Document contents:

1.0 Location

Juno Genetics offices and laboratory are located in:
The Sherrington Building,
The Oxford Science Park
Robert Robinson Ave
Oxford OX4 4GA
United Kingdom.

Telephone: +44 (0) 0203 7431944

2.0 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 chromosome rearrangements (PGT-SR), and products of conception testing.

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, sperm and oocytes and 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 the international standard ISO 15189. 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: https://www.junogenetics.co.uk/

3.0 Opening hours

Juno Genetics opening hours are:
Monday - Friday: 8.30 am – 5.30 pm.
Juno Genetics operates throughout the year except all UK bank holidays.

4.0 Services

Reproductive genetic services offered by Juno Genetics include:

4.1. **Preimplantation genetic testing for chromosome aneuploidy (PGT-A)** in human embryos produced during assisted reproductive treatment.

4.2. **Preimplantation genetic testing for monogenic disorders (PGT-M)**

4.3. **Preimplantation genetic testing for structural chromosome rearrangements (PGT-SR)**

4.1. PREIMPLANTATION GENETIC TESTING for ANEUPLOIDY (PGT-A)

4.1.1. 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 of aneuploid pregnancy, although some risk of these occurrences remains.

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

- Advanced reproductive 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 appreciate that PGT-A is not a replacement for routine prenatal testing. It is recommended that prenatal testing should still be undertaken when indicated.

4.1.2. 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 cells biopsied from preimplantation embryos, which have been placed in microcentrifuge 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 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 Juno's PGT-A platform for detection of full aneuploidies is above 98%.

Although most PGT-A results are available within seven days from when the samples are received in the Juno Genetics laboratory, it can take up to ten days for results to be produced. It is therefore necessary for embryos to be cryopreserved after biopsy.

4.1.3. PGT-A patient 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. A copy of the completed form should be sent to Juno Genetics along with the completed Requisition Form either via email, or via an upload in the Juno Genetics database.

Biopsy form: A completed Biopsy Form must be sent to Juno Genetics along with the samples or via email. This form will be used by Juno Genetics to confirm the specific test that should be carried out for each patient.

Sexual intercourse: It is strongly advised that couples refrain from having any intercourse during the course of their treatment in order 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.

4.1.4. PGT-A Reporting

At Juno Genetics, PGT-A results are reported according to either presence (Positive) or absence (Negative) of uniform aneuploidies (detected in the entirety of TE biopsy).
Juno Genetics offers aneuploidy reporting for either **PRIMARY outcomes** (full aneuploidies) or **PRIMARY + SECONDARY** outcomes (including mosaicism).

Below is a brief explanation about the difference in our reporting between the PRIMARY and PRIMARY+SECONDARY outcomes:

- A 'Positive' result indicates presence of a full aneuploidy/ non-mosaic, and a 'Negative' result indicates that aneuploidy was not detected

There are two options for reports:

- **Primary outcomes:** Mosaicism not reported. Only 'whole chromosomal' and 'full segmental aneuploidies' reported. A 'Negative' result in this category means that no 'whole chromosomal' or 'full segmental aneuploidy' was detected. The 'Negative' result is either 46,XY/ 46,XX or 'mosaic'.
- **Primary and secondary outcomes:** Mosaicism is reported. A 'Negative' result in this category means that no form of aneuploidy was detected (including mosaicism). The result is either 46,XY/ 46,XX

The PGT consent forms are different for both these categories.

4.2. PREIMPLANTATION GENETIC TESTING FOR STRUCTURAL REARRANGEMENTS (PGT-SR)

4.2.1. 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 genes 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 removal (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 to be an alternative to prenatal screening and it is strongly recommended that routine prenatal testing (amniocentesis or chorionic villus sampling) is carried in the event that a pregnancy occurs, in order to confirm that the fetus is chromosomally normal.

4.2.2. Methods used for PGT-SR at Juno Genetics

Next Generation Sequencing (NGS) is the principal method used for PGT-SR at Juno Genetics. It involves the lysis of cells biopsied from preimplantation embryos, which have been placed in microcentrifuge 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 in excess of 10Mb in size.

Although most PGT-SR results are available within seven days from when the samples are received in the Juno Genetics laboratory, it can take up to ten days for results to be produced. It is therefore necessary for embryos to be cryopreserved after biopsy.

4.2.3. 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.

Genetic report(s): 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 a test can be offered to the patient.

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 in order 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. A copy of the completed form should be sent to Juno Genetics along with the completed Requisition Form either via email, or via an upload in the Juno Genetics database.

Biopsy Form: A completed Biopsy Form must be sent to Juno Genetics along with the embryo biopsy samples. This form will be used by Juno Genetics to confirm the specific test that should be carried out for each patient.

Sexual intercourse: It is strongly advised that couples refrain from having any intercourse during the course of their treatment in order 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.

4.2.4. Sample preparation for PGT-A and PGT-SR

Biopsy kit

Juno Genetics provides IVF clinics with biopsy kits that contain sterile microcentrifuge 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. Cool packs should be placed in the coldest available freezer (these will be used to keep samples cool when sending them to Juno Genetics).

Instructions for sending samples for PGT-A and PGT-SR

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. 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. Having fewer cells than this increases the chances of failing to obtain a result and could potentially reduce accuracy. Juno Genetics recommends the use of trophectoderm biopsy at the blastocyst stage. If testing at stages other than the blastocyst stage is needed, or if the number of cells obtained from a blastocyst is lower than the required three cells, Juno Genetics must be notified.

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 microcentrifuge tubes provided in the biopsy kits by Juno Genetics. It is extremely important that the total volume of buffer in the microcentrifuge tube (containing the biopsied cells) is in the range of 1-2 microliters. If the amount of fluid in the tube exceeds 2 microliters DNA amplification will be less efficient, yielding poor quality results or causing a total failure of the test. The microcentrifuge 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 name, ID, embryo number and another embryo identifier where possible (e.g. culture dish ID). Tubes containing biopsied cells should be kept on ice or in a special ice rack (e.g. Eppendorf® PCR Cooler, iceless cold storage system for 96 well plates and PCR tubes).
The Plastic racks containing the tubed cells should be clearly labelled with the patient's name, DOB and clinic ID.

Dry runs/ Biopsy validation

Before initiation of clinical service, it is recommended that the referring IVF clinic `send 10 "dry run" samples per embryologist to Juno Genetics. These "dry run" samples should be TE samples (5-10cells), biopsied from embryos that are unsuitable for transfer, and/or have been donated for research. The TE sample should be collected in ~2 µl of wash buffer and should be placed into one of the supplied 0.2 ml PCR tubes. The sample should be clearly labelled with the embryo number. The chosen labelling scheme should be clearly transcribed on the biopsy form.

Shipping samples 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.

4.2.5. PGT-SR Reporting

At Juno Genetics, PGT-SR results are reported according to either presence (Positive) or absence (Negative) of uniform aneuploidies (detected in the entirety of TE biopsy).
Juno Genetics offers aneuploidy reporting for either **PRIMARY outcomes** (full aneuploidies) or **PRIMARY + SECONDARY** outcomes (including mosaicism).

Below is a brief explanation about the difference in our reporting between the PRIMARY and PRIMARY+SECONDARY outcomes:

- A 'Positive' result indicates presence of a full aneuploidy/ non-mosaic, and a 'Negative' result indicates that aneuploidy was not detected

There are two options for reports:

- **Primary outcomes:** Mosaicism not reported. Only 'whole chromosomal' and 'full segmental aneuploidies' reported. A 'Negative' result in this category means that no 'whole chromosomal' or 'full segmental aneuploidy' was detected. The 'Negative' result is either 46,XY/ 46,XX or 'mosaic'.
- **Primary and secondary outcomes:** Mosaicism is reported. A 'Negative' result in this category means that no form of aneuploidy was detected (including mosaicism). The result is either 46,XY/ 46,XX

The PGT consent forms are different for both these categories.

4.3. PREIMPLANATION GENETIC TESTING for MONOGENIC DISORDERS (PGT-M)

4.3.1. PGT-M General Information

The main aim of PGT-M is to help couples who are affected by a genetic disorder or are mutation carriers 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 of 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 are able to act as a 'reference' (see below)

Turnaround Time (TAT) for the development of a PGT-M test is 4-6 weeks, beginning from the date that all of the above are received.

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 of any intention to biopsy polar bodies, blastomeres, or fewer than the minimum recommended number of trophectoderm cells (i.e. less than three). Each biopsy specimen is placed in an individual microcentrifuge tube (provided), which is then shipped to Juno Genetics for testing. The embryos remain in the IVF clinic at all times.

PGT-M results are available within two weeks from when the embryo biopsy samples are received in the laboratory, and therefore it is necessary to cryopreserve the embryos after biopsy.

4.3.2. PGT-M patient preparation

Licenses for Monogenetic Disorder testing: Of note, in some countries a license may be required before PGT-M can be provided for a particular disorder or family**.** It is the responsibility of the IVF clinic to obtain any license required, although Juno Genetics will offer any assistance it can to support clinics applying for new licenses.

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 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 individual, 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 in order 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 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 non-adult 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 sampled 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 advised that couples refrain from having any intercourse during the course of their treatment in order 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.

4.3.3. Sample preparation for PGT-M

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

Blood samples from the couple are required for the test design (a minimum of 5 ml in EDTA tubes with lavender-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 in order 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 spongey applicators to soak up DNA from the cheek pouch. Such kits can be useful when DNA must be obtained from babies and elderly people.

It is important to notify Juno Genetics if the patients and other individuals who are providing samples have ever had a blood transfusion or bone marrow transplant 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 or buccal 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 ambient 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 microcentrifuge 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 shipping 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. Cool packs should be placed in the coldest available freezer (these will be used to keep samples cool when sending them to Juno Genetics).

4.3.4. 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 a minimum of three intact cells. Having fewer cells than this increases the chances of failing to obtain a result and could potentially reduce accuracy. Juno Genetics recommends the use of trophectoderm biopsy at the blastocyst stage, it. If testing at stages other than the blastocyst stage is needed, or if the number of cells obtained from a blastocyst is lower than the required three cells, Juno Genetics must be notified.

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 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 microcentrifuge tubes provided in the biopsy kits by Juno Genetics. It is extremely important that the total volume of buffer in microcentrifuge tube (containing the biopsied cells) is in the range of 1-2 microliters. If the amount of fluid in the tube exceeds 2 microliters DNA amplification will be less efficient, yielding poor quality results or causing a total failure of the test. The microcentrifuge 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 name, ID, embryo number and another embryo identifier where possible (e.g. culture dish ID). Tubes containing biopsied cells should be kept on ice or in special ice rack (e.g. Eppendorf® PCR Cooler, iceless cold storage system for 96 well plates and PCR tubes).

The Plastic racks containing the tubed cells should be clearly labelled with the patient's name, DOB and clinic ID.

Dry runs/ Biopsy validation

Before initiation of clinical service, it is recommended that the referring IVF clinic send 10 "dry run" samples per embryologist to Juno Genetics. These "dry run" samples should be TE samples (5-10cells), biopsied from embryos that are unsuitable for transfer, and/or have been donated for research. The TE sample should be collected in ~2 µl of wash buffer and should be placed into one of the supplied 0.2 ml PCR tubes. The sample should be clearly labelled with the embryo number. The chosen labelling scheme should be clearly transcribed on the biopsy form.

4.3.5. 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 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.

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 (see below). 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.

Please send the relevant samples to:
The Hayakawa Building,
The Oxford Science Park
Edmund Halley Rd, Littlemore
Oxford OX4 4GB
United Kingdom.

Please send all the forms to the email: pgtm@junogenetics.com

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

Once all DNA samples are received in the laboratory (along will all accompanying documentation), DNA will be extracted from the blood or buccal samples and we will begin our test design which can take 4-6 weeks to be completed. Please note that in order for a test to be signed off, the fully completed consent form needs to have also been received by the laboratory. Once a test design is completed, our team will notify the IVF clinic via email. It is essential that IVF clinics wait for this notification before starting the couple's treatment.

Fees for Testing: Please refer to the price list for related fees and contact the Juno Genetics Team if you have any queries (+44 203 743 1944 or accounts@junogenetics.com).

Sexual Relations: We strongly recommend that the patients be counselled not to have intercourse during the entire treatment cycle.

PERMISSION TO START THE CYCLE: ***It is essential that the patient does not begin her treatment cycle until our preliminary analyses are completed*** and we have confirmed that the test is working properly. We will notify you as soon as these analyses are complete so that the patient can begin her cycle.

Please note that we need a Biopsy or Requisition Form for each case, new or repeat.

5.0. SAMPLE ACCEPTANCE AND REJECTION CRITERIA – ALL TESTS

5.1. Sample acceptance criteria

Any sample received at the Juno Genetics laboratory should be clearly labelled with the patient's name, date of birth and clinic number (if applicable). Samples that belong to a patient should also be accompanied by a consent form, a test requisition form (only applicable for PGT-M, and POC tests), and a biopsy sheet for embryo biopsy samples for PGT-A, PGT-M or PGT-SR.

For samples provided by relatives for PGT-M or PGT-SR test design, the relationship to the patient and patient's details (including IVF centre where treatment is taking place) should be clearly provided to avoid any confusion. If any of the information provided is unclear, the IVF centre will be contacted by Juno Genetics staff to confirm these details.

It is important to inform Juno Genetics staff on the day of shipping any type of samples along with tracking information and the expected arrival date. This allows Juno Genetics staff to track the shipment and, in case of any delay, communicate with the shipping company.

5.2. Sample rejection criteria

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, taking into account 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 criteria will lead to a sample being considered for rejection:

- Broken specimen container
- Incorrect specimen container
- Leaking sample
- Inadequately labelled specimen container (this includes illegible writing)
- Incorrect sample received.
- Sample received thawed (if required to be sent frozen) or sent frozen (when required to be room temperature)
- Forms received with the sample are blood-stained.
- Missing information on requisition form
- If the sample looks to be in an unsuitable condition (e.g. clotted in the case of blood samples)
- Biopsy tubes not corresponding to those listed on the biopsy form.

5.3. 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 preimplantation genetic testing of their embryos receive genetic counselling about the procedure. Informed consent must be obtained from the patient in order to perform PGT and genetic counselling is an important part of this process. It is ideal that all patients speak to a genetic counsellor with previous experience of PGT, as all the risks, limitations, and potential outcomes should be discussed with the patient. It is also an HFEA requirement that all patients are appropriately counselled before undergoing treatment. Please contact Juno Genetics if any advice is needed in regard to seeking a genetic counsellor, or for further enquiries into the selected test.

6.0. GENERAL INFORMATION

6.1. Embryo biopsy and cell tubing training

Embryo biopsy and cell preparation and tubing are essential steps to ensure accurate PGT results. Therefore, Juno Genetics highly recommends that all IVF clinics perform biopsy and cell tubing trial runs using non-viable or discarded embryos (donated by the patients for training purposes). Juno Genetics will process these samples and issue a report for the clinic detailing the proportion of samples that successfully yield a result and the frequency of problems such as DNA contamination. This service is part of Juno Genetics' effort to support clinics in obtaining the best possible results and it is offered free-of-charge. In the case of sub-optimal results following a trial run, Juno Genetics personnel can advise on what measures might be undertaken in order to improve results.

6.2. Documentation

Upon clinic onboarding, Juno Genetics will provide documentation, including procedural instructions, consent form templates, and kit guidelines. For any support related to clinical onboarding, clinics can contact the Lab Director.

6.3. Data confidentiality and sample protection

Patient confidentiality will be maintained at all times. Only staff of Juno Genetics, the referring fertility clinic and agencies involved with monitoring, regulating or accrediting laboratories will have access to any records. Juno Genetics shall comply with the principles of the EU General Data Protection Regulation (GDPR); the UK's equivalent of which is the Data Protection act of (2018).

6.4. Provision of patient and family genetic examination results

To enable Juno Genetics staff to establish if a test for a particular single-gene disorder can be offered, we must be provided with the relevant documentation concerning the family's genetic disorder. This includes a family pedigree clearly showing the mutation status of each family member and all relevant DNA diagnostic reports [which must state the mutation(s) present in the couple]. Once this documentation has been received the clinic will be informed whether PGT-M can be offered for the particular mutation and from which family members blood or buccal samples will be needed.

Genetic reports are also required in advance for couples with a translocation looking to proceed with PGT-SR. A genetic report from the affected/carrier individual (stating the karyotype) should be emailed to Juno Genetics to allow us to establish if this translocation can be detected. Upon receipt of this documentation, the clinic will be informed whether the case can be accepted.

6.5. Turnaround Times

Turnaround times for the tests offered by Juno Genetics are as follows:

PGT-A (PGTSeq platform) – within 7 business days from receipt of embryo biopsy specimens by Juno Genetics

PGT-SR (PGTSeq platform) – within 7 business days from receipt of embryo biopsy specimens by Juno Genetics

PGT-M (test development) – 6-8 weeks (excluding bank holidays) beginning once all information and samples have been received by Juno Genetics (see section on PGT-M concerning required genetic reports and samples)

PGT-M (testing of embryo biopsy specimens) – within 10 business days from receipt of embryo biopsy specimens by Juno Genetics

6.6. Verbal requests

Any verbal requests to laboratory staff must be followed up by a written request to the laboratory team via email in order for it to be logged and acted on appropriately.

6.7. Juno Genetics Portal

Juno Genetics offers a clinical portal for submitting test requests, ordering kits, and accessing reports and documentation. Each clinic is granted a secure, unique login. Upon request, Juno will provide a demonstration and grant access to the portal.

6.8. Third Party Supplier Agreements

Clinics can request a Third-Party Agreement template from Juno Genetics.

6.9. User satisfaction and Complaint procedure

Juno Genetics is in regular communication with all clinics that refer samples. Customer comments, complaints and suggestions made during the course of routine communications are logged, discussed at laboratory meetings and, where appropriate, acted upon. Additionally, Juno Genetics requests that users of the service formally submit feedback once per year. A User Satisfaction Survey form is provided to clinics in order to facilitate this process. The results of the survey are reviewed by the Laboratory Director and senior staff and the findings are then disseminated to the rest of the Juno Genetics team at a laboratory meeting, along with explanation of any improvements that may be necessary.

The Juno Genetics management team continuously evaluates the selection of genetic tests which are offered to users and the way in which the results are reported. When feedback suggests that additional diagnostic tests may be desired, or that the reporting of results is not as clear as possible, these procedures will be examined to identify if these problems can be rectified without diminishing the quality of data or its clinical interpretation. Complaints can be made in writing directly to the laboratory director.

7.0. Reference to UKAS Accreditation Logo and Symbols

The laboratory's use of the relevant UKAS accreditation mark will follow the Office for Product Safety & Standards document, "Accreditation Logo and Symbols, the National Accreditation Logo and Symbols: Conditions for use by UKAS and UKAS Accredited Organisations".

Juno Genetics Ltd may use the relevant accreditation mark on stationery, quotations for work, reports and brochures, and other items relevant to the organisation's accredited activity, subject to the conditions set out in the publication.

Where a test performed by a non-accredited laboratory is reported as part of accredited results, the non-accredited test shall clearly be marked as 'Not UKAS accredited'.

Juno Genetics will not incorporate any reference to the laboratory's UKAS accreditation (either by use of the relevant national accreditation symbol or in words) when reporting results from non-accredited tests.