Inconclusive result**, for example due to **low fetal fraction**, **insufficient coverage**, **technical artifacts**, or **interpretative challenges**. In such cases, a **second blood draw may be requested;** if this also does not provide a conclusive result, genetic counseling may be recommended to discuss possible alternative diagnostic options.

Among the main factors that may affect the reliability of the result, it is important to consider:

- low fetal DNA fraction in maternal blood, more frequent in conditions such as high maternal BMI or early gestational age;
- twin pregnancies, in which it is not possible to reliably distinguish the condition of each individual fetus;

- vanishing twin, which may lead to false-positive or false-negative results;
- maternal neoplastic conditions, which may interfere with the analysis due to the presence of circulating tumor DNA, particularly in genes involved in carcinogenesis (e.g., BRAF, KRAS, NRAS);
- certain medically assisted reproduction scenarios, particularly those involving gamete donation, where additional information about the donor(s) may be required.

Limitations in twin pregnancies or in the presence of a vanishing twin

In twin pregnancies, it is not possible to distinguish the condition of each individual fetus; therefore, a **high-risk** result may refer **to one or both fetuses**. In any twin pregnancy with a high-risk result, prenatal or postnatal diagnostic confirmation should be considered for both fetuses/newborns. The test is not validated for pregnancies with more than two fetuses.

The presence of a vanishing twin may reduce the reliability of the screening, as DNA from the demised twin may persist in maternal blood even after the event. This may result in false-positive or false-negative results. In such cases, the optimal timing for performing GeneSAFE® may vary depending on ultrasound findings and the gestational age at which the loss occurred.

Specific limitations of the Inherited panel

For autosomal recessive conditions, cfDNA analysis has an intrinsic limitation: it does not allow determination of zygosity, i.e., it **cannot reliably establish whether a variant is present in a single copy (heterozygous state) or in two copies (homozygous state)** in the fetus. For this reason, when both parents are carriers of the same variant or of the same recessive condition, invasive prenatal diagnosis may be required to clarify the actual risk of disease manifestation in the fetus.

In the **absence of a paternal sample**, interpretation may be limited, and it is not possible to exclude with certainty that the fetus is homozygous for the maternal variant.

Some known variants are not covered by GeneSAFE®. By way of example, but not exhaustively:

- in the **CFTR** gene, poly-T/poly-TG tracts are not analyzed;
- for **GJB2/GJB6**, deletions at the DFNB1 locus are not detected;
- for the **HBB** gene, large rearrangements/deletions are not detected.

Specific limitations of the De Novo panel

The GeneSAFE® De Novo panel identifies only variants with **established pathogenic significance** in relation to the conditions explicitly listed in the table. It does not search for benign variants or variants of uncertain clinical significance (VUS). As with any genetic test, **variant interpretation is based on the scientific knowledge available at the time of analysis** and may be subject to future updates as new evidence emerges.

If the pregnant **woman carries a variant in one of the genes** included in the GeneSAFE® De Novo panel, it will not be possible to determine whether the fetus has inherited the maternal variant, and the test will yield a **partially inconclusive result.**

GeneSAFE® within the prenatal diagnostic pathway

GeneSAFE® is part of a broader prenatal diagnostic pathway, which includes ultrasound, biochemical screening, and, when indicated, invasive investigations. **First-trimester ultrasound** remains essential to confirm pregnancy viability, accurate gestational dating, number of fetuses, and chorionicity, as well as to identify any structural abnormalities.

Unlike traditional NIPT, which primarily assesses the risk of chromosomal abnormalities, GeneSAFE® focuses on the risk of selected fetal monogenic disorders. For this reason, it is generally proposed in the presence of specific indications, such as known family history, suspicious ultrasound findings, targeted clinical questions, or other conditions in which a non-invasive genetic assessment may be useful.

GeneSAFE® **does not replace invasive prenatal diagnosis** (chorionic villus sampling or amniocentesis), which remains indicated in the presence of high-risk results, ultrasound abnormalities, or when family or clinical history requires diagnostic confirmation.

The integration of different diagnostic tools (ultrasound, biochemical screening, NIPT for chromosomal abnormalities, GeneSAFE® for selected monogenic conditions, and invasive testing when indicated) allows for a more comprehensive prenatal diagnostic pathway, which can be tailored to the specific clinical needs of each pregnant woman.

Turnaround time

Results are generally available within **15 working days** of receiving the sample.

For GeneSAFE® analysis to be performed, the **test requesition form (TRF)** must be **correctly completed**. If required information is missing, the laboratory will request it, which may affect sample processing timelines and report issuance.

It is strongly recommended that the **paternal sample be provided at the time of maternal blood collection**, as it may be required for **additional genetic analyses** useful for result interpretation.

If the paternal sample is not available at the time of maternal sampling, the laboratory may request it at a later stage.

In any such case:

- additional analyses on the paternal sample may **delay reporting by up to 10 working days**;
- if the paternal sample is provided subsequently, this delay will be **calculated from the date the sample is received** by the laboratory.

In the **absence** of a paternal sample, result **interpretation** may be **limited**. In such circumstances, the test may yield:

- a high-risk result, or
- a partially inconclusive result.

These results may be re-evaluated if the paternal sample is subsequently provided, where deemed appropriate.

In pregnancies achieved through ART with gamete donation, it may be necessary to obtain information regarding the donor(s).

The absence of such additional information may limit result interpretation and may lead to:

- a high-risk result, or
- a partially inconclusive result.

Also in this case, results may be re-evaluated if the required information is subsequently made available.

Sample management

Samples are identified by an alphanumeric code and stored for a minimum of 3 and a maximum of 6 months after completion of the analysis. Residual samples may be used for further testing, subject to evaluation of their suitability for the specific additional analysis requested.

Informed consent

It is recommended that GeneSAFE® testing be performed following **pre-test counseling** with a specialist in medical genetics or fetal medicine. During this consultation, the patient receives clear and comprehensive information regarding the **purpose** of the test, its **benefits**, **limitations**, and **possible implications** of the results, with the opportunity to ask questions and clarify any doubts.

Based on this information, the patient may provide **written informed consent**, which represents an integral part of the prenatal screening pathway.