INFORMED CONSENT FOR PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)/STRUCTURAL REARRANGEMENTS (PGT-SR) AND FOR MITOSCORE

DESCRIPTION, PURPOSE AND ADVANTAGES OF PERFORMING THE ANALYSIS

Preimplantation Genetic Testing for Aneuploidy (PGT-A) is used in combination with in vitro fertilisation (IVF) treatment to detect chromosome abnormalities in embryos before their transfer. Chromosome abnormalities are often seen in embryos and, if transferred, frequently result in miscarriage and failed implantation, and may result in the birth of a child with a chromosome abnormality. The information obtained from PGT-A helps clinicians and patients decide which embryos to transfer in IVF treatment.

Every cell in the body has chromosomes, which are organised structures containing DNA and proteins. The information contained within the chromosomes is necessary for the body to develop and function. There are 24 different types of chromosomes in humans, numbered from 1 to 22, plus the sex chromosomes X and Y. Most human cells contain a total of 46 chromosomes: two copies each of chromosomes 1-22 and a pair of sex chromosomes (XX for a biological female and XY for a biological male). The sperm and the egg must have 23 chromosomes each to produce an embryo with the expected number of chromosomes; when a sperm fertilises an egg, the resulting embryo has 46 chromosomes in total.

Any biological female can produce chromosomally abnormal eggs and any biological male can produce chromosomally abnormal sperm. When fertilisation occurs, this results in an embryo with a chromosome abnormality. For example, if an egg with 24 chromosomes is fertilised by a sperm with 23 chromosomes, the resulting embryo has 47 chromosomes (one extra chromosome). A common example of a chromosome abnormality is Down syndrome, which is caused by the presence of three copies of chromosome 21 instead of two. Embryo aneuploidy (extra or missing chromosome material) may develop as a result of an abnormal egg (the risk of aneuploidy increases with maternal age), an abnormal sperm (more likely in men with severe male factor infertility), a biological parent carrying a balanced structural rearrangement of chromosomes (i.e. the expected amount of chromosome material is present but is arranged in an unusual way), or an error in cell division early on in the embryo's development..

Most chromosomally abnormal embryos are not distinguishable from normal embryos when assessed using a microscope in the IVF clinic. Therefore, assessment of embryo morphology cannot be used to detect the presence of chromosome abnormalities.

PGT-A looks at the chromosomes present in cells obtained via embryo biopsy. PGT-A detects extra or missing copies of the 24 chromosomes and large chromosome imbalances, and embryos with chromosome abnormalities are not recommended for transfer. The main benefits of PGT-A include reducing the risk of miscarriage, increasing the implantation rate, and reducing the chance of having a baby with a chromosome abnormality.. The type of chromosome abnormalities identified by PGT-A can be detected via invasive tests such as chorionic villus sampling (CVS) and amniocentesis. However, these procedures involve a risk of miscarriage and cannot be performed until after a pregnancy is achieved.

In addition, the MitoScore test can be performed, which measures the mitochondrial DNA (mtDNA) content of cells obtained via embryo biopsy to identify which of the patient's chromosomally normal (euploid) embryos might have a greater chance of implantation. Some studies have shown that an increase in the mtDNA content in euploid embryos is associated with a lower implantation potential and can be indicative of a reduced energy supply during the maturation of embryos. MitoScore is a value which shows the standard amount of mtDNA in euploid embryos and indicates the total amount of mtDNA in the sample (Diez-Juan A, Rubio C et al. 2015). Selecting chromosomally normal embryos with a lower MitoScore value can further increase the likelihood of implantation.

PROCEDURES, RISKS AND LIMITATIONS

The PGT-A process consists of several phases. The first three phases are carried out at your IVF clinic: in vitro fertilisation, embryo biopsy and cell preparation. The biopsied cells are then transferred to Igenomix for subsequent analysis.

In vitro fertilisation:

A PGT-A cycle needs an embryo cell or cells to analyse. Therefore, an IVF cycle is required regardless of fertility history. Your IVF clinic will advise you on this process and may require additional consent. An intracytoplasmic sperm injection (ICSI) is recommended as a method of fertilisation. It is also recommended that you abstain from sexual intercourse for at least two weeks before the egg retrieval and in the time leading up to the pregnancy test. Sperm can survive for several days in the body of a female and not all the eggs are removed during the egg retrieval process. A spontaneous pregnancy may lead to an incorrect diagnosis.

Embryo biopsy, cell preparation and transport:

The embryo (trophectoderm) biopsy can be carried out on day 5, 6 or 7 of embryonic development when the embryo is at the blastocyst stage. Several cells are removed from each blastocyst for PGT-A. After the biopsy, the embryos will remain at your IVF clinic. Depending on the stage of the cycle and your clinician's recommendations, the embryos may be frozen after the biopsy and before the results are received. This process is known as vitrification of embryos.

After performing the biopsy, the cells obtained are cleaned to eliminate any potential source of contamination and are transferred to a small tube supplied by Igenomix. These tubes containing the cells are then sent by courier to the laboratory, always under the strict temperature control and security conditions required by Igenomix. Depending on the location of the IVF clinic, the samples will arrive on the same or following day.

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Analysis and reporting of the results:

To process the sample, the test request form will need to be correctly filled out, or the analysis may be suspended until the information required has been provided to the laboratory.

Once the laboratory receives the samples, the genetic content (DNA) of the cells is amplified to increase the amount of genetic material present for evaluation. This sample is then prepared and analysed using next generation sequencing (NGS). If the sample contains extra or missing genetic material for one chromosome (i.e. an aneuploidy for one chromosome), the embryo is classified as 'aneuploid'; if aneuploidy is detected for two to five chromosomes, the embryo is classified as 'complex abnormal'; embryos with aneuploidy in more than five chromosomes are classified as 'chaotic abnormal'. Aneuploid, complex abnormal and chaotic abnormal embryos are not recommended for transfer. If aneuploidy is detected in some but not all of the cells tested, the embryo may be classified as 'mosaic aneuploid', depending on the proportion of aneuploid cells and the reporting preferences of your clinic; please see the section below entitled 'Mosaicism' for further details. The results are sent directly to your IVF clinic, therefore, you and your clinician can decide which embryo(s) to transfer based on this information.

The MitoScore test can be performed on the DNA obtained from the same embryo biopsy to identify which embryos may have a greater chance of achieving pregnancy. The amount of mitochondrial DNA (MitoScore) is measured using NGS.

Given the complexity of the genetic tests and the significant implications of the results, the results must be interpreted in conjunction with other clinical data by a suitably qualified healthcare professional. The results are strictly confidential.

The test results will be available within 10 working days following receipt of the sample at Igenomix for a deferred cycle. A small percentage of samples may be delayed due to unforeseeable circumstances. Should this occur, your clinic will be notified.

PGT-A cannot guarantee a healthy pregnancy nor eliminate the risk of failed implantation, miscarriage, or the birth of a child with genetic or non-genetic abnormalities. The main risks and limitations associated with PGT-A and MitoScore are:

1. **Risks due to the biopsy:**

It is possible that the implantation capacity of a chromosomally normal biopsied embryo is slightly reduced compared to a chromosomally normal embryo which has not been biopsied. However, there is evidence to suggest that the preferential selection of chromosomally normal embryos more than offsets any potential adverse effects of the biopsy and, in general, the implantation rate will be greater if the technique allows for the analysis of all the chromosomes.

It is possible that the embryo may become damaged during the biopsy and will stop developing or not be suitable for transfer. However, when handled by skilled embryologists, the risk of damage to the embryo is very low. Igenomix is not responsible for any potential damage to the embryo.

2. **Preparation of cells:**

Once the cells are removed from the embryo, they are transferred to a small tube. The cells may not be successfully transferred to the tube, in which case there would be no cellular material to perform genetic testing on. It is also possible that the cellular material may be impaired (of poor quality), in which case it would not amplify successfully. In both of these scenarios, PGT-A results would not be obtained from this particular embryo. Igenomix is not responsible in the event that cells are not present in the tube or the genetic material is of poor quality.

3. **Transport:**

The cells are sent via courier to Igenomix for analysis. Any unexpected events during transport may delay reception of the sample and, on rare occasions, cause damage to the cells. While unlikely, it is also possible that the sample may be lost. Igenomix is not responsible for any damage to or loss of a sample during transport.

4. **Limits in detection:**

PGT-A is a test designed to detect whole chromosome aneuploidies and it may also detect partial aneuploidies such as deletions, duplications and unbalanced structural rearrangements, depending on the segment size of the affected chromosome. PGT-A cannot detect gains or losses in chromosome segments smaller than 10 Mb (Megabases). These abnormalities are rare.

5. **Test limitations of PGT-A**

Accuracy: The accuracy of this test is greater than 98%.

Balanced structural rearrangements: PGT-A cannot detect balanced structural rearrangements (such as balanced translocations and inversions). In other words, abnormalities in the structure of the chromosomes which do not result in gains or losses of chromosome material will not be detected by PGT-A.

Single-gene disorders: Not all genetic abnormalities are due to chromosome abnormalities. Some genetic conditions may be caused by alterations in individual genes, such as cystic fibrosis, sickle cell anaemia, and haemophilia. PGT-A will not detect alterations within single genes; if there is a known family history of a single-gene disorder, targeted genetic testing may be appropriate and should be discussed with your clinician.

Uniparental disomy (UPD): Uniparental disomy – where both copies of a chromosome pair come from one parent – cannot be detected using PGT-A. Depending on the chromosome involved, UPD may result in health and/or developmental problems.

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Ploidy: PGT-A cannot detect haploidy, triploidy or tetraploidy – where an embryo has one, three, or four sets, respectively, of each chromosome instead of two. These types of chromosome abnormalities lead to miscarriage and/or partial molar pregnancies; their incidence in human preimplantation embryos is less than 1%.

The risk of birth defects (such as heart defects) in the general population is between 3-5% and may be due to genetic or non-genetic causes, which may not necessarily be due to an alteration in the number of chromosomes. Some conditions such as autism, schizophrenia, and diabetes, are multifactorial, meaning that they occur due to a combination of genetic and environmental factors. Currently, studies on embryos (or on foetuses during pregnancy) are not possible for the majority of these conditions as the exact cause is often unknown.

Given the above limitations, ultrasound monitoring is recommended if a pregnancy is achieved.

6. Mosaicism:

Mosaicism is defined as the presence of more than one type of chromosomally distinct cell line in an embryo. Mosaicism occurs randomly and spontaneously during an embryo's development. During the process of cell division, the chromosomes may not be distributed equally, giving rise to cells with an abnormal number of chromosomes; some cells may have too many chromosomes while others have too few. The impact of mosaicism on achieving and maintaining a pregnancy, and on the likelihood of having a healthy child, may depend upon the particular chromosome that is involved and the proportion of cells biopsied that have an abnormal number of chromosomes. For example, embryos with a chromosome abnormality in 30-50% of the biopsied cells are classified as 'low mosaic aneuploid' and data suggests they have a similar miscarriage risk and a similar chance of leading to a healthy live birth as normal (euploid) embryos. There is variation among IVF clinics with regards to their preferences for reporting mosaicism; for example, some clinics choose to report 'low mosaic aneuploid' results as euploid. Your IVF clinic can provide more information regarding their policy on the reporting of mosaic aneuploid embryos. Mosaic aneuploid embryos may be considered for transfer depending on the level of mosaicism and the policy of your IVF clinic.

If mosaicism is not detected in the cells analysed using PGT-A, this does not rule out the presence of mosaicism in the whole embryo. An embryo reported as normal may have chromosome abnormalities in other cells that were not biopsied.

7. Error in diagnosis:

PGT-A has a 1-2% chance of misdiagnosis. One possibility is a 'false positive'; this means that normal embryos are diagnosed as abnormal. Conversely, there is the possibility of a 'false negative', meaning that abnormal embryos are diagnosed as normal. False negatives may be due to the presence of mosaicism, as explained above, as the analysed biopsy cells may not always be representative of the whole embryo.

The embryo biopsy and all laboratory procedures are carried out under sterile conditions to avoid contamination or the presence of genetic material which does not come from the embryo and may lead to incorrect results. However, there is a very small possibility that contamination is introduced to the biopsy sample.

8. No diagnosis due to the absence of DNA:

It may not be possible to obtain results from an embryo biopsy; the likelihood of this is less than 4%. The most common reasons for this are the absence of cells in the test tube or poor-quality genetic material (common in cells damaged during the biopsy process). Some couples choose to transfer embryos with a result of 'no DNA detected'; when transferring these embryos, the benefits associated with PGT-A do not apply.

9. Non-informative results:

When the quality of a sample is insufficient, the statistical model used to determine the number of chromosomes does not allow for a conclusive result. In this instance, a 'non-informative' result will be reported. Some couples choose to transfer embryos with a 'non-informative result'; when transferring these embryos, the benefits associated with PGT-A do not apply.

10. Absence of embryos for transfer:

In some cases, all the embryos tested may be reported as abnormal, and no embryos would be recommended for transfer.

11. ICSI:

For embryos undergoing PGT-A, the recommended laboratory technique for the fertilisation of eggs is ICSI. If ICSI is not performed, the risk of an incorrect result increases due to possible contamination of the biopsy sample with sperm or granulosa cells adhering to the external surface of the embryo.

12. MitoScore:

High MitoScore values could indicate that the implantation potential of the embryo is low, but do not suggest abnormalities in the embryo. MitoScore does not provide information on the health of the embryo, and all embryos diagnosed as chromosomally normal are considered potential candidates for transfer, regardless of their MitoScore value.

ADDITIONAL INFORMATION CONCERNING PGT-SR

DESCRIPTION, PURPOSE AND ADVANTAGES OF PERFORMING THE ANALYSIS

Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR) is offered to patients who, before IVF treatment, find out that one of the reproductive partners is a carrier of a balanced chromosomal rearrangement, such as a translocation or inversion. The

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information regarding aneuploidies described for PGT-A also applies for PGT-SR (please see above sections), with the following modifications:

Translocations and inversions are two types of structural chromosome rearrangements:

- Translocations can occur when a piece of one chromosome swaps places with a piece of another chromosome ('reciprocal translocation'), or two chromosomes become attached ('Robertsonian translocation').
- An individual with an inversion has a segment of a chromosome which is reversed end-to-end.

Carriers of balanced structural rearrangements do not tend to have health problems related to the rearrangement, as there is not a gain or loss of chromosome material (i.e. the expected amount of chromosome material is present, it is just arranged in a different way). In some cases, these individuals may have difficulty conceiving.

A balanced chromosomal rearrangement may be suspected in cases where there is unexplained male infertility (low sperm count), implantation failure, recurrent miscarriages, and/or a family history of miscarriage or children with health problems, developmental delay, and/or learning disabilities.

In carriers of balanced structural rearrangements, there is a risk of producing eggs and sperm with unbalanced forms of the rearrangement (with extra or missing chromosome material). If the resulting embryos are transferred, this may lead to implantation failure, miscarriage, or the birth of children with health, developmental, and/or learning problems. If children inherit the balanced structural rearrangement, they should not have health problems, just like their carrier parent.

The identification of embryos with an unbalanced structural rearrangement may help patients and clinicians to decide which embryos to transfer.

PROCEDURES, RISKS AND LIMITATIONS

The PGT-SR process uses NGS and will be carried out in a similar manner to PGT-A (see previous sections), using the same biological sample. For the detection of chromosome imbalances, an optimised NGS platform is used to detect imbalances greater than 6 Mb, and fluorescence in situ hybridisation (FISH) technology is used – on biopsy samples from day-3 embryos – for imbalances smaller than 6 Mb, wherever possible. If using FISH technology, it is necessary to carry out an additional blood test on the carrier of the structural rearrangement.

Before planning a PGT-SR cycle, a karyotype report for the carrier of the balanced structural rearrangement must be provided, which will be reviewed by Igenomix staff who will then advise on the appropriate protocol and/or request additional tests if necessary.

The risks and limitations described above for PGT-A also apply to PGT-SR. There are, however, additional specific limitations of PGT-SR outlined below:

1. Chromosomal imbalances below 6 Mb cannot be detected with PGT-SR using NGS.
2. PGT-SR using FISH on blastomere cells taken from day-3 embryos will be done at our headquarters laboratory in Spain and has specific limitations, including:
    a. (i) It cannot detect gains or losses of chromosomes other than those involved in the structural rearrangement under study. If required, an additional biopsy from the developing embryo at day 5 may be requested for general aneuploidy screening (PGT-A) using NGS.
    b. (ii) Inconclusive results in the case of poor-quality nuclei fixed on a slide.
3. PGT-SR cannot differentiate between embryos without a structural rearrangement and embryos with a balanced structural rearrangement (same as the carrier parent).

The turnaround time for PGT-SR is 10 working days, the same as PGT-A.

DATA PRIVACY, STORAGE AND RESEARCH USE OF SAMPLES

Your privacy is a priority for the Igenomix Group ("**Igenomix**"). 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. You can find further information on the Igenomix Privacy Policy, along with your rights, at www.igenomix.co.uk, or this information may be provided to you upon request by contacting privacy@igenomix.com.

We would like to inform you that your personal data will only be processed to: (1) fulfil the obligations arising from the provision of the services contracted by you; (2) check and guarantee the quality of the services provided (internal audits, quality controls, laboratory validation studies); (3) for educational purposes, provided that it remains anonymous throughout and you cannot be identified during the analysis of the data; (4) for research purposes, scientific publications and presentations, provided that it remains anonymous throughout and you cannot be identified during the analysis of the data; (5) personally answer any questions or suggestions made by you during the process and to monitor the proper performance and resolution of the test, including the indefinite retention of your data, except where local laws of the applicable jurisdiction state otherwise; and (6) contact you in the future to request an evaluation of the services received, send commercial communications (including 'cross-selling' and 'upselling') from associated companies, and invite you to participate in market research and the development of new products.

You declare that you understand and accept that you will not obtain, either now or in the future, any economic benefit for any research carried out, and that there is no intention to compensate you for the products developed from any research.

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

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quality standards, or failing this, they will be periodically assessed by Igenomix. Any results obtained in this way will be inspected by Igenomix and this will be indicated in the final report.

In accordance with the laws on the Protection of Personal Data[i], the requesting party must have the patient's consent to perform the diagnostic tests requested and to process their data. You may, at any time, exercise your rights regarding access, rectification, opposition, erasure, automated decisions, restriction, portability, by contacting privacy@igenomix.com, providing proof of your identity.

HAVING READ AND UNDERSTOOD THE INFORMATION ABOVE, I (THE PATIENT) CONFIRM THAT:

I have been informed about the indication, procedure, success rate, risks and complications of the proposed treatment, as well as the financial cost of said test(s).

I understand that suitably qualified healthcare professionals are available to expand on any aspect of the information that is not sufficiently clear to me.

I have received appropriate counselling from a qualified person such as a physician or genetic counsellor. The explanations have been given to me in clear and simple language, and the clinician who consented me allowed me to make comments, clarified any issues I raised, and informed me that I may freely withdraw my consent at any time.

I am satisfied with the information received and I freely consent to an embryo biopsy being performed (PGT-A or PGT-SR) and to a blood sample being taken if required (PGT-SR), for the purpose of sending these samples to Igenomix facilities so that the analysis can be performed.

I understand and accept that the results of the test(s) will be sent to my referring clinician, so that they can advise me regarding a suitable treatment plan.

Patient consent

By signing this requisition form, I voluntarily request that Igenomix carries out the test(s) indicated above. I have read and received a copy of the informed consent included in the previous pages. The risks, benefits and limitations of the testing have been explained to me.

Patient's full name_____

Patient's signature_____ Date: _____/_____/_____

Partner consent (where applicable)

By signing this requisition form, I voluntarily request that Igenomix carries out the test(s) indicated above. I have read and received a copy of the informed consent included in the previous pages. The risks, benefits and limitations of the testing have been explained to me.

Partner's full name_____

Partner's signature_____ Date: _____/_____/_____

[i] **For non-US patients:** Customers residing outside the United States under certain jurisdictions may at any time request to have their personal information deleted from our active databases, subject to the applicable laws and regulations in each jurisdiction. Although we can delete your personal information from our active databases, part or all of your personal information may remain stored in back-up files for the purpose of complying with legal, regulatory or other requirements. Information that has already been coded and/or anonymised may not be recoverable or traceable for destruction, deletion or modification. If you wish to have your personal information removed from our active databases, please contact us at privacy@igenomix.com.

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