# **Instruction and Informed Consent for Couples for Pre-Implantation Genetic Testing for Monogenic Diseases (PGT-M)**

Dear clients,

Please read these instructions carefully and conscientiously in line with your decision to undergo pre-implantation genetic testing for monogenic diseases in embryos as part of your IVF cycle. Then sign this document before a witness (an employee of the IVF centre where you are receiving treatment) as an expression of your agreement with the information herein.

The team of the clinic for assisted reproduction wishes you much success during your treatment.

Name and surname of the female partner: ………………………….……………………………………………….

Personal Identification Number: .………………………………………………………………………..

Name and surname of the male partner: …………………………………………………………………………

Personal Identification Number: .………………….………………………………………………………

Reason for testing (name of monogenic disease): ……………………………….…………………………………..

**We have been informed of the following facts:**

1. **Pre-implantation genetic testing** (hereafter simply referred to as PGT) constitutes a set of testing methods that can only be used in association with the techniques of in-vitro fertilisation (hereafter simply referred to as IVF) for the targeted detection of certain serious chromosomal defects in ova (oocytes) and embryos. PGT is currently the only known technique that can act preventively prior to the commencement of the development of a pregnancy, i.e. in the period prior to the introduction of the embryo to the uterus (prior to embryo transfer).
2. **The risks of IVF and pregnancy:** The IVF method and every pregnancy come with certain specific risks. We declare that we have been informed of the risks of IVF and a pregnancy following IVF and we have confirmed our consent to perform the IVF by signing the appropriate informed consent form.
3. **Pre-implantation genetic testing for monogenic diseases (hereinafter “PGT-M”) in embryos**, which we are requesting, is a type of PGT method aimed at ruling out hereditary genetic abnormalities, so-called deoxyribonucleic acid (hereinafter “DNA”) mutations that have a high probability to result in illness in a child if it were transferred to the foetus. The frequency of the occurrence of hereditary monogenic disease in the population is 1-2%. The test will also focus on verifying the correct number of - tested chromosomes, and/or parts thereof. Chromosomes are structures created by a deoxyribonucleic acid (DNA) molecule that carry genetic information and are stored in the cellular nucleus. Under normal circumstances, each cellular nucleus (with the exception of spermatozoa and ova) contains 23 pairs of chromosomes, i.e. a total of 46 chromosomes. Spermatozoa and ova contain half the number of chromosomes, i.e. 23 each so that the newly formed cells of the embryo contain 46 once they have merged. Changes in the number and structure of the chromosomes may preclude the possibility of implanting an embryo and therefore of becoming pregnant or may lead to a miscarriage or the birth of a child with a developmental defect. Embryos in which the PGT does not find any chromosomal abnormalities have greater promise in terms of resulting in a pregnancy, and as such the probability of the success of the IVF treatment is higher. For the same reasons, the use of the PGT-M method--which also includes chromosomal abnormality testing--reduces the risk of a miscarriage.
4. **The purpose of the PGT-M procedure** is to select an embryo for embryo transfer in which mutation sets causing hereditary illness have been ruled out and is also a euploid embryo, i.e. an embryo that has not been found to have any abnormalities in the number or structure of the tested chromosomes or chromosomal parts. Points 9, 10, 11 and 16 deal with situations in which no such embryo is available.
5. **The risk of developmental defects in the population:** Testing using the PGT-M method cannot rule out other hereditary illnesses than those listed in “Reason for Testing” at the beginning of this consent form. It also cannot rule out other developmental defects than those which are the focus of the chosen PGT method. The frequency of developmental defects in the general population is 3-5%.
6. **Biopsies:** Embryo biopsies (the collection of material) are performed with great care using micro-manipulation devices, most frequently with aspiration (suction). Material for testing is obtained in the blastocyct stage (typically on the 5th- 6th day of development) by collecting several (usually 5 to 10) trophectoderm cells - trophoblasts (part of the embryo, which later develops into the amnion), meaning that the further development of the embryo is not interrupted. The removed sample is placed in a special solution in a test tube in order to isolate the DNA (deoxyribonucleic acid). This step renders the cells no longer viable and they cannot be returned to the embryo. It has been demonstrated that biopsy of groups of trophoblast cells (the trophectoderm) from blastocysts cannot in and of themselves cause any further developmental defects or illnesses to the embryo. The situation where a biopsy causes the cessation of embryonic development during the course of laboratory culture occurs at a frequency of less than 1%. To date, tens of thousands of children have been born after undergoing PGT testing without any increased frequency of developmental defects in comparison with the general population. I consent to the performance of a biopsy and, if it is necessary for achieving and optimising the diagnostic conclusion, I also consent to repeated biopsies (re-biopsies) of the embryos.
7. **Embryo vitrification:** Once the sample has been collected, each embryo is cryopreserved (frozen) using the vitrification method and then stored separately. The cryopreservation process does not reduce the probability of the implantation of the embryo after thawing. The couple has expressed their consent for the vitrification and further storage of the embryos as part of the consent for the performance of assisted reproduction (see point 16 for more). The tested embryo will then be transferred in one of the following menstrual cycles.

In the event that I am requesting the testing of embryos which have been frozen, I consent to and request that these embryos should be thawed for the purpose of the biopsy or re-biopsy (see point 6) and then subsequently revitrified.

1. **The procedure for testing samples using molecular-genetic methods:** DNA (deoxyribonucleic acid) is released from the collected sample in the form of trophectoderm cells in the laboratory and is then amplified using the methods of whole genome amplification (WGA). In the next process, various molecular-genetic methods (DNA chip - karyomapping, massive parallel sequencing - MPS, Sanger sequencing, qPCR, etc.) are used to detect mutations and deviations from the norm in terms of the number and structure of all chromosomes. The specific selection of methods used depends on the type of disease or mutation and the availability of DNA samples from the rest of the family. A set of genetic variants is then evaluated for each embryo in relation to the monogenic disease for which it is being tested and deviations from a normal state are then quantitatively evaluated either as excesses or losses of individual chromosomes or their parts.

PGT-M testing follows the principle of indirect diagnostics. This means that it is based on the observation of unique sets of markers that are incontrovertibly linked to a mutation or normal variant in the tested gene. Establishing the markers accompanying mutations or normal variants is possible based on a DNA analysis of the potential parents and a suitable reference sample. The reference sample may constitute DNA from a child, grandparent, or other close relative. If a reference sample is not available, an embryo can be used as a reference sample after direct detection of mutations has been carried out. The choice of reference and the decision to conducting direct mutation detection in the parents, reference sample, or embryo shall be made by the expert team based on an evaluation of all available clinical-genetic records and family tree. The treated couple will always be informed of the specific procedures and possible limits of the chosen process.

1. **The evaluation and interpretation of the PGT-M results**

The evaluation of the PGT-M results takes place in two stages. First, a set of mutations and normal variants in relation to the disease for which the PGT-M is being conducted is evaluated in the embryo. Embryos for which mutation sets causing hereditary diseases have been ruled out or embryos for which a set that allows for their transfer following genetic consultation advance to the second stage of evaluation.

* Embryos carrying a set of mutations causing hereditary disease are incontrovertibly unsuitable for embryo transfer, and their storage is usually terminated immediately (see point 16). It makes no sense to carry out any further biopsies or testing of this type of embryo.
* Embryos in which mutation sets causing hereditary diseases are ruled out are then evaluated based on the results of the second stage of evaluation, which focuses on deviations from the normal number of chromosomes.
* Embryos carrying mutation sets and normal variants allowing for their transfer following genetic consultation are then evaluated based on the results of the second stage of evaluation, which focuses on deviations from the normal number of chromosomes.

In the second stage of evaluation, the embryos are divided into the following four categories according to the significance of the deviation from the norm:

* **Euploid** embryos in which a deviation from a normal sample **was not detected** and in which a mutation set causing hereditary disease has been ruled out are incontrovertibly suitable for transfer into the womb (embryo transfer).
* Embryos containing at least one deviation at the level of an entire chromosome in the collected cells are designated as **aneuploid**. These are incontrovertibly unsuitable for embryo transfer and their storage is usually automatically terminated (see point 16). It makes no sense to carry out any further biopsies or testing of this type of embryo.
* If there is a deviation only in a part of the chromosome, this is categorised as **segmental aneuploidy**. This type of embryo is recommended to be stored furthermore (see point 16), as its repeated biopsy (rebiopsy) and reanalysis may be beneficial under certain circumstances. If a segmental aneuploidy is detected in an embryo, new studies have shown that this finding does not occur in the rest of the embryo in approximately half of embryos which therefore essentially classifies it as a mosaic embryo, in which the segmental aneuploidy has only been detected in the cells collected for testing. The collection of a second sample (a repeated biopsy of the embryo) and its testing in the case of a segmental aneuploidy is a very good predictor of its presence in the rest of the embryo (reliability > 90%).
* Embryos with more than one cell line present in the collected sample are categorised as **mosaic**. We most frequently find cells with deviations and cells with the normal number of chromosomes. This type of embryo is recommended to store further (see point 16). Mosaic embryos are capable of developing into healthy foetuses under certain circumstances, because they can rid themselves of any abnormal (aneuploid) cells using the mechanisms of selection and programmed cell death – apoptosis. Only chromosomally normal (euploid) cells then continue to develop. However, some mosaic embryos do not manage to eliminate these abnormal cells or the aneuploid cells may predominate. Once a mosaic embryo has been transferred, it is possible to expect a significantly lower share of implanted embryos, a significantly lower share of clinical pregnancies and at the same time an increased risk of miscarriage than in the case of the transfer of a euploid embryo.
1. **Embryo transfer after PGT-M:** The goal of the PGT-M is to transfer an embryo in which mutation sets responsible for the development of hereditary diseases have been ruled out as well as a euploid embryo, for the purpose of increased success and decreased risks and complications. The PGT-M method can reveal embryos that are unsuitable for embryo transfer due to mutations or genetic abnormalities in different treated couples. The following situations can occur:

• All tested embryos are abnormal - they carry mutation sets responsible for disease and/or are aneuploid:

It is possible that the testing may reveal that all of the embryos are abnormal. In that case, no embryo is recommended for the embryotransfer. However, this situation may not necessarily recur during the next IVF/PGT-M cycle and as such the couple may be encouraged to repeat the IVF cycle after a consultation with their doctor. If none of the embryos in the further cycles are suitable for transfer, it is possible to consider alternative treatment methods (oocyte donation, sperm donation or embryo donation) after consultation with a doctor.

• One or more embryos demonstrate neither set of mutations causing hereditary disease nor chromosome abnormalities (euploid embryo): these embryos are fully recommended for the embryotransfer (see point 13).

• The genetic testing of the embryo reveals a set of mutation and normal variant that require clinical-genetic consultation prior to its possible transfer and/or reveals mosaic embryos or embryos with segmental aneuploidy (see point 9): the embryotransfer options are specified further (see point 11).

1. **The transfer of a mosaic embryo or an embryo with segmental aneuploidy:** Under certain circumstances, a couple may decide to transfer a mosaic embryo or an embryo with segmental aneuploidy, especially under the following conditions:
2. the couple does not have any embryos designated as euploid and it only has mosaic embryos or embryos with segmental aneuploidy and/or
3. all of the couple’s euploid embryos have already been transferred without a pregnancy ensuing and the couple only has cryopreserved mosaic embryos or embryos with segmental aneuploidy and at the same time the detected deviation does not include chromosomes 13, 18, 21 and X, i.e. any of the chromosome groups in which aneuploidy is compatible with the carrying to full term and birth of the foetus, but at the same time cause serious developmental defects.
4. the testing of a repeat biopsy of an embryo with segmental aneuploidy has not confirmed the original finding and it has been categorised as euploid, or no repeat biopsy has been performed on the embryo at the express wish of the couple.
5. and at the same time there are serious grounds as to why the couple is not able to or does not wish to undergo a further IVF cycle to acquire further embryos.

Before the performance of the transfer of a mosaic embryo or an embryo with segmental aneuploidy, the couple will undergo a clinical genetic consultation and sign a separate informed consent form where they will confirm that they have been acquainted with the possible risks and despite that they have decided to request the performance of the transfer of the mosaic embryo or the embryo with segmental aneuploidy.

Once a mosaic embryo or an embryo with segmental aneuploidy has been transferred, it is not possible to rule out the risk of a serious developmental defect involving the further development of the embryo and the foetus and any such defect need not always be detectable using non-invasive prenatal diagnostic methods. Therefore, **genetic consultation and invasive prenatal diagnostics are recommended** in every pregnancy occurring after the transfer of such an embryo (see point 20 for more).

1. **The number of embryos for the performance of PGT-M and the accumulation of embryos from several cycles:** The successful use of PGT-M requires compliance with some fundamental conditions, one of which includes a sufficient number of acquired oocytes and a sufficient number of cleaving embryos. However, the suitable number of embryos may differ for each couple depending on the type of inheritance of the mutation and the frequency and occurrence of any chromosomal abnormalities in the couple. The situation must be evaluated for the couple on an individual basis according to known genetic factors as well as possible infertility factors. It depends on the agreement between the couple and the expert team. If the number of cultivated embryos is insufficient for the effective performance of the treatment procedure using PGT-M, it is recommended that the couple should undergo another cycle (or cycles) of IVF in order to “accumulate” the necessary number of embryos for genetic analysis. If it is not possible to accumulate any more embryos, it is possible to carry out PGT-M with any number of embryos, even if there is only one.
2. **The number of transferred embryos:** According to expert recommendations, it is advisable to always transfer solely 1 (in words: “one”) embryo into the uterus and the couple will confirm that they have been informed of this with their signatures. Any other vitrified embryos will remain in storage for eventual further transfer at a later time.
3. **When it is not possible to reach a diagnostic conclusion:** testing embryos using PGT-M may not lead to an incontrovertible diagnostic conclusion in approximately 3-5% of tested samples. This usually involves the following cases:
* the DNA becomes degraded before the performance of the testing
* the DNA amplification fails
* the finding cannot be unequivocally established
* a crossing over happens in the sperm or eggs in such close proximity of a mutation that a mutation set or normal variant cannot be reliably determined in the embryo (less than 2% of embryos)
* the analysis can be exceptionally influenced by the intervention of so-called force majeure (natural catastrophes, accidents, sudden equipment failure, states of emergency and so on)

If it proves impossible to reach a diagnostic conclusion in exceptional cases, it is possible to either biopsy the embryo again and subject it to subsequent testing or to transfer it with a clearly established level of risk of complications. These options will always be consulted with the couple and they will be able to make an individual decision based on all the known circumstances.

1. **Misdiagnosis:** The determination of a mutation status in cells obtained from embryos may be erroneous and may not always represent findings in the other cells of the embryo. Worldwide statistics indicate that the risk of a misdiagnosis by PGT-M is less than 0.5%, but this is even lower in laboratory practice. Similarly, a misdiagnosis may occur when detecting chromosomal aberrations during a PGT-M. This risk is unequivocally lower than the risk of the occurrence of chromosomal aberrations in an embryo resulting due to IVF techniques without the use of PGT-M and it is also lower than the risk of the occurrence of a chromosome aberration during natural conception in the general population.
2. **The subsequent storage of the vitrified embryos:** After the PGT-M has been performed and a finding has been detected in individual embryos, the general recommendations for storage of the embryos are:
* It Is not recommended to store the embryos containing a set of mutations causing hereditary disease or aneuploid embryos.
* It is recommended to store only euploid embryos and all conditionally transferable embryos, such as mosaic embryos and embryos with segmental aneuploidy. The couple may decide to terminate the storage of these embryos at any time.

The exact conditions for the subsequent storage of vitrified embryos, including consent to the storage termination are governed by the rules of the IVF clinic where embryo biopsy was performed.

1. **The limitations of the method:** The described method cannot reveal any losses or excesses of small parts of the chromosomes, the size of which is less than the resolution of the used methods. The reliability of testing for deviation and number of chromosomes can also be decreased due to a low quality of data acquired during testing. Reliable determination of mutation set or normal variants can also be decreased in various areas of the chromosomes (i.e. centromeric and telomeric parts) and due to low coverage of markers tied to mutations and normal variants. These circumstances can generally not be predicted in advance. The reliability of the testing is always determined for every embryo individually, and in the event that it decreases below 99.5%, it is always listed in the resulting protocol, which includes a detailed explanation. Simultaneously, PGT-M cannot rule out other hereditary diseases that are not specifically listed in the reason for testing. At the same time, it is not possible to rule out any other illnesses or developmental defects in the foetus that are not caused by a change in the number or structure of the tested chromosomes or chromosomal parts. The MPS method does not detect haploid chromosomal sets, triploidy 69, XXX, tetraploidy, uniparental disomy, and balanced chromosome rearrangements in the trophectoderm sample.
2. **Payment:** The payment and surcharges for other procedures associated with the use of the stimulation preparations and the performance of the IVF method are subject to the provisions of the valid law and the contractual conditions which apply at the assisted reproduction centre. Every treated couple will be acquainted with the calculation of the performance and the surcharges for the IVF and PGT-M in advance.
3. **Feedback on the course of the pregnancy, the birth and the health of the foetus:** The treated couple hereby consents that the assisted reproduction centre and laboratory carrying out the PGT-M analysis may be informed of all circumstances during the course of the pregnancy, the results of prenatal diagnostics, the course of the delivery, and the subsequent development of the child. This information will continue to be confidential and the assisted reproduction centre undertakes to protect this data from abuse in any form. This data will be used in an anonymised form for the publication of scientific work, monitoring the results of the PGT-M/PGT program and referring the results to the National Register of Assisted Reproduction (NRAR) and the register of the Consortium of Preimplantation Genetic Diagnostics at the European Society of Human Reproduction and Embryology (ESHRE). At the same time, the couple also undertakes to inform the assisted reproduction centre of the results of the treatment and to cooperate when providing any information on the course and results of the treatment.
4. **Prenatal diagnostics:** We recommend that every foetus conceived after the performance of the PGT-M testing should be duly tested during the course of the pregnancy using the methods of so-called non-invasive prenatal diagnostics (i.e. ultrasound testing of the foetus focussing on the occurrence of any innate defects, combined testing in the 1st trimester or possibly integrated testing). In the case of a pregnancy after the transfer of a mosaic embryo or an embryo with segmental aneuploidy (see point 11), a clinical - genetic consultation and **invasive prenatal diagnostics** (testing of collected amniotic fluid with a resolution corresponding to the character of the embryo’s laboratory chromosomal findings) at a special institution are always recommended, as is a genetic consultation at an institution specialising in prenatal diagnostics. The performance of invasive prenatal diagnostics in order to verify the results of the PGT-M is not indicated nor recommended provided that the reliability of the testing did not decrease below 99.5% or for any other reason listed in the resulting testing protocol. The decision on the performance of any invasive prenatal diagnostics is always made by the treated couple or by the pregnant woman.

**Final declaration:**

I hereby declare that I have read this form or that it has been read out to me.

I understand the currently known advantages and risks of testing using the PGT-M. I hereby voluntarily request the performance of PGT-M testing.

I confirm that I have been provided with consultancy on the PGT-M methods, their course and the conditions for their performance and I have had the opportunity to ask any supplementary questions pertaining to the PGT-M procedures and this consent.

Everything has been conveyed and explained to me clearly and comprehensibly. I have had the opportunity to consider everything properly, calmly and with sufficient time, I have had the opportunity to ask the doctor about everything I considered essential and I have been able to go through anything I did not understand with the doctor. I have received comprehensible answers to my questions.

I agree that according to the laboratory capacity, and unless otherwise stated in the consent of the IVF center performing embryo biopsy, the amplified DNA from the embryo biopsy shall be stored for further analysis performed to our advantage and that of our family for a period of at least five years from the transfer of the embryo. The amplified DNA from the biopsy of a cryopreserved embryo will be stored for a period of five years from the transfer of the embryo or five years from the completion of the cryopreservation.

I have also been instructed that I can withdraw my consent at any time, although I am aware that the process of embryo testing using the PGT-M method is a method which is performed over the course of several days and that, if I withdraw my consent, the testing will admittedly not continue, but I will still have to pay for all of the steps that were undertaken prior to the withdrawal of my consent and to do so at my own expense.

The signature of the female partner – client The number of the female partner’s - client’s identification card The date

The signature of the male partner – client The number of the male partner’s - client’s identification card The date

Signature verified by\*:

 Name Signature Date

\* The document must be officially authenticated, if it is signed outside the premises of the IVF centre. Otherwise, the signature will be authenticated by a staff member from the IVF centre using the presented personal identification document.

**The doctor’s declaration:**

I hereby declare that I have clearly and comprehensibly explained the purpose, nature, anticipated benefit, consequences and possible risks of PGT-M testing to the treated individual. At the same time, I have also acquainted the treated individual with the possible results and the consequences of the testing not being able to be performed for the aforementioned reasons (it would fail) or of it not having the necessary predicative ability to fulfil the monitored purpose. I have acquainted the treated individual with the possible risks and consequences associated with rejecting this testing. The results of the laboratory testing will be confidential and will not be provided to any third parties without the consent of the treated individual, unless the legal regulations state otherwise.

 Name Signature