Informed consent for the Carrier Genetic Testing (CGT)

DESCRIPTION, PURPOSE AND ADVANTAGES OF THE TESTS, INCLUDING GENETIC MATCHING

The main objective of the Carrier Genetic Test (CGT) is to identify individuals—including gamete donors—or couples who are at increased risk of having offspring affected by certain genetic disorders. This analysis enables the consideration of alternative reproductive options with a reduced risk of transmitting autosomal recessive or X-linked (in females) conditions included in the test panel.

Scientific studies estimate that most healthy people, on average, are carriers of 1 or 2 mutations which can, in some cases depending on the gene or mutation, lead to severe genetic disorders in offspring. In most cases, the carriers show no symptoms, nor do they have any known family medical history of any disorder.

This test aims to detect the presence of mutations in genes linked to autosomal recessive inheritance in a couple (and/or gamete donors) as well as mutations in X-linked genes in females (including female donors). If both gametes are carriers of mutations in the same autosomal recessive inheritance gene, there is a greater risk (25%) of having offspring with an inherited disorder. In females, if the analysis includes the study of X-linked genes (consult with prescribing doctor) and the results detect a mutation in these genes, it means a direct risk for future male offspring (1 in 2 males, or 50%) and counselling will be given on ways to prevent this risk. For potential female gamete donors, a positive result for X-linked genes will limit or prevent them from being accepted on gamete donation programmes.

The genetic matching test is a comparative analysis of carrier screening results. It identifies whether both individuals (including gamete donors) carry pathogenic variants in the same gene associated with an autosomal recessive disorder. If no mutations are found in the same gene, the risk of having an affected child with those conditions is considered low. However, if both individuals carry mutations in the same autosomal recessive gene, there is a 25% risk (1 in 4 pregnancies) of having an affected child, regardless of sex. Therefore, genetic matching results are critical for assessing the suitability of combining male and female gametes based on their associated reproductive genetic risk.

It is worth highlighting that although the genes under analysis present mostly recessive inheritance, some may also be linked to a dominant condition or phenotype. Genetic counselling for dominant phenotypes is outside the scope of this test.

This test is recommended for assisted reproductive treatments but is also clinically relevant for couples conceiving naturally.

PROCEDURES, RISKS AND LIMITATIONS OF THE CGT TEST AND GENETIC MATCHING

CGT TEST

To process the sample, the test request form must be completed correctly. If not, the analysis may be delayed until the required information is provided to the laboratory.

The test process includes:

1. Collection of blood and/or saliva sample.
2. Extraction of DNA from the biological sample.
3. NGS *(Next Generation Sequencing, short read type)* of the **[clinical or whole] exome,** including enrichment and library preparation of genetic areas of interest, (exons and adjacent regions).
4. Bioinformatic analysis of NGS data, which may be re-used for related tests (such as CGT Sync re-analyses or data upgrades).
5. Clinical interpretation of the variant(s) based on the standards and recommendations of professional colleges (Richards et al., 2015).
6. Occasionally, the analysis of certain genes may require complementary studies through other methods to study frequent mutations which cannot be detected using NGS technology. Information on these studies is included in the methodology section of the results report.
7. Results report, including pathogenic or likely pathogenic variants, **depending on the carrier test requested.**

Given the complexity of the genetic tests and the significant implications of the test results, the results obtained must be interpreted in conjunction with other clinical and family data, within the general context of a medical practice run by health professionals. The result reports are strictly confidential.

The results of the CGT test will be available within 20 working days. A small percentage of samples may be delayed due to unforeseeable causes. Should this occur, the corresponding clinic in charge will be notified.
Carrier tests are genetic screening tests typically performed on individuals without a personal or family history of the genetic disorders or diseases included in the test. The clinical sensitivity of a screening test may be lower than that of a diagnostic test, which is performed on individuals who are clinically affected. As a result, a negative screening test result does not completely rule out the possibility that the person is a carrier of a pathogenic variant. This is due to inherent limitations of the test, including technical, biological, and procedural factors.

The limitations of CGT carrier testing are outlined below:

a. Every CGT test only reports on the genes corresponding to the analysis requested and does not include others (genes listed on the results reports -list of analysed genes section- and webpage -see https://cgt-panels.igenomix.eu/). In this sense, the set of reported genes may differ depending on the type of variant. Essentially, part of the gene content will include information on SNV/indel variants (single-nucleotide variation) and CNVs (copy number variations), while another group of genes will include only SNV/indel variants. Therefore:
   i. Not all known monogenic diseases are considered (recessive and X-linked conditions).
   ii. Mitochondrial and multifactorial diseases are not included; dominant genes and digenic inheritance (two heterozygous mutations in two different genes) are neither evaluated, unless otherwise indicated. However, some recessive genes may also be linked to phenotypes with dominant inheritance. Therefore, a positive result in one of these genes (regarding potential personal risk) may require individualized genetic counselling.

b. The NGS technique has the following technical limitations:
   i. When reading (sequencing) DNA, some regions may have low or very low coverage, typically below 20 (<20x); the detection of variants in these positions may be limited or they may not be detected.
   ii. There are complex regions in DNA, such as tandem sequence or nucleotide repeats, that cannot be efficiently detected using the short-read NGS technology employed. A clear example of this is mutations associated with triplet or trinucleotide repeat expansions.
   iii. Regarding CNV-type changes (deletions and duplications of genes or parts of them), a validated algorithm is used to determine the most likely copy number for each region of interest. However, it is important to note:

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(1) The detection of CNVs is associated with their size; in general, sensitivity is higher the larger the deleted or duplicated gene fragment is. In addition, performance is better for deletions than for duplications.
(2) Although standardized and validated procedures are followed in the laboratory, sample processing—including the different sequencing steps—may generate artifacts that affect the ability to detect CNVs.

iv. Bioinformatic tools limitations: During the validation of NGS raw data the correct use of bioinformatic tools was confirmed. However, it's important to highlight some limitations:
(1) Different software tools used for bioinformatic analysis can produce slightly different results from the same data;
(2) The accuracy of how the sequencing data is matched to a reference genome can vary depending on the quality of that reference;
(3) The ability to identify the functions of genes and genetic changes is still limited, which can make it difficult to fully understand the biological meaning of the results
(4) Interpreting genetic variants is an evolving process that relies on information stored in databases and on advanced algorithms. Since these databases are updated at different times, there can be differences between the latest information available online and the versions stored in downloaded files, which are updated less frequently but used for automated analysis.
(5) Only pathogenic or likely pathogenic variants are reported according to the MANE Select transcript for each gene, or, if unavailable, the transcript with the highest biological relevance. Since other transcripts may exist for each gene, any variants located in exons not included in the MANE Select transcript will not be included in the analysis.

c. The CGT is very accurate, but as with all genetic tests, there are different factors which can influence the result, including genetic knowledge limitations. Therefore, a negative result reduces but does not eliminate the risk of having affected offspring due to:
i. The appearance of a genetic disorder caused by a de novo mutation in the gametes of one of the individuals analysed.
ii. It is estimated that there is a 3-5% risk among the general population of congenital defects caused by genetic factors (may include the previous point about de novo DNA changes) not detected by this test, and by non-genetic factors.
iii. The presence of mosaicism in the DNA may affect the analysis. These forms of mosaicism arise after the fusion of the parental gametes that gave rise to the individual being tested, and they may involve specific regions, entire tissues, or be confined to the germline. Bone marrow transplants can also result in mosaicism.
iv. The presence of low-frequency polymorphisms, low complexity regions, as well as pseudogenes and homopolymers which can give rise to false positives and negatives.
v. SMN1 gene copy number testing: it is possible the test finds a normal result due to a duplicated gene in one allele (copies in cis) with no copies in the other allele. This is known as silent carrier. The frequency of silent alleles varies depending on different populations and ethnic groups, but it is estimated for general population to represent between 3-5% of mutated alleles due to exon 7 deletion.
vi. The CYP21A2 gene analysis presents unique challenges due to its high sequence homology (~98%) with the pseudogene CYP21A1P, which leads to frequent gene rearrangements and complex mutations. These challenges can cause difficulties in distinguishing CYP21A2 from CYP21A1P, increasing the risk of misdiagnosis (false or inconclusive results).
vii. The evidence obtained (analytical results) are displayed in the reports delivered through an IT reporting system using automatic variant interpretation and diagnostic rules. This IT system counts on automated parts, but also parts that require review by the diagnostic team. In general, all validated processes ensure that all automated parts generate reports without incidences; however, the possible updates of the IT system, regardless of the different quality controls established, could lead to disruptions in the reporting process.
viii. Similar for all laboratory tests, there is a small chance that a result will be inaccurate for procedural reasons, an error during collection or with the sample label, an error in the processing, or the collection or interpretation of data.
ix. There may be other medical reasons that contraindicate the test and which should be researched by the doctor before proposing the test, such as the presence of congenital or acquired chimera, temporarily through blood transfusion, or permanently through bone marrow transplant. Congenital chimerism develops when two twin embryos become one; the likelihood of this happening is low or very low. Allogenic bone marrow transplants generate chimera or coexistence of the patient's own cells with others from the marrow donor, including the possibility of an exclusive donor cell colonisation. It is important to consider that these circumstances will lead to discrepant findings if the analysed sample comes from peripheral blood. Scientific literature outlines the possibility of working with a skin punch biopsy to avoid possible discrepancies in genetic findings when it comes to bone marrow transplants. However, this type of sample has not been validated for CGT analysis.

GENETIC MATCHING

Genetic matching uses the genetic information obtained in each of the carrier tests performed on the individuals referred to in the matching request form. This analysis will provide a report on the genetic reproductive risk (low or high) based on the findings of each individual carrier test (as described above).

The process for performing the match analysis is as follows:

1. Genetic matching consists of a direct assessment of the results obtained in each carrier test report. In certain cases, and only for samples taken in Igenomix, the **genetic data stored in Igenomix will be updated in the form of sequencing reads** prior to the match analysis. This update generally does not require a biological sample or additional sequencing, except if after the update, and prior to the match analysis, the sequencing results are incomplete (this is estimated in a very low number of cases, less than 1%). Therefore, occasionally, depending on the genes analysed in each test, the match analysis may require a new biological sample to complete the genetic information.
2. In females, the **update of stored sequencing reads,** and subsequent analysis **will only include autosomal recessive genes.**
3. A reduced genetic reproductive risk or **favourable match** result means that the **risk of having offspring affected** by any of the disorders or diseases included in the carrier tests **has decreased but is not zero** due to the intrinsic features of the test and its limitations (see limitations below, as well as in the relevant sections of the respective reports).
4. An increased genetic reproductive risk or **unfavourable match** result means that **there is a high risk** — compared to the general population — of having **offspring affected** by any of the disorders or diseases analysed. In couples using their own gametes, the result will be accompanied by genetic counselling and possible preventive measures. In cases where the match analysis is for donor assignment, an unfavourable result implies a recommendation to find another donor or the adoption of preventive measures.

On the other side, regarding the **genetic matching analysis, certain limitations may apply.** For any recessive gene included in both tests (male and female gametes), the analysis coverage or mutations analysed in each of the tests could be qualitatively and/or quantitatively different; in addition, while it is easy to know if a given gene has been analysed in both tests, it is not easy, or always possible to determine if both tests have analysed the same mutations. These tests differences can occur when comparing results from a given panel/test of one laboratory -including Igenomix tests with different versions of the same product- but especially when comparing results from tests produced by different laboratories. Due to these possible differences, it could be the case that an individual is a carrier of a mutation not tested for in the other test. Generally, this does not prevent the match analysis from being carried out if certain rules are followed, as it does not usually entail a very high clinical risk. Exceptions to the above would be (i) if it is a gene with a high rate of carriers (CFTR, SMN1, etc.); (ii) if it is a frequent mutation; (iii) in relation to the above, if the people belong to a certain population, ethnic group, etc.; (iv) if there is consanguinity between the couple. In this sense, Igenomix is not responsible or liable for the results obtained by means of a test carried out in an external laboratory, or for any consequences arising from using said data to perform the match, should the data prove to be erroneous.

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DATA PRIVACY

Your privacy is a priority for the Igenomix Group. Your identity and all data referring to your personal information will be confidential and only Igenomix personnel will be permitted access to this information, along with the relevant authorities when required by the laws of the applicable jurisdiction. Below are listed the different purposes for which your personal data will be processed either by the Clinic or the healthcare provider, or by Igenomix:

Processing of your personal data for the purpose of carrying out the genetic test.

For this purpose, Igenomix Spain Lab S.L. ("Igenomix") is processing your personal data exclusively on behalf of your clinician/healthcare provider. Your clinician/healthcare provider is the data controller and Igenomix is the data processor. This entails that for this purpose, Igenomix will only process your personal data following the instructions and directions of your clinician/health care provider.

Hence, the purpose and means for the processing of your personal data to carry out the test is mainly determined by your clinician/healthcare provider, who will have provided you with all relevant privacy information regarding the processing of your personal data in line with the applicable regulations.

Therefore, if you have any queries regarding the processing of your personal data for this purpose, you may contact the data controller (your clinician/healthcare provider) at the address it has provided you in its own privacy policy. If you need to contact Igenomix, you can reach out to our Data Protection Officer through the following e-mail address privacy@igenomix.com or with postal mail to the address Igenomix Spain Lab S.L Edificios Europark, Parque Tecnológico, Ronda de Narcís Monturiol, nº11, B, 46980 Paterna, Valencia
The sample will be analysed by Igenomix or an associated group selected by Igenomix on an international level. Igenomix reserves the right to carry out part or all of the analyses included in the test through third party laboratories certified with recognised international quality standards, and with the same level of data protection security measures. Any results obtained in this way will be inspected by Igenomix and this circumstance will be indicated in the final report issued.

Use of Pseudonymized Information for Continuous Improvement, Internal Training and Knowledge Development (Not Biomedical Research)

In accordance with applicable international standards (ISO 15189, CAP, CLIA) and national legislation, we are required to carry out ongoing validations, quality controls, internal audits, and staff training. These activities are essential to ensure the accuracy, reliability, and safety of the services provided by Igenomix.

As part of this, test results generated from Igenomix's marketed services may be used in a key-coded (pseudonymized) form, meaning they cannot be directly linked to your identity, and access to identifying information is strictly restricted. The data may be used internally for: (1) Continuous Improvement; Optimise protocols, techniques and tools, ensuring increasingly effective and safe care. (2) Team Training; To help our staff maintain a high level of competence using real but de-identified case data (3) Internal Knowledge Development; To deepen the understanding of genetic and reproductive processes, to support learning and refinement of our laboratory systems and practices.4) Regulatory Post-Marketing Surveillance – to conduct retrospective statistical analyses required by the EU In-Vitro Diagnostic Regulation (IVDR) to confirm the ongoing safety and performance of our tests. Findings may be disclosed to competent authorities and to the scientific community only in aggregated or anonymised form (e.g. conference presentations or peer-reviewed publications).

Note: These activities are not considered biomedical or scientific research. The use of your data is strictly for internal operational purposes, such as quality assurance, training, and process improvement, and does not involve the creation of new generalizable scientific knowledge, publication of research results, or use of your sample/data in external studies.

These internal uses in no way compromise the confidentiality or integrity of your personal information. Your identity cannot be accessed by the staff conducting these activities, and all processing is subject to strict safeguards.

Sample storage

Samples collected as part of Igenomix services will be stored temporarily in accordance with applicable laws and standards (e.g., ISO 15189). Samples are kept under controlled conditions to maintain suitability for testing.

Samples are retained only for as long as necessary for the intended purpose after which it will be securely destroyed or anonymized in a manner that protects your privacy.

Exceptions to this process shall only be made where longer retention is legally required or explicitly authorized by the individual, with their informed consent.

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HAVING READ AND UNDERSTOOD THE FOREGOING, I AM AWARE OF:

The indication, procedure, success rate, risks and complications of the proposed treatment, as well as the financial cost of said test(s).
The fact that medical staff are at my disposal to expand on any aspect of the information that is not sufficiently clear to me.

I understand the explanations given to me in clear and simple language, and the doctor who saw me allowed me to make comments, clarifying any issues I raised and informing me that I may freely withdraw my consent at any time.

I am satisfied with the information received and I freely consent to giving a blood/saliva sample at the Centre/Clinic which I have attended. I also consent to the sample being sent to Igenomix (Vitrolife Group) facilities for the purpose of carrying out the aforementioned test(s).

The CGT result is based on the gene panel version referred to in the results report.

In any event, I declare that I have received suitable genetic counselling from qualified personnel **(DOCTOR/CLINIC/GENETIC COUNSELLOR)** who have informed me about the importance of the test, including possible alternatives that I could choose in view of the results thereof, and who are available to me for any questions or additional genetic counselling that I may require once the results of the CGT test are known. I also accept that the results of the test(s) may be passed on to my doctor, so that he or she can advise me correspondingly on the suitable treatment to follow.

I am aware that I may withdraw this consent at any time. However, if withdrawal occurs after the CGT test has been completed, IGENOMIX may use my genetic data within their information systems only if all identifying information is removed and the data are used solely for research purposes.

AUTHORIZATION AND RESULTS OPTIONS, INCLUDING UPDATING DATA: (Choose the correct option)

Request for CGT test (for all individuals)

☐ **I do not wish to receive information:** I do not wish to have access to the results of the carrier test (indicate with regards to whom). I understand that the matching test may establish that there is a high genetic risk of having affected offspring and in this case, the medical staff will take appropriate steps.

☐ Individual 1 ☐ Individual 2

Request for CGT test on donor (updating of CGT results after data update for use in matching test; only donors)
I am aware that my DNA will be sequenced for a greater gene content than those initially included in the CGT test given that this may be necessary for gene matching purposes. I am aware that if I require further genetic information, I must contact the Igenomix Support Department directly for a new CGT results report. I accept and acknowledge that these results do not rule out the risk of transferring hereditary disorders to my offspring.

SIGNATURES

Signature of the Individual 1 or Legal Representative	Date
Name of Individual 1 or Legal Representative	Relationship of the legal representative with the patient 1 (Complete only if necessary)
Signature of the Individual 2 or Legal Representative	Date
Name of Individual 2 or Legal Representative	Relationship of the legal representative with the patient 2 (Complete only if necessary)

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