The information in this document relates to the CarrierTest Custom (IM) for Donors panel, which was prepared by GNTlabs by GENNET for Institut Marquès (Spain) and being performed from 1st of January 2026.

Limitations, Methodology and Considerations

Like any laboratory test, CarrierTest Custom (IM) for Donors has certain limitations. While it is a reliable screening method that provides valuable information on genetic carrier status, it cannot offer 100% certainty. The following considerations should be taken into account when interpreting the results.

General Considerations and Possible Sources of Uncertainty

The test result is valid assuming the sample belongs to the tested individual. CarrierTest Custom (IM) for Donors is performed under strict laboratory quality standards; however, as with any medical test, a very rare possibility of sample mix-up or technical issue during collection, labeling, or processing can never be completely excluded.

Additional Information on Residual Risk

The CarrierTest Custom (IM) for Donors is intended for healthy individuals (gamete donors) who show no signs of a genetic disorder. The aim of the test is to reduce the risk of the donor being an undetected carrier, not to eliminate the overall genetic risk entirely. The remaining (residual) risk of being a carrier after a negative CarrierTest Custom (IM) for Donors result depends on the structure of the specific gene, the laboratory method used, and the carrier frequency in the European population, which may differ for individuals of other ethnic origins (see Table 2).

If a mutation is detected in one partner/donor, the residual carrier risk of the other partner/donor is used to calculate the risk of the offspring being affected. The risk that both the recipient/partner and the donor are carriers of a mutation in the same gene is very low following a negative CarrierTest Custom (IM) result for both parties. For the recipient couple using this donor, the risk of having a child with an autosomal recessive disorder is therefore very low. The risks of diseases arising from new mutations in germ cells or multigenic mutations may not be detected by this test.

Technical and Analytical Aspects

CarrierTest Custom (IM) for Donors uses whole-exome sequencing (WES), which analyzes a virtual panel of the (primarily) coding regions of selected human DNA genes (refer to Table 2) with overlaps into introns up to 50 bp. This test is based on massive parallel sequencing technology using short reads (SBS sequencing, Illumina), which is primarily suitable for analyzing SNP and small InDel variants. The sequencing data for each sample is subject to quality control, which ensures that all evaluable samples will have a minimum of 55M paired-end reads (clusters) after the removal of optical and PCR duplicates. The technical parameters of this method do not guarantee 100% coverage of all target regions.

Detecting certain variants or gene parts may not be possible due to local sequence characteristics, high/low genomic complexity, or the presence of closely related pseudogenes. Variants in promoter or deep intronic regions (unless specified otherwise), repetitive expansions (trinucleotide, hexanucleotide, or other), structural variants like inversions and gene conversions, and low-level mosaic variants may not be detected by this technology.

With the exception of specified genes or regions (see Table 1), CarrierTest Custom (IM) for Donors does not analyze changes in the number of copies of genes or their parts (CNVs). The test is focused on germline mutations (mutations in germ cells). Somatic mutations are not examined. The test analyzes DNA and therefore does not investigate possible interactions between different genes or epigenetic factors.

For samples with lower quality (e.g., blood from patients with hematological disorders or highly degraded DNA), the quality of the NGS data may be reduced, which can lower the method's sensitivity for variant detection.

Variant Classification and Pathogenicity

CarrierTest Custom (IM) for Donors is a screening method that only detects selected pathogenic variants of classes 4/5 (pathogenic/likely pathogenic). Variant pathogenicity is evaluated based on current scientific and clinical knowledge (ClinVar/ClinSign databases) and may change over time. Should the classification of a detected variant change, GNTlabs will inform about this fact and offer an updated interpretation. The test cannot rule out mutations in other (uncovered, unanalyzed, or unevaluated) genes. GNTlabs, at its own discretion and in line with its commitment to the highest quality results, verifies NGS findings usi complementary methods such as Sanger sequencing, long-range PCR, fragment analysis, or MLPA and StripAssay methods.

Conclusion

CarrierTest Custom (IM) for Donors is an internally developed and validated diagnostic tool accredited according to the standard ČSN EN ISO 15189:2013. Despite meeting the highest scientific and analytical standards, a residual risk cannot be excluded. Consultation of the results with a qualified geneticist is beneficial, as they will consider the aforementioned limitations, as well as the family history, clinical picture, and all available information.

Suggestion

The results of CarrierTest Custom (IM) for Donors findings could be reviewed with a clinical geneticist, who may interpret the results for the patient and suggest possible treatment, monitoring, and preventive measures for the patient and their family.

Table 1: **Notes on the analysis of selected genes**

Gene	***Notes***
AR	The current testing method does not evaluate CAG trinucleotide repeat expansions in this gene.
CFTR	Single exon deletion/duplication analysis is limited to deletions of previously reported exons: 1, 2, 3, 11, 19, 20, 21.
DMD	Single exon deletion/duplication analysis is limited to regions repeatedly published in the UMD database (http://www.umd.be/DMD/W_DMD/).
F8	The current testing method does not include the detection of intron 1/22 inversions in the *F8* gene, which account for up to 45% of severe hemophilia A cases. This detection can be ordered as an additional test.
FMR1	The analysis is limited to determination of CGG expansions in the *FMR1* gene. The analysis was performed using the LabGscan™Fraxa PCR Kit (CE IVD certified). The method detects a CGG expansion in the FMR1 gene, but does not detect the expansion if there is a sequence change at the primer binding site. The method also fails to detect rare point mutations and deletions in the FMR1 gene that can cause X-chromosome fragility syndrome. This test does not detect methylation, deletions, or point mutations responsible for fragile X syndrome.
HBA1/2	This is an analysis of the most common alpha-thalassemia deletions in the *HBA1* and *HBA2* genes (types 3.7, 4.2, 20.5, FIL, MED) using the additional StripAssay method.
HBB	The test is optimized for the detection of small variants (SNVs and indels) in coding regions and adjacent intron-exon boundaries. Large deletions/duplications may not be reliably detected by this method due to high sequence homology and the presence of segmental duplications. For these types of variants, complementary testing using MLPA is recommended.
SMN1	The current testing method detects sequence variants in exon 7 and copy number variations in exons 7-8 of the SMN1 gene (NM_022874.2). Sequencing and deletion/duplication analysis are not performed on any other regions in this gene. Approximately 5% to 8% of the population has two copies of SMN1 on one chromosome and a deletion on the other, which is known as a [2+0] configuration (PubMed: 20301526). The current testing method cannot directly detect carriers with an SMN1 [2+0] configuration.

Table 2: **List of genes analyzed within CarrierTest Custom (IM) for Donors, associated diseases and residual risks**

Gene	Disorder	Ethnicity	Detection Rate (%)	Individual Carrier Risk (a priori)	Individual Residual Risk After Negative Result	Risk of Affected Fetus When One Partner is a Carrier and the Other Partner Has a Negative Result
ABCD1	Adrenoleukodystrophy, X-linked	General population (Females)	99%	~1 in 20000	Negligible	Negligible
AR	Androgen Insensitivity Syndrome (X-linked)	General population (Females)	99%	1 in 320	1 in 31900	1 in 127600
ATP7A	ATP7A-related copper transport disorders(incl. Menkes disease)	General population (Females)	99%	~1 in 17000	Negligible	Negligible
ATRX	Alpha-thalassemia X-linked intellectual disability	General population (Females)	99%	~1 in 15000	Negligible	Negligible

BTK	Agammaglobulinemia, X-linked	General population (Females)	99%	Negligible	Negligible	Negligible
CFTR	Cystic fibrosis	Non-Finnish European/White	98%	1 in 21	1 in 1000	1 in 4000
COL4A5	Alport syndrome	General population (Females)	99%	~1 in 5000	Negligible	Negligible
CYBB	Chronic granulomatous disease	General population	99%	Negligible	Negligible	Negligible
DKC1	Dyskeratosis congenita, X-linked	General population (Females)	99%	Negligible	Negligible	Negligible
DMD	Dystrophinopathies, including Duchenne and Becker	General population (Females)	99%	~1 in 3000	Negligible	Negligible
EDA	Hypohidrotic ectodermal dysplasia	General population (Females)	99%	~1 in 5000	Negligible	Negligible
EMD	Emery-Dreifuss muscular dystrophy	General population	99%	Negligible	Negligible	Negligible
F8	Hemophilia A (X-linked)	Non-Finnish European/White (Females)	80%	1 in 3 500	Negligible	Negligible
F9	Factor IX deficiency (hemophilia B)	General population (Females)	99%	~1 in 20000	Negligible	Negligible
G6PD	Glucose-6-phosphate dehydrogenase deficiency	General population (Females)	99%	1 in 150	1 in 14900	1 in 30000 (Male)
GJB1	Charcot-Marie-Tooth, X-linked 1	General population (Females)	90%	1 in 667	1 in 6660	1 in 26640
GJB2	Deafness and hearing loss, nonsyndromic	Non-Finnish European/White	99%	1 in 18	1 in 1700	1 in 6800
GLA	Fabry disease	General population (Females)	99%	~1 in 5000	Negligible	Negligible
HBA1/HBA2	alpha-thalassemia	Non-Finnish European/White	97%	1 in 20	1 in 630	1 in 2500
HBB	Beta-hemoglobinopathies, includes sickle cell disease	Non-Finnish European/White	99%	1 in 256	1 in 25500	1 in 102000
CHM	Choroideremia	General population	95%	<1 in 500	<1 in 9980	<1 in 39920
IDS	Mucopolysaccharidosis type II	General population	99%	Negligible	Negligible	Negligible
IL2RG	Severe combined Immunodeficiency (SCID), X-linked	General population (Females)	99%	~1 in 25000	Negligible	Negligible
MECP2	Rett syndrome (XL)	General population (Females)	99%	Negligible	Negligible	Negligible
MTM1	Myotubular myopathy	General population (Females)	99%	~1 in 25000	Negligible	Negligible
NR0B1	Congenital adrenal hypoplasia, X-linked	General population (Females)	99%	Negligible	Negligible	Negligible
OCRL	Dent disease	General population	99%	Negligible	Negligible	Negligible
OTC	Ornithine transcarbamylase deficiency	General population (Females)	99%	~1 in 20000	Negligible	Negligible
PDHA1	Pyruvate dehydrogenase deficiency	General population (Females)	99%	~1 in 10000	Negligible	Negligible
PRPS1	Arts syndrome (XL)	General population (Females)	98%	Negligible	Negligible	Negligible
RS1	Juvenile retinoschisis, X-linked	General population (Females)	99%	~1 in 12500	Negligible	Negligible
SLC6A8	Cerebral creatine deficiency syndromes	General population (Females)	99%	~1 in 5000	Negligible	Negligible
SMN1	Spinal muscular atrophy	General population	91%	1 in 54	1 in 590	1 in 2360
WAS	Wiskott-Aldrich syndrome	General population	99%	Negligible	Negligible	Negligible