

JUNO

GENETICS

USER MANUAL



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1. LOCATION AND CONTACT DETAILS

JUNO GENETICS offices and laboratory are located in:

Parque Tecnológico Paterna
Ronda de Guglielmo Marconi, 11-A, 2º, A1-2, A2-2. 46980 Paterna, VALENCIA

MAIN TELEPHONE NUMBERS

General contact telephone:
(+34) 96.069.48.00

MAIN CONTACT EMAIL ADDRESSES

1. **General email contact address:**
supportspain@junogenetics.com
2. **Specific inquiries for PGTseq related test (PGT-A, PGT-SR and POC):**
pgtseq.es@junogenetics.com

Specific inquiries for PGTseq cases from clinics in ITALY please use the following email address instead:
pgtseq.ita@junogenetics.com

3. **Specific inquiries for PGT-M test:**
genetica.es@junogenetics.com

Specific inquiries for PGT-M cases from clinics in ITALY please use the following email address instead:
pgtseq.ita@junogenetics.com

4. **Specific inquiries for GeneSeeker related tests:**
geneseeker@junogenetics.com

2. ABOUT US

JUNO GENETICS is a private laboratory specialising in reproductive genetics. Our services include preimplantation genetic testing for chromosome abnormalities (PGT-A), monogenic disorders (PGT-M) and structural chromosomal rearrangements (PGT-SR), products of conception testing (POC) and Carrier Genetic Screening (GeneSeeker).

Our dedicated team includes some of the most experienced scientists in the field of reproductive genetics. We continually invest in research aimed at improving genetic analyses of embryos and other human tissues. Our scientists have consistently been at the forefront of advances in technology and accuracy. Research led by scientists at **JUNO GENETICS** has resulted in the publication of more than 500 scientific papers and has been recognised by the receipt of multiple awards. The focus on innovation and continual improvement has helped **JUNO GENETICS** to achieve published accuracy rates for embryo assessment that are amongst the highest in the world. **JUNO GENETICS** has a strong emphasis on quality, as evidenced by the laboratory's work towards obtaining recognized accreditation/certification laboratory quality standards. A key aim of the company is to be accessible to its clients, providing a rapid response to queries and giving support whenever needed. More information about the company can be found on the **JUNO GENETICS** website.

WEB ADDRESSES

www.junogenetics.eu (info available in English)

www.junogenetics.es (info available in Spanish)

www.junogenetics.it (info available in Italian)

3.

OPENING HOURS

JUNO GENETICS opening hours are:

Monday - Friday: 8.30 am – 5.30 pm.

JUNO GENETICS Spain operates throughout the year except all Spanish bank holidays.

For PGT-M cases:

PLEASE NOTIFY THE TEAM WHEN THE SAMPLES ARE TO BE SENT Ideally samples should be sent MONDAY-THURSDAY

4.

COMPLAINT PROCEDURE

At JUNO GENETICS we understand that you may sometimes feel that we could do something better, that we are not meeting your needs and expectations, or that we have even made a mistake.

We want to know whether you think we are not great, whether we have done something wrong or whether we can improve something.

To formally process a complaint, we only ask you to send it to us through the channel that we have enabled on our website to better address these matters. To do so, you must click on the link available on our website and fill in the form available for this purpose (<https://www.junogenetics.es/contactenos/>).

In case you have any doubt where to find the form you can contact us by e-mail to supportspain@junogenetics.com for assistance.

NOTE: Don't forget to indicate what you expect from JUNO for solving your inquiry.

After receipt, complaints will be reviewed. We will do our best to try to give you an answer within 3 working days. Take into account that in some cases, an effective complaint resolution may require longer time periods.

5.

LABORATORY COMMITMENT ON DATA PRIVACY OF PERSONAL INFORMATION

The laboratory follows strict policies on Information Governance and maintains a data protection infrastructure in line with European REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL ('GDPR') and Spanish regulation in addition to the Spanish data protection laws, such as Organic Law 3/2018 of 5 December on Personal Data Protection and the guarantee of digital rights applicable in Spain.

Further information on Data Privacy can be found on JUNO's webpage.

6. SENDING SAMPLES TO JUNO GENETICS LABORATORY

6.1 RELEVANT INFORMATION FOR REFERRAL CASES

Due to the complexity of the genetic tests and due to these tests only being performed within a medical context case, the test can only be requested by an authorized health professional. This role may be different depending on the local regulations but generally this role is assigned to clinicians with a professional license in force.

JUNO GENETICS Spain does not offer any kind of clinician service and do not offer services as a self-testing product. Requests from direct patients and lay user will be not accepted.

Authorized health professionals cannot refer tests for themselves, as the aforementioned case, tests will only be accepted when referred by other authorized health professional.

JUNO GENETICS Spain does not offer any kind of medical service, due to this reason only requests referred by authorized external health professionals to JUNO can be accepted.

6.2 RELEVANT INFO FOR SAMPLE COLLECTION AND ACCEPTANCE

JUNO GENETICS provides you with sample collection kits to ensure that the specimen is collected and preserved in optimal conditions until it arrives to the laboratory. Instructions set for ensuring an optimal sample collection and shipping are also provided along with the collection kits. You may already ask for sample collection and shipping instructions to our customer support service. See contact details section for further information.

For ensuring accurate results and an adequate test performance all received samples are checked for sample acceptance. Any samples that are to be considered for rejection are brought to the attention of the laboratory director (or if not available, the most senior member of staff), who will decide if the sample can be recovered, considering the difficulties in obtaining a replacement sample. The referring clinic will be contacted as soon as possible to inform them of a sample rejection and a repeat sample will be requested, if necessary. If a sample is received in an inadequately labelled container, the clinic will be contacted to either supply the missing information or provide a repeat sample.

The following events may lead to a sample being considered for rejection:

- Received specimen container is broken.
- Commingled or possibly contaminated samples that may affect examination results.
- Incorrect specimen container (e.g., blood not in an EDTA tube).
- Leaking sample from specimen container.
- Insufficient sample volume or quantity.
- Inadequately labelled specimen container (this includes illegible writing).
- Reception of a non-validated specimen type (e.g., Receive blood drop in blood cards instead whole blood in EDTA tube).
- Sample received at inadequate temperature (e.g., sample received at room temperature when refrigerated conditions during shipping are mandatory).

- Mandatory information on requisition form missing.
- Non-approved requisition forms by Juno are sent with the sample.
- Sample looks to be in an unsuitable condition (e.g., cloudy or clotted).
- Specimen containers not corresponding to those listed on the requisition form.
- Samples received without any patient identifier.
- Blood received in expired sample containers.
- The sample/patient does not meet any pre-examination requirement required for ensuring the test performance (medication status of the patient, minimum gestational week...).

Very important:

Any received specimen must be labelled with at least two identifiers and an associated document with the same identifiers. That ensures the specimen traceability with the appropriate patient data.

For PGT cases sending embryo specimens, Juno ID labels are included within the sample collection kits provided by JUNO. One copy of this QR labels must be stuck in the PCR tube and the other one tube in the biopsy collection form.

6.3 RELEVANT INFO FOR REQUESTING A TEST

JUNO GENETICS only accepts test requests from clinics and Health professionals. Although paperwork forms can be accepted to request a test, JUNO prefers to operate through electronics means integrated into the JUNO laboratory information system (LIMS). This ensures that JUNO GENETICS receives test, patient and clinic data in a secure and reliable manner.

The level of the LIMS integration may differ from one clinic to another in one of the following tiers:

- **Tier1:** LIMS from clinic is fully integrated with the LIMS of JUNO. Customer request the tests using its own LIMS platform and receives the test results and any other associated information in the same way.
- **Tier2:** A middle-platform is used for the test requesting and receiving the test results. This middle-ware software is offered by JUNO GENETICS (CLINICS PORTAL). This platform offers additional functionalities to the users as: 1) possibility of ordering sample collection kits and other consumables, 2) access to the most up-to-date test-related documentation (informed consents, requisition forms, instructions...) and 3) download relevant Key Performance indicators about the services provided.
- **Tier 3:** Clinic requests are not directly transmitted to the LIMS of JUNO GENETICS in any way. Hardcopies of the test request along with sample itself come together and the information is lately registered on the LIMS of the JUNO GENETICS during sample reception stage by JUNO's staff.

Regardless the tier of integration a hardcopy of the electronic request is required to be shipped along with the specimen container. Not sending this documentation may led to reject the sample at reception, or delay test results.

Verbal test request are not accepted by JUNO GENETICS as a way for requesting a test.

6.4 CLINICAL ADVICE

While we try to explain procedures and their limitations as concisely as possible within our consent forms, we strongly advise that all patients having a genetic test receive genetic counselling. Informed consent must be obtained from the patient to perform any genetic test. It is ideal that all patients speak to a genetic counsellor with previous experience on the test that is being requested to discuss the associated risks, limitations, and potential outcomes with the patient. Please contact JUNO GENETICS if any advice is needed in regard to seeking a genetic counsellor, or for further enquiries into the selected test.

6.5 TURNAROUND TIMES

Turnaround times for the tests offered by JUNO GENETICS are as follows:

- **PGT-A** - 7 working days from receipt of embryo biopsy samples and associated test documentation (test request and patient's informed consent).
- **PGT-SR** - 7 working days from receipt of embryo biopsy samples and associated test documentation (test request and patient's informed consent and genetic reports).
- **POC** - 7 working days from receipt of maternal and fetal/embryo samples and associated test documentation (test request and patient's informed consent).
- **PGT-M (test design)** - 40 working days from the date on which JUNO GENETICS has received all the patient's samples and information (test requests, informed consents and genetic reports).
- **PGT-M (testing of embryo biopsy specimens)** - 10 working days from receipt of embryo biopsy samples and associated test documentation (test request and patient's informed consent).
- **GeneSeeker** - 25 working days from receipt of blood sample and associated test documentation (test request and patient's informed consent).

7. SERVICES

Reproductive genetic services offered by JUNO GENETICS Spain include:

7.1 PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)

7.1.1 PGT-A General Information

The main intention of PGT-A for patients undergoing IVF treatment is to increase the likelihood that embryos chosen for transfer to the uterus have the correct number of chromosomes in their cells. In theory, the transfer of chromosomally normal embryos should be associated with a higher probability of viable pregnancy in comparison to embryos that are aneuploid. Published data confirms that embryos classified as 'abnormal' using JUNO GENETICS' PGT-A method have extremely low potential for producing a baby. The transfer of chromosomally normal embryos is expected to significantly reduce the frequency of miscarriage and aneuploid pregnancy, although some risk of these occurrences remains.

Traditionally, the main indications for PGT-A have been:

- Advanced Maternal age (35 years and older).
- Repeated implantation/IVF failure (3 or more failed cycles).
- Repeated miscarriage (3 or more miscarriages).
- Severe male factor.

However, any patient may wish to consider PGT-A, especially if they would like to minimise the number of embryo transfers required to obtain a viable pregnancy, or if they are particularly concerned about risks of miscarriage and aneuploid conceptions. It is important for all patients to understand that while risks such as an early spontaneous miscarriage or an aneuploid conception may be reduced, they cannot be completely eliminated. Furthermore, it is important that patients understand that PGT-A is not a replacement for routine prenatal testing. It is recommended that prenatal testing should still be undertaken when indicated.

7.1.2 Methods used for PGT-A at JUNO GENETICS

Next Generation Sequencing (NGS) is the principal method used for PGT-A at JUNO GENETICS. It involves the lysis of the biopsied cells from preimplantation embryos, which have been placed in PCR tubes (supplied by JUNO GENETICS) by embryologists in the referring fertility clinics. The DNA (from all chromosomes) that has been released from the cells is then amplified, leading to the generation of large quantities of DNA from each tested sample. Amplified products are labelled with unique molecular barcodes and are then subjected to sequencing, resulting in hundreds of thousands of fragments of DNA sequence from each sample tested. These sequences, known as 'reads' are compared to the sequence of the reference human genome, allowing identification of the chromosome from which each read was originally derived. Statistical evaluation of the relative number of reads from each part of the genome enables the copy number of each chromosome to be determined with high accuracy. For example, the presence of a trisomy is associated with a relative increase in the number of reads for the affected chromosome, while a monosomy is associated with a lower number of reads than expected. Additionally, during the process of sequencing, thousands of polymorphisms (variations in the DNA sequence that exist in the population) are detected. These polymorphisms have the potential to provide additional verification of the copy number of each chromosome. The accuracy rate of NGS is above 95%.

7.1.3 Requirements for accepting a case

No specific requirements must be fulfilled by the patients to ensure an adequate PGT-A test performance.

7.1.4 PGT-A patient and sample preparation

Counselling:

Appropriate counselling for all patients who are having their embryos genetically tested is strongly advised. It is the responsibility of the IVF clinic to ensure that all patients receive required counselling. JUNO GENETICS can be contacted if any advice is needed.

Consent form:

After receiving appropriate counselling about the procedure, patients undergoing PGT-A must sign an appropriate Consent Form. This Consent Form can be provided by JUNO GENETICS, or it can be prepared by the referring IVF clinic, after consultation with JUNO GENETICS. The signed Consent Form confirms that patients give their permission for the test to be carried out on their samples and understand all associated benefits and limitations. A copy of the completed form should be sent to JUNO GENETICS along with the completed Requisition Form. These documents usually are sent by transferring them in the JUNO GENETICS database, although alternatively it can be sent via email to pgtseq.es@junogenetics.com or even can be shipped a hardcopy along with the sample.

Biopsy form:

A completed Biopsy Form must be sent to JUNO GENETICS along with the samples or by email to pgtseq.es@junogenetics.com. This form will be used by JUNO GENETICS to confirm the specific test that should be carried out for each patient and to confirm that the received specimens belong to the studied case.

Sexual intercourse:

It is strongly recommended that couples refrain from having any unprotected intercourse during their treatment to avoid any chance of a natural pregnancy. Embryos resulting from natural conception will not have undergone genetic testing and therefore none of the potential benefits of PGT assay will apply in such cases.

Biopsy kit

JUNO GENETICS provides IVF clinics with biopsy kits that contain sterile PCR tubes placed in a biopsy rack and wash buffer labelled with the batch number and the expiry date. These are provided in a plastic bag shipped in a polystyrene box that also contains cool packs. The biopsy kit can be stored in its provided plastic bag, away from any possible contamination, at room temperature. The wash buffer should be stored in the refrigerator (2-8°C) upon arrival. Cool packs should be placed in the coldest available freezer (these will be used to keep samples cool when sending them to JUNO GENETICS).

Embryo biopsy

Each IVF centre should follow their own established procedure for embryo biopsy. Most blastocyst biopsy strategies involve the sampling of approximately five cells. The standard PGT-A method employed by JUNO GENETICS require a minimum of three intact cells in a maximum volume of wash buffer of 2.5 µl. Having fewer cells or higher volume than this increases the chances of failing to obtain a result and could potentially reduce accuracy. If the number of cells obtained from a blastocyst is lower than the required three cells, JUNO GENETICS must be notified so that alternative techniques can be employed.

Cells washing and tubing

Once cells are removed from the embryo, it is recommended to wash them through three microdroplets of wash buffer (consumable provided by JUNO GENETICS as part of the biopsy kit), pipetted onto a clean Petri dish. It is very important that the drops are not overlaid with oil as this often contains molecules that inhibit the DNA amplification, which is an essential part of all PGT methods. Washing of the sample will help to remove DNA contaminants, such as those derived from sperm or cumulus cells. Even when ICSI is used for fertilisation and all cumulus cells are carefully removed, there remains a possibility that DNA from these cells as well as from other sources of contamination may be present. As the biopsy sample is moved from one drop to the next, any contaminants will be diluted. The pipette used for moving

the sample should be cleaned by flushing with a few microliters of clean wash buffer between each of the different microdroplets used for washing. Cell washing should be done thoroughly but at the same time gently in order to avoid damaging the biopsied cells. If cells lyse, they are less likely to give a PGT result.

After the third wash, the biopsy specimen should be placed in one of the sterile PCR tubes provided in the biopsy kits by JUNO GENETICS. It is extremely important that the total volume of buffer in the PCR tube (containing the biopsied cells) is in the range of 1-2.5 µl. If the amount of fluid in the tube exceeds 2.5 µl DNA amplification will be less efficient, yielding poor quality results or causing a total failure of the test. The PCR tubes should be kept closed as much as possible and the transfer of the biopsy specimen should be performed in a sterile environment. In some cases, it is possible to confirm that the biopsied cells have been successfully transferred to the tube, by observation under a microscope. If attempting visualisation of cells, it is recommended that the biopsy specimen is pipetted onto the side of the tube, 2-3 mm from the bottom of the tube. Tubes should be labelled with the patient' initials and embryo number. Additionally, the unique QR labels provided by JUNO GENETICS shall be stuck in the wall of the PCR tube. The second copy of this QR label must be attached in the paperwork of "Embryo biopsy form".

Tubes containing biopsied cells should be kept at cold temperature.

Shipping samples to JUNO GENETICS

There is no need to inform JUNO GENETICS when any samples are being sent to its laboratory. The IVF clinic just has to request to the provided courier the pick-up order with the customer details provided by the Customer Support area of JUNO GENETICS.

Biopsied cells from embryos should be sent in the kit provided by JUNO GENETICS. Samples from different patients should not be stored in the same "Biopsy collection" box, but in order to optimise shipping, different Biopsy boxes can be included in the same shipping cooler.

A minimum of 2 frozen cool packs should be added to the box and the lid closed 30 minutes before the samples are inserted to cool down the inside of the box. Once the biopsy is completed and the box is ready to be collected, the cardboard box should be closed and sealed with tape. Overnight shipment with guaranteed next day delivery is recommended.

7.1.5 Dry runs

Before starting a clinical service, all embryologists from the new referring IVF clinic that are routinely involved in embryo biopsy and loading should send 10 "dry run" samples to JUNO GENETICS. These "dry run" samples should be cells (ideally TE) biopsied from embryos that are unsuitable for transfer, and/or have been donated for research. There is no need to send negative controls as the presence or absence of any contaminating extraneous DNA can be detected. The samples should be labelled with the embryologist initial and embryo/dry run samples number. The chosen labelling scheme should be clearly explained on the biopsy form.

7.2 PREIMPLANTATION GENETIC TESTING FOR STRUCTURAL REARRANGEMENTS (PGT-SR)

7.2.1 PGT-SR General Information

PGT-SR is used for couples, where one or both partners are carriers of a structural chromosome rearrangement (for example, a translocation or an inversion). Such couples have a high chance of producing gametes (sperm or eggs) in which some of the regions situated on the rearranged chromosomes are either lost or duplicated. Embryos produced from chromosomally abnormal gametes may lead to pregnancies, but these frequently miscarry or produce children affected by congenital abnormalities and/or mental disability. PGT-SR aims to examine the cells of embryos produced using IVF and distinguish abnormal embryos that have lost or gained pieces of chromosome from those that have a normal amount of chromosomal material (having an entirely normal set of chromosomes or having a balanced form of the chromosome rearrangement, essentially the same as the carrier parent). The intention is that only embryos classified by PGT-SR as chromosomally normal or balanced rearrangement carriers should be considered for transfer to the uterus. Embryos that carry a balanced form of rearrangement cannot be distinguished from those who do not carry the translocation. Typically, the PGT-SR procedure requires the biopsy of approximately five cells from the trophectoderm of embryos at the blastocyst stage of development. The cells are placed into small test tubes and sent to JUNO GENETICS for analysis.

It is important to note that although PGT-SR reduces the risk of a pregnancy affected by chromosome abnormality, no test performed on small numbers of cells from preimplantation embryos can be 100% accurate. There remains

a possibility that an embryo classified as 'normal/balanced' could contain deleted or duplicated parts of chromosomes, or suffer from other forms of chromosome abnormality which may be undetectable using the PGT-SR method. For this reason, PGT-SR should not be considered an alternative to prenatal screening, and it is strongly recommended that routine prenatal testing (non-invasive prenatal testing, amniocentesis or chorionic villus sampling) is carried out if a pregnancy occurs, to confirm that the fetus is chromosomally normal. present. As the biopsy sample is moved from one drop to the next, any contaminants will be diluted. The pipette used for moving.

7.2.2 Methods used for PGT-SR at JUNO GENETICS

Next Generation Sequencing (NGS) is the main method used for PGT-SR at JUNO GENETICS. It involves the lysis of cells biopsied from preimplantation embryos, which have been placed in 0.2 ml PCR tubes (supplied by JUNO GENETICS) by embryologists in the referring fertility clinics. The DNA (from all chromosomes) that has been released from the cells is then amplified, leading to the generation of large quantities of DNA from each tested sample. The amplified products are labelled with unique molecular barcodes and are then subjected to DNA sequencing, resulting in hundreds of thousands of fragments of DNA sequence from each sample tested. These sequences, known as 'reads' are compared to the sequence of the human genome, allowing identification of the chromosome from which each read was originally derived. Statistical evaluation of the relative number of reads from each part of the genome enables the copy number of each chromosome to be determined with high accuracy. For example, if part of a chromosome is duplicated there will

be a relative increase in the number of reads derived from the affected region of the genome. Conversely, loss of part of a chromosome is associated with a lower number of reads for that area than expected. Additionally, during the process of sequencing, thousands of polymorphisms (variations in the DNA sequence that exist in the population) are detected. These polymorphisms have the potential to provide additional verification of the copy number of each chromosome. The accuracy rate of NGS is expected to be above 95% for the detection of losses and gains affecting whole chromosomes and should be similar for pieces of chromosome larger than 10Mb.

7.2.3 Requirements for accepting a case

For patients, non-specific requirements must be fulfilled by the patients or embryos in order to ensure an adequate PGT-A test performance.

Prior to offering PGT-SR to patients, genetic reports describing the chromosome rearrangement the patient carries should be sent to JUNO GENETICS. A senior member of staff will review the report and decide whether or not a test is technically feasible. The genetic counsellor or PGT coordinator at the IVF clinic will then be notified of this decision. JUNO GENETICS may decline to offer PGT-SR if the nature of the chromosome rearrangement is not clear or if, after review, the accuracy of the test is predicted to be low. This is particularly likely to happen if the pieces of chromosome involved in the rearrangement are very small in size. It is essential that patients do not start their IVF treatment until JUNO GENETICS has communicated with the clinic and confirmed that the test can be offered to the patient/s.

7.2.4 PGT-SR patient preparation

Counselling:

Appropriate counselling for all patients who are having their embryos tested using PGT-SR is extremely important. It is the responsibility of the IVF clinic to ensure that all patients receive required counselling. JUNO GENETICS can be contacted if any advice is needed

Required samples:

For most PGT-SR cases referred to JUNO GENETICS, the chromosomal fragments involved in the rearrangements are sufficient in size to be detected by the NGS platform used. In the case of reciprocal translocations three out of the four chromosome fragments should fall within the detection limits of the NGS method. In such cases, no patient blood samples are required and there is no preliminary work-up before starting a cycle. However, for some cases where further investigation is necessary to assess whether the fragments are detectable, DNA samples from the patient and/or from an affected family member (prenatal sample, miscarriage specimen, child or parent) may have to be tested before a final decision can be made on whether PGT-SR can be offered to the couple.

Consent form:

After receiving appropriate counselling about the procedure, patients undergoing PGT-SR must sign an appropriate Consent Form. This Consent Form can be provided by JUNO GENETICS, or it can be prepared by the referring IVF clinic, after consultation with JUNO GENETICS. The signed Consent Form confirms that patients give their permission for the test to be carried out on their samples and understand all associated benefits and limitations. A copy of the completed form should be sent to JUNO GENETICS along with the completed Requisition Form. These documents are usually sent via email to pgtseq.es@junogenetics.com or alternatively can be shipped a hardcopy along with the sample.

Biopsy form:

A completed Biopsy Form must be sent to JUNO GENETICS along with the samples or by email to pgtseq.es@junogenetics.com. This form will be used by JUNO GENETICS to confirm the specific test that should be carried out for each patient and to confirm that the received specimens belong to the studied case.

Sexual intercourse:

It is strongly recommended that couples refrain from having any unprotected intercourse during their treatment to avoid any chance of a natural pregnancy. Embryos resulting from natural conception will not have undergone genetic testing and therefore none of the potential benefits of PGT will apply in such cases.

Biopsy kit

JUNO GENETICS provides IVF clinics with biopsy kits that contain sterile PCR tubes placed in a biopsy rack and wash buffer labelled with the batch number and the expiry date. These are provided in a plastic bag shipped in a polystyrene box that also contains cool packs. The biopsy kit can be stored in its provided plastic bag, away from any possible contamination, at room temperature. The wash buffer should be stored in the refrigerator upon arrival (2-8°C). Cool packs should be placed in the coldest available freezer (these will be used to keep samples cool when sending them to JUNO GENETICS).

Embryo biopsy

Each IVF centre should follow their own established procedure for embryo biopsy. Most blastocyst biopsy strategies involve the sampling of approximately five cells. The standard PGT-A and PGT-SR methods employed by JUNO GENETICS require a minimum of three intact cells in a maximum volume of wash buffer of 2,5 µl. Having fewer cells or higher volume than this increases

the chances of failing to obtain a result and could potentially reduce accuracy. Then, if the number of cells obtained from a blastocyst is lower than the required three cells, JUNO GENETICS must be notified so that alternative techniques can be employed.

Cells washing and tubing

Once cells are removed from the embryo, it is recommended to wash them through three microdroplets of the wash buffer (provided by JUNO GENETICS in the biopsy kit), pipetted onto a clean Petri dish. It is very important that the drops are not overlaid with oil as this often contains molecules that inhibit the DNA amplification, which is an essential part of all PGT methods. Washing of the sample will help to remove DNA contaminants, such as those derived from sperm or cumulus cells. Even when ICSI is used for fertilisation and all cumulus cells are carefully removed, there remains a possibility that DNA from these cells as well as from other sources of contamination may be present. As the biopsy sample is moved from one drop to the next, any contaminants will be diluted. The pipette used for moving the sample should be cleaned by flushing with a few microliters of clean wash buffer between each of the different microdroplets used for washing. Cell washing should be done thoroughly but at the same time gently in order to avoid damaging the biopsied cells. If cells lyse they are less likely to give a PGT result.

After the third wash, the biopsy specimen should be placed in one of the sterile PCR tubes provided in the biopsy kits by JUNO GENETICS. It is extremely important that the total volume of buffer in the PCR tube (containing the biopsied cells) is in the range of 1-2.5 µl. If the amount of fluid in the tube exceeds 2.5 µl DNA amplification will be less efficient, yielding poor quality results or causing a total failure of the test. The PCR tubes should be kept closed as much as possible and the transfer of the biopsy specimen should be performed in a sterile

environment. In some cases, it is possible to confirm that the biopsied cells have been successfully transferred to the tube, by observation under a microscope. If attempting visualisation of cells, it is recommended that the biopsy specimen is pipetted onto the side of the tube, 2-3 mm from the bottom of the tube. Tubes should be labelled with the patient's initials and embryo number. Additionally, the unique QR labels provided by JUNO GENETICS shall be stuck in the wall of the PCR tube. The second copy of this QR label must be attached in the paperwork of "Embryo biopsy form".

Tubes containing biopsied cells should be kept at cold temperature.

Shipping samples to JUNO GENETICS

There is no need to inform JUNO GENETICS when any samples are being sent to its laboratory. The IVF clinic just has to request to the provided courier the pick-up order with the customer details provided by the Customer Support area of JUNO GENETICS.

Biopsied cells from embryos should be sent in the kit provided by JUNO GENETICS. Samples from different patients should not be stored in the same "Biopsy collection box", but in order to optimise shipping, different Biopsy boxes can be included in the same shipping cooler.

A minimum of 2 frozen cool packs should be added to the box and the lid closed 30 minutes before the samples are inserted in order to cool down the inside of the box. Once the biopsy is completed and the box is ready to be collected, the cardboard box should be closed and sealed with tape. Overnight shipment with guaranteed next day delivery is recommended.

7.2.5 Dry runs

Before starting a clinical service, all embryologists from the new referring IVF clinic that are routinely involved in embryo biopsy and loading should send 10 "dry run" samples to JUNO GENETICS. These "dry run" samples should be cells (ideally TE) biopsied from embryos that are unsuitable for transfer, and/or have been donated for research. There is no need to send negative controls as the presence or absence of any contaminating extraneous DNA can be detected. The samples should be labelled with the embryologist initial and embryo/dry run samples number. The chosen labelling scheme should be clearly explained on the biopsy form.

7.3 ANEUPLOIDY ANALYSIS OF PRODUCTS OF CONCEPTIONS (POC)

7.3.1 POC testing General Information

Depending on the age of the mother, miscarriage occurs in 10-40% of pregnancies. An incorrect number of chromosomes (aneuploidy) is the most common cause of miscarriage, being implicated in ~65% of all losses. However, it is even more common in miscarriages from older mothers and less frequent amongst younger women. In order to help clarify whether aneuploidy is likely to have contributed to a miscarriage, JUNO GENETICS offers a test that assesses cells from the embryo/fetus or associated extraembryonic tissues (e.g. placenta). This material is collectively referred to as products of conception (POC).

Traditional methods of testing POCs for aneuploidy have involved karyotyping. However, this technique requires cell culture, which takes time, delaying results. Additionally, up to a quarter of the POC samples fail to grow in culture and therefore provide no data concerning the cause of the miscarriage. Another problem is that some samples are contaminated with maternal cells, which tend to grow better than those from the embryo/fetus, meaning that the karyotypes obtained might be derived from the mother rather than the POC.

JUNO GENETICS has developed a test that overcomes many of these limitations by avoiding the need to culture cells from the POC. Consequently, results are obtained from the vast majority of samples tested and more rapidly. The test also assesses hundreds of DNA polymorphisms in the POC and in a sample of DNA from the mother in order to reveal whether any maternal contamination is present.

Traditionally, the main indications for POC has been:

- Gestational loss with no clear indication of the pregnancy loss.

7.3.2 Methods used for POC testing at JUNO GENETICS

A POC sample and a maternal blood sample are collected using the kit supplied by JUNO GENETICS. The genetic material is extracted from each of these samples and subjected to Next Generation Sequencing (NGS), which produces hundreds of thousands of fragments of DNA sequence. These sequences, known as 'reads' are compared to the sequence of the human genome, allowing identification of the chromosome from which each read was originally derived. Statistical evaluation of the relative number of reads from each part of the genome enables the copy number of each chromosome to be determined with high accuracy. For example, the presence of a trisomy is associated with a relative increase in the number of reads for the affected chromosome, while a monosomy is associated with a lower number of reads than expected. Additionally, during the process of sequencing, thousands of polymorphisms (variations in the DNA sequence that exist in the population) are detected. These polymorphisms are essential for revealing some types of abnormality that involve duplication or loss of an entire set of chromosomes (e.g. triploidy). Additionally, the polymorphisms tested can reveal whether or not the POC sample is genetically identical to the mother's blood sample (indicative of contamination) or genetically distinct (free of contamination).

7.3.3 Requirements for accepting a POC case

No specific requirements must be fulfilled by the patients for accepting a POC case.

7.3.4 POC patient preparation

Counselling:

Appropriate counselling for all patients who are having their POC genetically tested is strongly advised. It is the responsibility of the IVF clinic to ensure that all patients receive required counselling. JUNO GENETICS can be contacted if any advice is needed.

Required samples:

A sample of the foetus and a sample of maternal blood are required to rule out contamination of the sample and to ensure that the sample tested belongs to the foetus and not to the mother.

The following types of specimens that can be tested include:

- a. Foetus specimen obtained following curettage using vacuum aspiration procedure. This is the preferred sample type.
- b. Empty gestational sac.
- c. Delayed miscarriages (more than 2 – 4 weeks).
- d. Foetal remains expelled spontaneously and collected at home.
- e. In cases of multiple pregnancies, remains from each foetus should be collected in separate pots.

Consent form:

After receiving appropriate counselling about the procedure, the patient that performs POC test must sign an appropriate Consent Form. This Consent Form can be provided by JUNO GENETICS, or it can be prepared by the referring IVF clinic, after consultation with JUNO GENETICS. The signed Consent Form confirms that patient gives their permission for the test to be carried out on their samples and understand all associated benefits and limitations. A copy of the completed form should be sent to JUNO GENETICS along with the completed Test Requisition Form. These documents usually are sent by transferring it in the JUNO GENETICS database, although alternatively it can be sent via email to pgtseq.es@junogenetics.com or alternatively can be shipped a hardcopy along with the sample.

Requisition form:

A completed Test Requisition Form must be sent to JUNO GENETICS along with the samples or by email to pgtseq.es@junogenetics.com. This form will be used by JUNO GENETICS to confirm the specific test that should be carried out for each patient and to confirm that the received specimens belong to the studied case.

Sample collection kit:

JUNO GENETICS provides IVF clinics with sample collection biopsy kits that contain a sterile sample collection canister and EDTA blood tube. The JUNO GENETICS POC kit can be stored at room temperature, away from any possible contamination, at room temperature.

In a case where a JUNO GENETICS POC collection kit is not available, please use any sterile container (of reasonable size) and a non-expired EDTA tube for collecting mother's blood.

POC specimen handling:

Please ensure that maternal decidua is properly separated from the fetal tissue. A description of the collected tissue should be provided at the bottom of the attached consent form. It will be useful to mention if any difficulty was encountered while locating the fetal tissue. Additional steps will be taken at JUNO GENETICS to maximise the likelihood that only fetal tissue is tested.

The collected POC tissue should be placed in the sterile container provided in JUNO GENETICS POC collection kit. The tissue should be immersed in sterile salt solution (saline or PBS) or in IVF culture medium.

In a case where a JUNO GENETICS POC collection kit is not available, please use any sterile container (of reasonable size).

In order to avoid any possible leakage, it is very important that the container lid is firmly closed and then tightly sealed with tape or parafilm.

The container with the POC sample along with the blood tube should be clearly labelled with the patient's identifying information (name, date of birth and patient number). The samples should then be placed in the supplied kit along with the completed and

signed “PRODUCTS OF CONCEPTION (POC) CONSENT FORM”.

The collected POC and blood samples should be sent on the same day as collection. If this is not possible, the samples should be stored in a fridge (2-8°C) overnight then shipped the following day.

Shipping samples to JUNO GENETICS

The kit should be properly sealed and shipped at room temperature using first-class postal service or via a courier company.

Contact by email or phone to notify the laboratory of the shipment of the POC and blood sample. Customer support will organise the pick-up from the courier of the POC and blood samples.

7.4 PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISORDERS (PGT-M)

7.4.1 PGT-M General Information

The main aim of PGT-M is to help couples who are affected by a genetic disorder or are carriers of a mutation to reduce their risks of having a child affected by the condition. This is accomplished by collecting small numbers of cells from embryos produced during an IVF treatment cycle, subjecting the cells to genetic testing and only transferring to the uterus those embryos estimated to be at low risk of the inherited condition being tested. It is very important to note that although PGT-M attempts to reduce the likelihood of having an affected pregnancy or child, it cannot entirely eliminate this possibility. PGT-M is not 100% accurate and for this reason it is strongly recommended that any pregnancy established after PGT-M should undergo prenatal testing to confirm that the fetus is unaffected.

There are two stages to PGT-M –

1. Test design.
2. Test.

Test design is carried out at JUNO GENETICS and begins once all the following have been received:

- Requisition form.
- Genetic reports from the couple and any other family members whose samples are being used during test development, specifying their disease status and which mutations (if any) they carry.
- Consent forms from the couple and any other family members who have provided samples.
- Blood or DNA samples from the couple.
- Where possible, blood/saliva/DNA sample from one or more additional family members who can act as a ‘reference’ (see below)

The mutation(s) responsible for the disorder should already have been identified before patients are referred, as knowledge of the underlying cause of the condition is essential in order for JUNO GENETICS to assess the feasibility of PGT-M and to design a test. JUNO GENETICS does not offer a service to identify mutations in patients, so this needs to have been done by a laboratory specialising in mutation characterisation. Genetic reports from such laboratories, confirming the exact mutation in each patient, is a requirement in order for a PGT-M referral to be accepted. In most cases, JUNO GENETICS will carry out confirmatory testing as part of the test development for specific families. However, this is not always possible and consequently it is very important that patients have been thoroughly evaluated prior to referral, receiving an accurate medical diagnosis and a genetic evaluation of whether they carry a mutation and characterisation of the nature of the genetic alteration.

JUNO GENETICS recommends that embryo biopsy is performed at the blastocyst stage of development (involving the removal of 3-10 cells from the trophectoderm). It is important that JUNO GENETICS is notified whether the biopsy contains fewer trophectoderm cells than the minimum recommended (e.g., less than three). Each biopsy specimen is placed in an individual PCR tube (provided), which is then shipped to JUNO GENETICS for testing. The embryos remain in the IVF clinic at all times.

7.4.2 PGT-M patient preparation

Counselling:

Appropriate counselling for all patients who are having their embryos tested using PGT-M is extremely important. JUNO GENETICS strongly recommends that IVF clinics make counselling mandatory for all patients requesting PGT-M. It is the responsibility of the IVF clinic to ensure that patients receive adequate counselling. JUNO GENETICS can be contacted if any advice is needed.

Genetic report(s):

Prior to offering PGT-M to patients, genetic reports describing the mutation(s) responsible for the disorder being tested should be sent to JUNO GENETICS. A senior member of staff will review the reports and decide whether or not a test is technically feasible. The genetic counsellor or PGT coordinator at the IVF clinic will then be notified of this decision. JUNO GENETICS may decline to offer PGT-M if the nature of the mutation is not clear or if, after review, the accuracy of the test is predicted to be low.

Required samples:

In all cases a blood, saliva or DNA samples will be required from the male and female patients. A sample is needed from both patients even if only one of them carries a mutation. Furthermore, it is usually necessary to collect DNA samples from one or more additional family members (as well as the couple requesting testing). This/these individual/s, referred to as the 'reference' will ideally be a close relative (e.g., children of the couple; parents or siblings of the patients; samples from previous pregnancies). In order to determine which members of the family would be the most useful to obtain a sample from, information should be made available on which individuals have been tested, their clinical diagnosis, their relationship to the patients and their availability and willingness to provide a sample. Formal genetic reports must be provided for the couple and the family member(s) serving as the reference. Upon review of the genetic reports, JUNO GENETICS will inform the IVF clinic which individuals' samples will be required to design a test. Please note, it is essential that patients

do not start their IVF treatment until JUNO GENETICS has communicated with the clinic and confirmed that a test can be offered to the patient.

Consent form:

After receiving appropriate counselling about the procedure, patients undergoing PGT-M must sign an appropriate Consent Form. This Consent Form can be provided by JUNO GENETICS, or it can be prepared by the referring IVF clinic, after consultation with JUNO GENETICS. The signed Consent Form confirms that patients give their permission for the test to be carried out on their samples. A copy of the completed form should be sent to JUNO GENETICS along with the completed Test Requisition Form either via email, or via an upload in the JUNO GENETICS database. Moreover, any relative whose sample will be used for test verification must complete a consent form or have one completed by an appropriate individual on their behalf (for example, parents can sign on behalf of their minor children).

Requisition and Biopsy forms:

A completed requisition form indicating the type of test required for each patient must be sent to JUNO GENETICS before the patient begins their treatment cycle. Additionally, a completed Biopsy Form must be sent to JUNO GENETICS before-hand or along with the samples. This form is used by JUNO GENETICS to confirm the specific test that should be carried out for each patient.

Fertilisation technique:

The use of conventional IVF for oocyte fertilisation often results in surplus sperm attaching to the zona pellucida, as well as the persistence of small numbers of cumulus cells. During embryo biopsy, there is a risk that some of these cells, or their genetic material, might be taken along with the biopsied cell(s). This can lead to DNA contamination and an error in determining the genetic status of the corresponding embryo. Therefore, it is strongly recommended to carefully remove all cumulus cells and use ICSI for fertilisation (even in the absence of a male factor).

Sexual intercourse:

It is strongly recommended that couples refrain from having any intercourse during their treatment to avoid any chance of a natural pregnancy. Embryos resulting from natural conception will not have undergone genetic testing and therefore none of the potential benefits of PGT will apply in such cases.

7.4.3 Sample preparation for PGT-M

Samples required for PGT-M protocol design (Pre-PGT-M)

lood samples from the couple are required for the test design (collected in EDTA tubes with purple-coloured cap). Moreover, blood samples from relatives (children/parents/siblings of the couple) who have been identified by JUNO GENETICS scientists as a 'reference' might also be required to maximise the accuracy of testing. If it is not possible to collect a blood sample from an individual serving as a reference, JUNO GENETICS can provide a saliva collection kit to the clinic or the patient directly. Upon request, JUNO GENETICS can also provide a special type of saliva collection kit with spongy applicators to soak up saliva from the cheek pouch (buccal swabs). Such kits can be useful when DNA must be obtained from babies, the elderly or people where it is a problem to obtain a blood sample from them.

It is important to notify JUNO GENETICS if the patients and other individuals who are providing samples have received a bone marrow transplant or a recent blood transfusion (less than 60 days), since this may affect the results obtained from their blood samples, potentially leading to a misdiagnosis. In such cases, JUNO GENETICS may specifically request saliva samples from the individuals in question.

All patient samples collected should be clearly labelled with their full name, date of birth, patient number and date of collection. Samples from relatives, should also include full name, date of birth and date of collection. Additionally, it is very important to state the relationship to the patient receiving PGT-M.

Collected samples (blood, saliva, buccal swab or DNA) should be sent to the JUNO GENETICS laboratory at room temperature using a mail service that guarantees delivery within two working days. Please note that the terms and conditions of postal/courier service for sending biological samples, including packaging and labelling requirements should be carefully followed.

Biopsy kit

JUNO GENETICS provides IVF clinics with biopsy kits that contain sterile PCR tubes placed in a plastic rack and wash buffer

labelled with the batch number and the expiry date. These are provided in a plastic bag shipped in a thermally insulated shipping box that also contains cool accumulators. The biopsy kit can be stored in its provided plastic bag, away from any possible contamination, at room temperature. The wash buffer should be stored in the refrigerator (2-8°C) upon arrival. Cool packs should be placed in the coldest available freezer (these will be used to keep samples cool when sending them to JUNO GENETICS).

7.4.4 Instructions for sending samples for PGT-M

JUNO GENETICS should be informed via email when any samples are being sent to its laboratory.

Embryo biopsy

Each IVF centre should follow their own established procedure for embryo biopsy (see the sections on PGT-A and PGT-SR for information about how JUNO GENETICS can assist with the evaluation of biopsy and cell tubing procedures). Most blastocyst biopsy strategies involve the sampling of approximately five cells. The standard PGT-M method employed by JUNO GENETICS requires at least three intact cells. Having fewer cells than this increases the chances of failing to obtain a result and could potentially reduce accuracy. It is important that JUNO GENETICS is notified whether the biopsy contains fewer trophectoderm cells than the minimum recommended (three cells).

Cells washing and tubing

Once cells are removed from the embryo, it is recommended to wash them through three microdroplets of the wash buffer (provided by JUNO GENETICS in the biopsy kit), pipetted onto a clean Petri dish. It is very important that the drops are not overlaid with oil as this often contains substances that inhibit the DNA amplification, which is an essential part of all PGT methods. Washing of the sample will help to remove DNA contaminants, such as those derived from sperm or cumulus cells. Even when ICSI is used for fertilisation and all cumulus cells are carefully removed, there remains a possibility that DNA from these cells as well as from other sources of contamination may be present. As the biopsy sample is moved from one drop to the next, any contaminants will be diluted. The pipette used for moving the sample should be cleaned by flushing with a few microliters of clean wash buffer between each of the different microdroplets used for washing. Cell washing should be done thoroughly but at the same time gently enough to avoid damaging the biopsied cells. If cells lyse, they are less likely to give a PGT result.

After the third wash, the biopsy specimen should be placed in one of the sterile PCR tubes provided in the biopsy kits by JUNO GENETICS. It is extremely important that the total volume of buffer in PCR tube (containing the biopsied cells) is in the range of 1-2.5 µl. If the amount of fluid in the tube exceeds 2.5 µl DNA amplification will be less efficient, yielding poor quality results or causing a clinical outcome cannot be obtained for the biopsied embryo. The PCR tubes should be kept closed as much as possible and the transfer of the biopsy specimen should be performed in a sterile environment. In some cases, it is possible to confirm that the biopsied cells have been successfully transferred to the tube, by observation under a microscope. If attempting visualisation of cells, it is recommended that the biopsy specimen is pipetted onto the side of the tube, 2-3 mm from the bottom of the tube.

Tubes should be labelled with the patient's initials and embryo number. Additionally, the unique QR labels provided by JUNO GENETICS shall be stuck in the wall of the PCR tube. The second copy of this QR label must be attached in the paperwork of "Embryo biopsy form".

Tubes containing biopsied cells should be kept on ice or in special cold rack (e.g., Eppendorf® PCR Cooler, iceless cold storage system for 96 well plates and PCR tubes).

7.4.5 Dry runs

Before starting a clinical service, all embryologists from the new referring IVF clinic that are routinely involved in embryo biopsy and loading should send 10 "dry run" samples to JUNO GENETICS. These "dry run" samples should be cells (ideally TE) biopsied from embryos that are unsuitable for transfer, and/or have been donated for research. There is no need to send negative controls as the presence or absence of any contaminating extraneous DNA can be detected. The samples should be labelled with the embryologist initial and embryo/dry run samples number. The chosen labelling scheme should be clearly explained on the biopsy form.

7.4.6 Samples provided for PGT-M

Blood, saliva or buccal samples used for test design

Blood withdrawal should be performed either at the IVF clinic or at an appropriate health care centre where a trained phlebotomist will be able to perform the sample collection.

Saliva collection kits can be sent directly to the patient or any family member from JUNO GENETICS. They can also be provided to the IVF centre for delivery to patients. The collection kits are always accompanied by instructions for use.

DNA samples

In some cases, archived DNA samples may be available, extracted from the patients, their relatives, or from previous pregnancies (e.g., prenatal samples). These samples are often held by third-party health care centres (hospitals, genetics laboratories where previous diagnosis was performed, etc). Where such samples are potentially available, JUNO GENETICS will arrange with the IVF centre and/or patient on how to obtain such samples.

Embryonic samples

Biopsy specimens (trophectoderm cells) are provided by the IVF centre and shipped to JUNO GENETICS. Biopsied cells from embryos should be sent in the kit provided by JUNO GENETICS in the shipping box. A minimum of 2 frozen cool packs should be added to the box and the lid closed 30 minutes before the samples are inserted in order to cool down the inside of the box. Once the biopsy is completed and the box is ready to be collected, the cardboard box should be closed and sealed with tape. Overnight shipment with guaranteed next day delivery is recommended.

7.5 GENESEEKER TEST (CARRIER GENETIC SCREENING)

7.5.1 General Information about GeneSeeker Test

GeneSeeker test, performed on a blood sample, examines a large number of genes, looking for alterations in the DNA (mutations). The test can be performed in couples to reveal whether they are at high-risk of transmitting certain mutations associated with serious autosomal recessive or X-linked genetic disorders. The test may also be of value to IVF patients who are using donor gametes, discussed in more detail below.

Some of the medical conditions that can affect a foetus during pregnancy, or are diagnosed after the birth of a child, are not inherited, while others may have a genetic basis that is not fully understood. However, many conditions are known to be caused by specific mutations. There are around 5,000 disorders associated with the inheritance of mutations in individual genes. Couples that have such mutations in their genes may have a high-risk of producing children affected by serious inherited disease.

Every individual has two copies of each gene, except for the genes located on the sex chromosomes (X and Y). Men only have one copy of the genes found on the X-chromosome, whereas women have two copies of those genes. In many cases, having a single defective copy of a gene does not cause serious medical problems because the second copy of the gene is able to compensate. A person who has one gene with a mutation and one normal copy, and has no symptoms of disease, is referred to as a 'carrier'. When eggs or sperm are produced, they receive only one of the two copies of each gene. This means that an egg or a sperm produced by a mutation carrier could inherit their normal gene copy or their defective copy. If a carrier has children with another person who carries a mutation in the same gene, there is a risk that a child could inherit a defective copy of the gene from each of the parents and therefore have no functional copies of the gene. On average, this is expected to happen in 25% of children produced by parents who have mutations in

the same gene. This may cause the child to develop a serious inherited disorder. Diseases that require inheritance of two defective copies of the gene and cause little or no harm as long as an individual has at least one normal copy, are known as 'recessive' diseases. There are around 2,000 recessive diseases known.

Being found to be a carrier of a mutant gene is not unexpected. Indeed, it is estimated that we are all carriers of recessive genetic mutations. Data from scientific studies have shown that most healthy people carry an average of 1 or 2 mutations that could cause severe genetic diseases in their offspring if they had children with someone who carries a defective copy of the same gene. However, these mutations rarely cause any health problems for the person carrying them because they are recessive, and they still have one fully functional copy of the gene.

It should be noted that mutations affecting genes on the X-chromosome often behave in a recessive manner in women (because women have two copies of the X-chromosome and therefore two copies of each gene). However, men only have one copy of the X-chromosome and consequently if they inherit a defective gene on that chromosome, they will not have any copies of the gene that work properly and may therefore have symptoms of an inherited disorder. When a woman is a carrier of a mutation on the X-chromosome, on average 50% of her male children will be affected with an inherited condition.

In many cases, the presence of a recessive mutation can be identified in the DNA of an individual before they have a family. If their partner is also tested, they might be found to have no mutations or, more likely, they also have mutations but in different genes. Such couples are at low risk of having a child affected by an inherited recessive condition. However, if both partners are found to have a mutation in the same gene they will be at high-risk.

The GeneSeeker test allows to detect thousands of mutations responsible for serious inherited diseases. When applied to men and women who are planning to start a family, GeneSeeker can help to reveal couples who are at particularly high-risk of having a child

affected by a genetic disorder. Such couples can consider various options to minimise the chance of having an affected child, including preimplantation genetic testing (PGT), prenatal testing, or other strategies that aim to reduce risk.

In the case of IVF patients who are using sperm or egg donors, it is possible to screen the patient who is using their own gametes to identify recessive mutations that they carry and then screen potential donors too. The donor chosen for the patient can be carefully selected, avoiding any who have mutations detected in the same gene (or genes) as the patient. This helps to reduce the risk of combinations of gametes (sperm and eggs) that are at especially high-risk of producing an affected child.

Traditionally, the main indications for GeneSeeker have been:

- Prior pregnancy with an affected child.
- Patients of an ethnicity with higher risk of being carriers of some mutations (e.g., ashkenazi jewish).
- Patients (also using gamete donor) that want to ensure that they are genetically compatible (meaning that they are not carriers of the same recessive mutations) decreasing the risk of having an affected child associated with those mutations.

Due to that knowledge about genes and their variants is constantly growing and expanding, Geneseeker test and its associated panels are constantly being updated with the most up to date clinical information.

If you are interested in our test, we encourage to visit our webpage to see the current Geneseeker tests offered, and the included genes and variants that can be identified.

<https://junogenetics.eu/our-tests/gene-seeker/>
(information in English)

<https://www.junogenetics.es/nuestros-tests/gene-seeker/>
(information in Spanish)

May you have any doubt about the included information, don't hesitate to get in touch with our Geneseeker specialists team:
e-mail contact: geneseeker@junogenetics.com

They will provide first-hand information about the genes and variants included in our tests.

7.5.2 Requirements for accepting a GeneSeeker case

Although no specific patient requirements are needed for accepting a GeneSeeker case is important to notify JUNO GENETICS if the patient who is providing samples:

- has had a recent blood transfusion, less than 60 days, or
- has ever had a bone marrow transplant.

All these conditions may affect the results obtained from their blood samples, potentially leading to a misdiagnosis. In such cases, JUNO GENETICS may specifically request saliva or buccal samples from the individuals in question.

7.5.3 GeneSeeker patient preparation

Counselling:

Appropriate counselling for all patients who are being tested with GeneSeeker is extremely important. JUNO GENETICS strongly recommends that IVF clinics that may offer this genetics test make counselling mandatory for all patients requesting GeneSeeker. It is the responsibility of the IVF clinic to ensure that patients receive adequate counselling. JUNO GENETICS can be contacted if any advice is needed.

Required samples:

Usually a whole blood sample of the patient is needed to perform the GeneSeeker test. In case that additionally a couple wants to perform a matching study, samples from both patients are needed.

Consent form:

After receiving appropriate counselling about the procedure, patients undergoing GeneSeeker must sign an appropriate Consent Form. This Consent Form can be provided by JUNO GENETICS, or it can be prepared by the referring IVF clinic, after consultation with JUNO GENETICS. The signed Consent Form confirms that patients give their permission for the test to be carried out on their samples. A copy of the completed form should be sent to JUNO GENETICS along with the completed Test Requisition Form either via email, or via an upload in the JUNO GENETICS database.

Test requisition forms:

A completed Test Requisition form, for individuals or for couples, indicating the panel of the test to be tested must be sent to JUNO GENETICS. This info is used by JUNO GENETICS to confirm the specific test that should be carried out for each patient.

Sexual intercourse:

For maximizing the benefits of the GeneSeeker test it is strongly recommended that couples that aim to get pregnant refrain from having any unprotected intercourse until obtaining the results of the GeneSeeker test and obtaining appropriate advising about what implications may have to their offspring.

7.5.4 GeneSeeker sample preparation

Blood withdrawal of 3-5ml of blood should be collected in an EDTA tube. This blood draw should be performed either at the IVF clinic or at an appropriate health care centre where a trained phlebotomist is able to perform the sample collection.

Saliva and buccal collection kits can be sent directly to the patient or any relatives from JUNO GENETICS or they can be provided to the IVF centre. Instructions on how to use the collection kits always accompany the kit.

The sample collection container (blood tube, buccal swab, saliva container...) should be clearly labelled with the patient's identifying information (name, date of birth and patient number). The samples should then be placed in the supplied kit along with the completed and signed GeneSeeker Test Request Form and/or Informed consent.

The collected sample should be sent on the same day as collection. If this is not possible, the samples should be stored in a refrigerator (2-8 °C) and shipped within 2-3 days.

The kit should be properly sealed and shipped at room temperature using postal service or via a courier company JUNO GENETICS

JUNO

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