Information sheet PrenatalSAFE®

What is PrenatalSAFE®

PrenatalSAFE® is a **non-invasive prenatal screening test (NIPT)** aimed at assessing the risk that the fetus is affected by certain chromosomal abnormalities. Th 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.

Who PrenatalSAFE® is intended for and when to perform it

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

- **singleton pregnancies**, both spontaneous and obtained through assisted reproductive technique (ART);
- **twin pregnancies**, both **dichorionic** and **monochorionic**;
- pregnancies with a **vanishing twin** (pregnancies initially twin, with subsequent loss of one of the two).

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

PrenatalSAFE® may be requested in various situations, including:

- advanced maternal age;
- intermediate-risk result at first-trimester combined screening;
- personal choice of the pregnant woman who wishes to undergo an early, accurate and non-invasive screening;
- other indications provided by the referring physician based on clinical history or ultrasound findings.

Which conditions PrenatalSAFE® can identify

Depending on the selected version, PrenatalSAFE® can detect the following conditions.

Common autosomal trisomies, in particular **Trisomy 21**, clinically associated with Down syndrome; **Trisomy 18**, clinically associated with Edwards syndrome; and **Trisomy 13**, clinically associated with Patau syndrome.

Rare autosomal aneuploidies, including trisomies (presence of an extra copy of an entire chromosome) involving chromosomes other than those classified as “common trisomies”. This group includes abnormalities affecting all autosomes, such as trisomies of chromosomes 9 and 16, as well as trisomies 7, 8, 11, 14, 15 and 22.

Sex chromosome aneuploidies, including **Monosomy X**, clinically associated with Turner syndrome; **XXY** (Klinefelter syndrome); **XYY** (Jacobs syndrome); and **XXX** (Triple X syndrome).

Chromosomal deletions and duplications ≥ 7 Mb, i.e. segmental chromosomal abnormalities involving a portion of a chromosome. Deletions correspond to a loss of chromosomal material, while duplications correspond to a gain (also referred to as triplication). Examples include 18p deletion, 12p duplication, 9p deletion and 7q deletion.

Microdeletions <7 Mb include the following specific conditions (loss of chromosomal material smaller than 7 Mb but greater than 3 Mb): 1p36 deletion syndrome, 4p- (Wolf-Hirschhorn syndrome), 5p- (Cri-du-Chat syndrome), 15q11.2 (Prader-Willi / Angelman syndrome), 22q11.2 deletion syndrome (also known as DiGeorge syndrome), 8q24 (Langer-Giedion syndrome), 11q23 (Jacobsen syndrome), and 17p11.2 (Smith-Magenis syndrome).

Fetal sex can also be determined with high accuracy (>99.9%).

Available PrenatalSAFE® testing levels

PrenatalSAFE® is available in different levels of analysis, which vary according to the type of chromosomal abnormalities investigated. The more basic levels focus on the most common aneuploidies, while the more comprehensive levels allow the assessment of rare chromosomal abnormalities, structural alterations, and selected microdeletion syndromes.

The choice of the **most appropriate level** should be made together with the **referring physician** or **healthcare professional**, taking into account the clinical history, ultrasound findings, and the desired level of detail in the evaluation.

Table 1 Available levels of PrenatalSAFE®

Test	Common autosomal trisomies	SCA	RAA	DEL/DUP ≥ 7 Mb	Microdeletions <7 Mb*	Dichorionic twin	TAT
PrenatalSAFE® 3	✓	X	X	X	X	✓	3 wd
PrenatalSAFE® 5	✓	✓	X	X	X	X	3 wd
PrenatalSAFE® 5 DiGeorge	✓	✓	X	X	*✓ (22q11.2)*	X	5 -7 wd
PrenatalSAFE® Plus	✓	✓	*✓ (trisomies 9 and 16)*	X	*✓ (panel 6 microdeletions)*	X	5 -7 wd
PrenatalSAFE® Karyo	✓	✓	✓	✓	X	✓ (no SCA)	4 - 5 wd
PrenatalSAFE® Karyo Plus	✓	✓	✓	✓	✓	X	5 - 7 wd

RAA: Rare autosomal aneuploidies; SCA: Sex chromosome aneuploidies; DEL/DUP ≥ 7 Mb: Chromosomal deletions and duplications ≥ 7 Mb; ; TAT: turnaround time

*Microdeletions included: 1p36 deletion syndrome, 4p- (Wolf-Hirschhorn syndrome), 5p- (Cri-du-Chat syndrome), 15q11.2 (Prader-Willi / Angelman syndrome), 22q11.2 deletion syndrome (also known as DiGeorge syndrome), 8q24 (Langer-Giedion syndrome), 11q23 (Jacobsen syndrome), and 17p11.2 (Smith-Magenis syndrome).

Tests that can be performed in combination with PrenatalSAFE®

In some cases, PrenatalSAFE® may be combined with additional tests in order to expand the range of information that can be obtained during pregnancy.

GeneSAFE® is a non-invasive prenatal test that assesses the risk of **selected fetal monogenic disorders**, both **inherited** and **de novo**, through the analysis of circulating fetal DNA in maternal blood. The combination of PrenatalSAFE® with GeneSAFE® therefore allows the evaluation not only of the main chromosomal abnormalities, but also of the risk of specific monogenic genetic conditions.

GeneScreen® is instead a test performed on the **parents**, aimed at identifying **carrier status** for certain inherited genetic conditions. It is therefore not a fetal test, but an additional investigation that may help to better define the couple's reproductive risk.

RhSafe® is a non-invasive prenatal test that allows determination of the fetal Rh(D) status through the analysis of circulating fetal DNA in maternal blood. It is an optional test, which can be 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 sheets.**

Table 2: Possible integrations of PrenatalSAFE® 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).

What results can be obtained with PrenatalSAFE®

PrenatalSAFE® may yield one of the following results.

A **low-risk** result indicates that the probability that the fetus is affected by one of the investigated abnormalities is very low, although not zero. In this case, the pregnancy will continue with the standard ultrasound and clinical follow-up.

A **high-risk** 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 stages of gestation, namely **chorionic villus sampling** (CVS), typically between the 11th and 13th weeks, or **amniocentesis**, typically between the 16th and 18th weeks. After the end of the pregnancy, diagnostic testing is generally 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 counselling and/or with a specialist in prenatal diagnosis, taking into account the PrenatalSAFE® result.

An **inconclusive** result indicates that the test did not provide an interpretable outcome. The most common causes may include insufficient fetal fraction, inadequate sequencing coverage, or the presence of artefacts or noise in the analyzed regions.

- If an inconclusive result occurs at the **first blood draw**, repetition of the analysis on a **second sample** is generally recommended.
- If the result remains inconclusive after the **second blood draw**, the test is **not repeated further**; in such cases, **genetic counselling** is recommended in order to discuss the most appropriate prenatal diagnostic pathway and, where appropriate, the possible options for invasive prenatal diagnosis.

A **partially inconclusive** result may rarely occur; in this case, the test did not provide an interpretable result for one or more specific regions among those analyzed, while the remaining regions yielded one of the results described above.

Analytical method and performance of PrenatalSAFE®

All samples are analyzed using the **VeriSeq™ NIPT Solution v2** platform (Illumina), a CE-IVD–certified genome-wide massively parallel sequencing test. The entire workflow, from DNA extraction to bioinformatic analysis, is standardized within Eurofins Genoma laboratories. Data analysis is further supported by **NIPTflow™,** an internally developed algorithm that enables the evaluation of chromosomal abnormalities and, in particular, small microdeletions, thereby improving result interpretation and reducing discordant cases.

The performance of PrenatalSAFE® has been described in a retrospective study including **71,883 consecutive pregnancies**, published in *Prenatal Diagnosis* (Faieta et al., 2024), which demonstrated a high level of test reliability. Clinical sensitivity was greater than 99% for common trisomies, with similarly high values observed for sex chromosome aneuploidies, and specificity consistently exceeding 99.9% across all analyzed categories. Integration with NIPTflow™ contributed to a reduction in discordant cases and further supported the clinical utility of the genome-wide approach, including for rarer conditions.

The following table summarizes the performance of PrenatalSAFE® across the different categories of chromosomal abnormalities investigated, reporting the observed sensitivity and specificity values for each category.

Table 3 Performance of NIPT analysis for common aneuploidies, SCAs and other abnormalities in 71,883 pregnancies (Ref: Faieta M, Falcone R, Duca S, Corsetti E, Giannico R, Gigante L, Diano L, Calugi G, Spinella F, Pizzuti F. Test performance and clinical utility of expanded non-invasive prenatal testing: experience from 71,883 consecutive routine cases in a single center. Prenat Diagn. 2024 Jul;44(8):936–945. doi: 10.1002/pd.6580. Epub 2024 Apr 30. PMID: 38686956.)

Positives: 1011 Follow-up : 868	Trisomy 21	Trisomy 18	Trisomy 13	Common Trisomies
True positive	437	93	37	567
False positive	3	1	8	12
True negative	71368	71716	71765	71229
False negative	2	0	0	2
Sensitivity (95% CI)	**99.54%** (98.36% - 99.94%)	**100%** (96.11% - 100.00%)	**100%** (90.51% - 100.00%)	**99.65%** (98.74% - 99.96%)
Specificity (95% CI)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**99.99%** (99.98% - 100.00%)	**99.98%** (99.97% - 99.99%)
PPV (95% CI)	**99.32%** (98.02% - 99.78%)	**98.94%** (94.21% - 99.97%)	**82.22%** (67.95% - 92.00%)	**97.93%** (96.41% - 98.92%)
NPV (95% CI)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)

CI: Confidence intervals; positive cases without follow-up were excluded from the positive case count in Table 3 (n): T21 (49); T18 (14); T13 (10).

Table 4 Performance of NIPT for sex chromosome aneuploidies (SCAs) (Ref: Faieta M, Falcone R, Duca S, Corsetti E, Giannico R, Gigante L, Diano L, Calugi G, Spinella F, Pizzuti F. Test performance and clinical utility of expanded non-invasive prenatal testing: experience from 71,883 consecutive routine cases in a single center. Prenat Diagn. 2024 Jul;44(8):936–945. doi: 10.1002/pd.6580. Epub 2024 Apr 30. PMID: 38686956.)

SCA	X0	XXX	XXY	XYY	SCA
True positive	52	27	51	26	156
False positive	13	0	3	1	17
True negative	65706	65745	65718	65745	65598
False negative	1	0	0	0	1
Sensitivity (95% CI)	**98.11%** (89.93% - 99.95%)	**100%** (87.23% - 100.00%)	**100%** (93.02% - 100.00%)	**100%** (86.77% - 100.00%)	**99.36%** (96.50% - 99.98%)
Specificity (95% CI)	**99.98%** (99.97% - 99.99%)	**100%** (99.99%- 100.00%)	**99.99%** (99.99% - 100.00%)	**99.99%** (99.99% - 100.00%)	**99.97%** (99.96% - 99.99%)

PPV (95% CI)	**80%** (68.23% - 88.90%)	**100%** (87.23% - 100.00%)	**94.44%** (84.61% - 98.14%)	**96.3%** (81.03% - 99.91%)	**90.17%** (84.73% - 94.17%)
NPV (95% CI)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)

CI: confidence intervals; SCA: sex chromosome aneuploidies.
Positive cases without follow-up were excluded from the positive case count in Table 4 (n): X0 (18); XXX (6); XXY (7); XYY (5); SCA (36).

Table 5 Performance of NIPT analysis for rare autosomal aneuploidies, segmental abnormalities and microdeletions (Ref: Faieta M, Falcone R, Duca S, Corsetti E, Giannico R, Gigante L, Diano L, Calugi G, Spinella F, Pizzuti F. Test performance and clinical utility of expanded non-invasive prenatal testing: experience from 71,883 consecutive routine cases in a single center. Prenat Diagn. 2024 Jul;44(8):936–945. doi: 10.1002/pd.6580. Epub 2024 Apr 30. PMID: 38686956.)

Other anomalies	RAA	DEL/DUP ≥ 7 Mb	Microdeletions <7 Mb*
True positive	33	20	5
False positive	36	16	2
True negative	46623	46656	28743
False negative	0	0	1
Sensitivity (95% CI)	**99.99%** (89.42% - 100.00%)	**99.99%** (83.16% - 100.00%)	**83.33%** (35.88% - 99.58%)
Specificity (95% CI)	**99.92%** (99.89% - 99.95%)	**99.97%** (99.96%- 99.99%)	**99.99%** (99.99% - 100.00%)
PPV (95%CI)	**47.83%** (35.65% - 60.20%)	**55.56%** (38.10%- 72.06%)	**71.43%** (37.40% - 91.27%)
NPV (95%CI)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)

CI: confidence intervals; RAA: rare autosomal aneuploidies. Positive cases without follow-up were excluded from the positive case count in Table 5 (n): RAA (25); segmental abnormalities >7 Mb (7); microdeletions (2).
* Microdeletion syndromes investigated include: DiGeorge syndrome, Cri-du-Chat syndrome, Prader-Willi syndrome, Angelman syndrome, 1p36 deletion syndrome, Wolf-Hirschhorn syndrome, Jacobsen syndrome, Langer-Giedion syndrome, and Smith-Magenis syndrome.

Limitations of PrenatalSAFE®

PrenatalSAFE® is a **screening test** and not a diagnostic test: the results indicate a level of risk rather than absolute certainty, and a **residual risk of false-negative and false-positive results** always remains (<0.1%). A low risk result significantly reduces the probability of the investigated abnormalities but does not completely exclude it.

The main factors that may lead to **unexpected or discordant results** include confined placental mosaicism, the presence of a vanishing twin (resorbed co-twin), and maternal chromosomal abnormalities or conditions such as benign or malignant tumors, or organ transplantation.

PrenatalSAFE® is intended to provide information only on the chromosomes and abnormalities included in the selected level of analysis and reported in the results table; therefore, it **does not detect chromosomal abnormalities** that are **not included** in the **chosen** analysis **level**. PrenatalSAFE® is not able to detect abnormalities other than those described in the dedicated section of this document. By way of example, but not exhaustively,

the test does not detect chromosomal mosaicism, balanced chromosomal rearrangements, triploidy and polyploidy in general, uniparental disomy, congenital or genetic conditions not related to chromosomal abnormalities, structural malformations, or prenatal infections.

The ability of the test to detect an abnormality depends on factors such as fetal fraction and sequencing depth. In approximately 2–3% of cases, the test may yield an inconclusive result (for example due to low fetal fraction or other technical reasons).

Additional factors may influence the reliability of the result, including high maternal body weight, which may reduce the fetal fraction (FF), defined as the proportion of fetal DNA relative to total circulating cell-free DNA; previous blood transfusions or organ transplantation; and the presence of maternal conditions such as neoplasms, including those of benign significance (e.g. uterine fibroids).

Limitations in twin pregnancies or in the presence of a vanishing twin
In **dichorionic twin pregnancies**, the result cannot be attributed to a single fetus: a high-risk result may refer to one or both fetuses. In addition, analysis of **sex chromosome aneuploidies** and **microdeletions** is **not available** in this setting.

In **monochorionic twin pregnancies**, a high-risk result is considered to apply to both fetuses. In the presence of the Y chromosome, both fetuses are considered male; in the absence of the Y chromosome, both are considered female.

In any twin pregnancy, whether monochorionic or dichorionic, in the presence of a high-risk result, invasive prenatal diagnosis or postnatal **diagnostic testing** should be performed **on both fetuses/newborns**.

The test is not validated for pregnancies with more than two fetuses. In all twin pregnancies, confirmation by invasive prenatal diagnosis is recommended in the case of a high-risk result.

The presence of a vanishing twin may reduce the reliability of the screening, as DNA from the non-viable twin may persist in maternal circulation even after fetal demise. Since chromosomal abnormalities are a common cause of first-trimester pregnancy loss, this condition may increase the likelihood of false-positive results. In cases of vanishing twin, the optimal timing for performing PrenatalSAFE® may vary depending on ultrasound findings and on the gestational age at which the fetal loss occurred.

PrenatalSAFE® within the prenatal diagnostic pathway

PrenatalSAFE® is part of a broader prenatal diagnostic pathway, which includes ultrasound and biochemical assessments and, when indicated, invasive investigations.

First-trimester ultrasound remains essential to confirm pregnancy viability, accurate dating, the number of fetuses and chorionicity, as well as to identify any structural abnormalities.

Traditional **first-trimester screening** (ultrasound and biochemical tests) continues to represent an important step and, in some clinical protocols, NIPT is offered as a second-line test in cases of intermediate or high risk (the so-

called “contingent approach”). In other settings, PrenatalSAFE® may be offered to all pregnant women as a first-line screening test (the “universal approach”).

PrenatalSAFE® does not replace invasive prenatal diagnosis (chorionic villus sampling or amniocentesis), which remains indicated in cases of high-risk results or when ultrasound abnormalities or specific clinical risk factors are identified.

The integration of different diagnostic tools (ultrasound, biochemical screening, NIPT, and invasive diagnosis) allows for a more comprehensive prenatal diagnostic pathway, which can be tailored to the specific clinical needs of each pregnant woman.

Sample management

Samples are identified by means of an alphanumeric code and are stored for a period ranging from a minimum of 3 months to a maximum of 6 months after completion of the analysis. Any residual sample may be made available for further investigations, subject to evaluation of its suitability for the specific additional testing requested.

Informed consent and pre-test counselling

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

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

References

1. Faieta M, Falcone R, Duca S, et al. Test performance and clinical utility of expanded non-invasive prenatal test: experience on 71,883 unselected routine cases from one single center. Prenat Diagn. 2024;44(8):936–945. doi: 10.1002/pd.6580
2. **Società Italiana di Genetica Umana (SIGU).** Checklist per la consulenza pre-test NIPT. Rev.1, 2021.
3. **Ministero della Salute.** Screening del DNA fetale non invasivo (NIPT). Indicazioni e raccomandazioni per l’utilizzo in gravidanza fisiologica e in gravidanza a rischio.