Information Sheet for GeneScreen® Focus Test

What is GeneScreen® Focus

GeneScreen® Focus is a genetic test performed on the **biological parents** during an **ongoing pregnancy**, aimed at better clarifying the **couple's reproductive risk** for certain inherited genetic conditions included in the analyzed panel.

The test does not analyze fetal DNA and is **not a diagnostic test** for the fetus. Its purpose is to identify whether one or both parents are **carriers** of genetic variants associated with specific inherited conditions, thus contributing to the overall genetic assessment of the pregnancy.

GeneScreen® Focus within the prenatal pathway

GeneScreen® Focus is part of a **broader prenatal pathway** aimed at obtaining as comprehensive a genetic evaluation of the ongoing pregnancy as possible.

In particular, the test may be performed within the **PrenatalSAFE® Full Risk** pathway, which combines:

- assessment of **fetal chromosomal abnormalities** risk through **PrenatalSAFE®**
- assessment of risk for certain **fetal monogenic disorders** through **GeneSAFE®**
- assessment of **parental carrier** status through **GeneScreen® Focus**

In this context, **GeneScreen® Focus** contributes to a more precise definition of the **couple's reproductive risk** and represents **complementary information** to other non-invasive prenatal tests.

Who is it intended for and when to perform GeneScreen® Focus

GeneScreen® Focus can be requested **during pregnancy** to better define the couple's reproductive risk and is particularly indicated in:

- presence of a **family history** of genetic disorders
- **consanguinity**
- couples at their **first pregnancy**, as results remain informative for **future conceptions**
- when a **broader prenatal genetic evaluation** is desired (Full Risk pathway)

Applicable pregnancies

GeneScreen® Focus can only be performed in pregnancies with **homologous conception**, either **spontaneous** or through **assisted reproductive technique** (ART).

The test is **not indicated** in cases of **heterologous conception**, as proper interpretation requires that analyzed individuals correspond to the **biological parents**.

Which conditions can be identified by GeneScreen® Focus

GeneScreen® Focus is a **carrier screening test** aimed at identifying genetic variants associated with specific inherited diseases included in the panel.

The **list of genes and associated conditions** is reported in the table 1.

Tabella 1 List of genes and condition analysed in Genescreen Focus

Gene	Disorder	Inheritance*
ACADM	Medium-chain acyl-CoA dehydrogenase deficiency	AR
AGXT	Hyperoxaluria, primary, type 1	AR
ARSA	Arylsulfatase A deficiency	AR
ATP7B	Wilson disease	AR
BTD	Biotinidase deficiency	AR
CBS	Homocystinuria due to cystathionine beta-synthase	AR
CFTR	Cystic fibrosis	AR
DHCR7	Smith-Lemli-Opitz syndrome	AR
EMD	Emery-Dreifuss muscular dystrophy, type 1 X-linked	XL
FMR1	Fragile X syndrome	XL
GAA	Glycogen storage disease, type II	AR
GALC	Krabbe disease	AR
GALT	Galactosemia	AR
GBA	Gaucher Disease	AR
GJB1	Charcot-Marie-Tooth neuropathy, X-linked dominant, type 1	XL
GJB2	Deafness, autosomal recessive, type 1A; Deafness, digenic, GJB2/GJB6	AR, DD
GJB6	Deafness, autosomal recessive, type 1B; Deafness, digenic GJB2/GJB6	AR, DD
GLA	Fabry disease	XL
HADHA	Long-chain 3-hydroxyl-CoA dehydrogenase (LCHAD) deficiency; Mitochondrial trifunctional protein deficiency	AR
HBA1	Alpha-thalassemia	AR
HBA2	Alpha-thalassemia	AR
HBB	Beta-thalassemia, Sickle cell anemia and other HBB-related hemoglobinopathies	AR
HEXA	Tay-Sachs disease	AR
MEFV	Familial Mediterranean fever	AR
MMACHC	Combined methylmalonic acidemia and homocystinuria, cblC type	AR, DD
PAH	Phenylalanine Hydroxylase Deficiency	AR
PMM2	Congenital disorder of glycosylation, type 1A	AR
SERPINA1	Alpha-1 antitrypsin deficiency	AR
SLC26A2	SLC26A2-related disorders	AR
SMN1	Spinal muscular atrophy	AR

* XL: X-Linked; AR: autosomal recessive; DD: digenic inheritance. *XL conditions are evaluated only in females

Possible results of GeneScreen® Focus

GeneScreen® Focus may provide one of the following results:

No pathogenic variants detected - No pathogenic variants detected

Carrier - A pathogenic variant was identified in one gene. This generally indicates carrier status and usually has no clinical impact on the individual's health but must be interpreted at the couple level. If **both** partners are **carriers** of pathogenic variants in the **same autosomal recessive gene**, the couple has **an increased reproductive risk** for that condition. If the **female** partner is carrier of a pathogenic variant in X linkeda **X linked condition**, the couple has **an increased reproductive risk** for that condition.

Inconclusive - In some cases, the test may not provide a reliable or interpretable result due to technical or sample-related issues.

Implications when both partners are carriers

When both partners are carriers of pathogenic variants in the same autosomal recessive gene, each pregnancy carries:

- a 25% probability that the child is affected;
- a 50% probability that the child is a healthy carrier;
- a 25% probability that the child is unaffected.

This result **does not represent a diagnosis** but indicates a clinically significant reproductive risk that may require **further investigation**.

Possible confirmatory diagnostic options include:

- **chorionic villus sampling** (CVS), typically between the 11th and 13th weeks of gestation;
- **amniocentesis**, typically between the 16th and 18th weeks of gestation;
- **postnatal testing** on blood or buccal swab.

The most **appropriate follow up** should be discussed during **genetic counselling**.

Clinical significance for the current and future pregnancies

Since GeneScreen® Focus analyzes the genetic profile of the biological parents, the result is informative **not only for the ongoing pregnancy** but also for **future pregnancies** of the same couple.

However, interpretation remains subject to possible future updates in scientific knowledge and/or changes in the composition of the analyzed gene panel.

Method of analysis

GeneScreen® Focus is performed on biological samples collected from both partners and involves molecular genetic analysis of selected genes associated with inherited conditions.

The test allows the identification of **pathogenic and likely pathogenic** variants according to current international classification criteria.

Variants classified as benign or likely benign are not reported. Variants of uncertain significance (VUS) are generally not reported, except in specific situations where they may have clinical relevance.

For the analysis of the FMR1 gene (associated with **Fragile X syndrome**), a specific methodological approach is used, and testing is typically performed in the **female partner**.

Further technical details are reported in the **technical summary** of the final report.

Limitations of GeneScreen® Focus

GeneScreen® Focus is **a screening test** and not a diagnostic test: the results allow the evaluation of the couple's reproductive risk with respect to the genetic conditions included in the analyzed panel, but they do not provide absolute certainty, nor do they completely exclude the possibility of a genetic disorder in the conception.

The test is intended to provide information **exclusively on the genes and conditions included in the selected panel** and reported in the documentation supporting the report; therefore, it does not detect genetic conditions not included in the panel, nor other abnormalities that are not specifically investigated. A negative result **reduces** the **reproductive risk** for the investigated conditions, **but does not completely eliminate** it, since there may be variants that cannot be detected by the methodology used, variants located in regions not analyzed, or genetic conditions caused by genes not included in the test.

The analysis is aimed at identifying **pathogenic or likely pathogenic** variants in the genes examined. Benign or likely benign variants are generally not reported, nor are all variants of uncertain clinical significance, except where their interpretation is relevant in the couple's clinical and genetic context. As with any genetic test, the **interpretation** of variants is based on the **scientific knowledge available at the time** of analysis and may be **subject to revision over time** as new evidence becomes available.

GeneScreen® Focus assesses the carrier status of both partners and therefore allows an estimate of the couple's reproductive risk, but it **does not by itself establish whether the fetus is affected or unaffected** by a specific genetic condition. Where a result is consistent with a couple at increased reproductive risk, the clinical significance of the finding must be discussed in **genetic counselling**, and any prenatal or postnatal follow-up investigations must be evaluated in the context of the ongoing pregnancy.

Since the test is performed in the setting of a pregnancy conceived through **homologous conception**, correct interpretation of the result requires that the two analyzed samples actually belong to the **biological parents** of the conception. For this reason, GeneScreen® Focus is not indicated in cases of heterologous conception.

Some genetic conditions require a specific analytical approach. In particular, for the female partner, the **assessment** of **Fragile X syndrome** is performed **separately** by dedicated analysis of the FMR1 gene, because this condition is related to a molecular mechanism different from the sequence variants analyzed in the main carrier screening panel. Also in this case, the result contributes to the definition of reproductive risk but must be interpreted within the overall context of the couple and the family history.

The test may also present intrinsic technical limitations, related, for example, to the quality and quantity of the biological sample, the coverage of certain gene regions, or the complexity of specific genomic areas. In rare cases, these conditions may lead to an inconclusive result or require further analytical verification.

Finally, GeneScreen® Focus **does not replace genetic counselling**, which remains recommended both before and after the test, especially in the presence of a positive family history, consanguinity, previous adverse reproductive events, or results showing an increased risk for specific inherited conditions.

GeneScreen® within the prenatal diagnostic pathway

GeneScreen® Focus is part of a broader **prenatal diagnostic pathway**, which includes ultrasound and biochemical assessments, non-invasive genetic tests, and, when indicated, invasive investigations.

Unlike **PrenatalSAFE®** and **GeneSAFE®**, which analyze **circulating fetal DNA** to assess the risk of chromosomal abnormalities and certain fetal monogenic disorders, respectively, GeneScreen® Focus is a test performed on the biological parents and is aimed at better defining the couple's reproductive risk with respect to specific inherited genetic conditions.

For this reason, GeneScreen® Focus may represent a useful complement within the prenatal pathway, particularly in the presence of a family history of genetic conditions, consanguinity, previous adverse reproductive events, or when a broader genetic evaluation of the ongoing pregnancy is desired.

In the presence of a result indicating an increased reproductive risk, **the test does not replace invasive prenatal diagnosis** but may contribute to **guiding** the **most appropriate follow-up pathway**. In such cases, genetic

counselling is recommended in order to discuss the meaning of the result, its implications for the ongoing pregnancy, and the possible diagnostic options.

The integration of the different diagnostic tools (ultrasound, biochemical screening, **PrenatalSAFE®, GeneSAFE®, GeneScreen® Focus,** and, when indicated, invasive diagnosis) allows the construction of a more comprehensive prenatal diagnostic pathway, tailored to the specific clinical and reproductive needs of the couple.

Sample management

Samples are identified through an alphanumeric code and are stored for at least six 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

It is recommended that GeneScreen® Focus be performed following appropriate pre-test counselling with a professional experienced in medical genetics. During this consultation, the couple 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, patients may provide their written informed consent, which represents an integral part of the prenatal genetic evaluation pathway.