IGENOMIX LABORATORY
USER MANUAL

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1 IGENOMIX LABORATORY

1.1 INTRODUCTION

IGENOMIX LABORATORY (onwards Igenomix), is a private medical testing laboratory (License Number 10800) specialising in reproductive genetic services and is part of Vitrolife group a company with headquarters in Göteborg, Sweden.

Igenomix currently performs different tests in-house that can summarized as the following: Endometrial Receptivity Analysis (ERA), Endometrial Microbiome Metagenomic Analysis (EMMA), Analysis of Infectious Chronic Endometritis (ALICE), Preimplantation Genetic Testing for Monogenic Disorders (PGT-M), Preimplantation Genetic Testing for Aneuploidy (PGT-A), and Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR); Genetic Testing for Products of Conception (POC), Carrier Genetic Test (CGT), Sperm Aneuploidy Testing (SAT), and Embryo priority test (EMBRACE).

OPENING TIMES

The laboratory facilities for sample reception are open:

+ from Monday to Friday 8:00am to 6:00pm

Customer Support service is available:

+ From Monday to Friday 8:00am – 6:00pm

1.2 CONTACT DETAILS

General Enquiries contact details:

+ by email to supportspain@igenomix.com

+ by Tel: +34 96 390 53 10 (ext 1)

Kit request contact details:

+ by email to pickup@igenomix.com

+ by Tel: +34 96 502 34 60

1.3 LAB ADDRESS

IGENOMIX LABORATORY

Parque Tecnológico de Paterna

Calle Narcís Monturiol Estarriol nº11 Parcela B, Edificio Europark

46980 – Paterna. Valencia, SPAIN

2 MAIN ACTIVITIES

2.1 GENERAL INFORMATION

All genetic tests are carried out as clinically appropriate. Additional information regarding the different tests offered is available to users on the IGENOMIX's Europe website and can also be requested by email to: supportspain@igenomix.com. In addition, test can also be requested by our clinic portal, a platform where you can request the test and also have access to the results of all the reports, for further information, write us to supportspain@igenomix.com or call us in +34 96.390.53.10 (ext 1).

Further interpretation of reports is available to users by calling the laboratory (+34 96 390 53 10) and requesting to speak with a test senior member of staff. Clinical advice on ordering of examinations and on interpretation of examination results is available to users.

2.2 COMPLAINT PROCEDURE

The laboratory is committed to delivering service of the highest quality at all times to ensure patient safety and customer satisfaction. To process a complaint, you can contact us through different channels:

+ by Email write us to supportspain@igenomix.com

+ by phone: call us in +34 96.390.53.10 (ext 1)

+ through our "request information" section

+ through the satisfaction survey included in the Quality section, accessible on our website.

All complaints will be answered in less than 2 working days. Take into account that in some cases, an effective complaint resolution may require some additional days.

2.3 LABORATORY POLICY ON PROTECTION OF PERSONAL INFORMATION

The laboratory follows strict policies on Information Governance and maintains a data protection infrastructure in line with REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL ('GDPR').

Further information about Igenomix Spain's Privacy Policy can be found on the IGENOMIX's Europe webpage

2.4 REQUIREMENTS PRIOR TO SENDING A SAMPLE

Given the complexity of the genetic tests and the significant implications of the test results, the tests must be prescribed by competent healthcare professionals (usually doctors) and the results obtained must be interpreted in conjunction with other clinical data, within the general context of a medical practice run by healthcare professionals.

Before referrals can be made, users need to complete the "Clinic Enrollment Form" (for healthcare professionals), or the "Customer Enrollment Form" (for patients) which can be requested by email from supportspain@igenomix.com. Once the form is completed it should be returned by email to supportspain@igenomix.com.

The Test Requisition Form and the Informed Consent Form (if applicable) need to be completed, placed into the provided return courier envelope, and included in the kit box along with the sample to be sent to the laboratory.

Any Test Requisition Form or Test Informed Consent can be requested by email from supportspain@igenomix.com.

For ERA, EMMA, ALICE and ENDOMETRIO test cases, these forms are included into the EndomeTRIO kit, provided for the sample taking.

Igenomix highly recommends that the test instructions, which can be found on the Igenomix webpage or requested from our Customer Support Service by email or phone (see section **Error! Reference source not found.**), are carefully read prior to sending samples. These documents provide relevant information about sample requirements, patient preparation, test documentation, sample collection and sample shipping for the different tests offered.

2.5 LABORATORY CRITERIA FOR ACCEPTING AND REJECTING SAMPLES

The following cases may lead to sample rejection:

- Samples not accompanied with their documentation (Test Requisition Form and Informed Consent).
- Sample documentation (Test Requisition Form and Informed Consent) has not been correctly completed.
- Mandatory fields in sample documentation, identified on the forms with an asterisk (*), have not been completed.
- Missing patient and/or clinician signature on the Test Requisition and Informed Consent.
- Incorrectly labelled, unlabeled or damaged sample containers (usually tubes).
- Using an outdated version of the Test requisition Form and/or Informed Consent form may delay the report or lead to sample rejection.
- Failure to meet the specific test requirements indicated in the test

instructions (for example, specific timings of sample collection, minimum amounts of sample, specific biological status of patient, etc.)

2.6 INSTRUCTIONS FOR COMPLETION OF REQUEST DOCUMENTATION

All the forms clearly state the mandatory fields to be completed. The Test Requisition Form must be signed by the referring clinician. The Informed Consent form must be signed by the patient.

In most of the Igenomix tests, the Test Requisition Form and the Informed Consent are combined within the same document. In those cases, you can find the signature boxes for both the clinician and the patient at the end of the combined form. For PGT family tests (PGT-A, PGT-SR and PGT-M) and some other tests the signature box for patients can be found at the end of the informed consent form.

Please review carefully the documents associated to each test. Feel free to contact Igenomix Customer Support if you have any concerns about the appropriate completion of these forms.

Additionally, note that verbal request is not accepted.

3 TESTS OFFERED

3.1 Tests performed in-house

The laboratory currently performs the following major tests in-house: Preimplantation Genetic Testing for Monogenic Diseases (PGT-M), Preimplantation Genetic Testing for Aneuploidy (Smart PGT-A), Preimplantation Genetic Testing for Chromosomal Rearrangements (PGT-SR), Endometrial Receptivity Analysis (ERA), Endometrial Microbiome Metagenomic Analysis (EMMA); Analysis of Infectious Chronic Endometritis (ALICE), Carrier Genetic Test (CGT), Sperm Aneuploidy Testing (SAT), Testing for Products of Conception (POC), and Embryo priority test (EMBRACE),

In addition to the above tests, the laboratory offers the following tests performed in-house:

- Molecular studies: Fragile-X syndrome (CCG expansion), Cystic fibrosis (study 50 frequent mutations); Y Chromosome Microdeletions; G20210A Prothrombin (FII) Analysis; G1691A Factor V Leiden Analysis; C677T and A1298C MTHFR Analysis; Spinal muscular atrophy (deletion exons 7/8).

If you require additional information about our test portfolio, please contact our Customer Support service.

3.2 Preimplantation Genetic Testing for Monogenic Diseases (PGT-M)

PGT-M test description:

PGT-M may be performed on embryos during in vitro fertilization (IVF) treatment to test for single gene diseases or to perform HLA matching. PGT-M, requiring only a small number of cells, identifies which embryos are not at an increased risk of developing the disease tested. The goal of PGT-M is to help couples start a "healthy" family and avoid the difficult choice of having to terminate a pregnancy if a "positive" result is obtained through prenatal diagnosis. PGT- M is performed by using PCR.

Pre-requirements for accepting a PGT-M case:

Prior to offering PGT-M, the genetic reports for the affected partner and for certain family members with known disease status must be available and sent to the laboratory of Igenomix. The report must clearly identify the gene and the mutation responsible for the disease/disorder to be tested by PGT-M. Family history information relating to the disease is also necessary to assess the case properly. With this information, Igenomix will give an answer about the technical viability of PGT-M and will require the samples needed for the PGT-M workup (pre-PGT-M) test. A case discussion with a senior member of laboratory staff will be required in certain instances. The scenarios where PGT-M can be considered include autosomal dominant disorders, autosomal recessive disorders, X-liked disorders and HLA matching.

NOTE: Embryo sex will be revealed when reporting PGT-M for X-linked disorders.

PGT-M test sample requirements:

For pre-PGT-M, a minimum of 1x3 ml of peripheral blood (in EDTA tubes) and/or a buccal swab (less recommended) from the prospective parents and other relevant family members is needed. Based on the outcome of pre-PGT-M, the laboratory will inform the IVF clinic by email whether PGT-M can be offered or not. The patients can then start their treatment towards PGT-M or seek alternative treatment which can be further discussed with a senior member of laboratory staff.

A trophectoderm biopsy on day five or six is needed for the PGT-M. Ask the lab about the possibility of performing PGT-M on day 3 biopsy if needed.

The solution used for "washing/tubing" the biopsied cells is provided by Igenomix. The biopsied cells must be "tubed" in sterile 0.2ml microcentrifuge tubes provided by Igenomix. The lid of these tubes must be labelled with the female patient initials followed by the embryo number. The "plate/rack" in turn is placed in a sterile plastic bag in a cooler with "ice packs" also provided by the laboratory.

Further information on how to prepare a sample can be found and downloaded from the Igenomix website or requested by email from our Customer Support service, see section 1.3. The "Embryo Biopsy Worksheet" and the "Test Requisition Form" (included within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and placed in a plastic sleeve inside the cooler prior to transport.

Professional user validation for PGT tests (DRY RUN'):

Following the enrolment of a new clinic (see section 2.4), we recommend performing a "validation" or "dry run" for every embryologist involved in the embryo biopsy/tubing for PGT-M. This process aims to provide reduce the likelihood of difficulties with clinical cases that could lead to a failure to determine a result(s) for the sampled embryo(s). Instructions on how to complete a "validation run" can be requested by email. A validation/dry run report is issued after the analysis and signed by a senior member of laboratory staff or the Laboratory Director.

PGT-M sample transportation to the laboratory:

For PGT-M workup (pre-PGT-M), blood samples and/or buccal swabs should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by Igenomix but outsourced to a third-party logistics company). Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack.

For PGT-M, the clinic needs to notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The PGT kit provided by Igenomix must be used for the shipment, including the cooler box. **Freeze the ice packs, cool-rack and biopsied samples before shipment.** The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled as 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples, please review the test instructions included on the Igenomix website or contact Igenomix Customer Support service (see section 1.3).

PGT-M test turnaround time (TAT):

The clinician that has requested the test will receive the results.

Pre-PGT-M results will be available **within 3 weeks** for common mutations and **6 weeks** for the non-frequent mutations, from receipt of samples by Igenomix.

PGT-M results will be available **within 10 working days** from receipt of samples by Igenomix.

PGT-M Reporting: diferente en otra versión

For pre-PGT-M the following results can be obtained:

- PGT-M for the familiar mutation can be offered.
- PGT-M for the familiar mutation cannot be offered.

For PGT-M the following results can be obtained, for each embryo, as a result of performing this test:

Cases with Recessive Inheritance

- **Non-carrier:** Embryo not found to have inherited either of the "at-risk haplotypes". This embryo is at reduced risk of being affected by the indicated genetic condition.
- **Carrier:** Embryo found to have inherited one parental "at-risk haplotype". This embryo is at reduced risk of being affected by the indicated genetic condition. Please refer to the original genetic report, a medical geneticist, and/or a clinical genetic counsellor for additional information regarding the potential implications, if any, of carrier status.
- **At-risk:** Embryo found to have inherited both "at-risk haplotypes". This embryo is at-risk of being affected by the indicated genetic condition. Please refer to the original genetic report, a medical geneticist, and/or a clinical genetic counsellor for additional information regarding clinical features of this condition. PGT-M cannot determine clinical presentation or severity of symptoms.

Cases with Dominant Inheritance

- **Low-risk:** Embryo not found to have inherited the parental "at-risk haplotype". This embryo is at reduced risk of being affected by the indicated genetic condition.
- **At-risk:** Embryo found to have inherited the parental "at-risk haplotype". This embryo is at-risk of being affected by the indicated genetic condition. Please refer to the original genetic report, a medical geneticist, and/or a clinical genetic counsellor for additional information regarding clinical features of this condition. PGT-M cannot determine clinical presentation or severity of symptoms.

Cases with X-linked Inheritance

- **Low-risk:** Embryo not found to have inherited the maternal "at-risk haplotype". This embryo is at reduced risk of being affected by the indicated genetic condition.
- **Carrier:** Embryo found to have inherited the maternal "at-risk haplotype". The clinical presentation for carriers of X-linked conditions can vary broadly. The possibility of a carrier presenting with some features of the condition due to skewed X-inactivation cannot be eliminated. Please refer to the original genetic report, a medical geneticist, and/or a clinical genetic counsellor for additional information regarding the potential implications, if any, of carrier status.
- **At-risk:** Embryo found to have inherited the maternal "at-risk haplotype". This embryo is at-risk of being affected by the indicated genetic condition. Please refer to the original genetic report, a medical geneticist, and/or a clinical genetic counsellor for additional information regarding clinical features of this condition. PGT-M cannot determine clinical presentation or severity of symptoms. For conditions with X-linked dominant inheritance, male and female embryos inheriting the at-risk haplotype will be at risk for

symptoms.

No DNA detected

DNA was not detected, due to the absence of, or degraded DNA.

Non-informative

A reliable result could not be achieved due to factors such as Allele Drop Out (ADO), parental/external contamination, recombination and others.

3.3 Preimplantation Genetic Testing for Aneuploidy (Smart PGT-A)

Smart PGT-A test description:

Smart PGT-A is a genetic test that may be performed on embryos during IVF treatment to screen for numerical chromosomal abnormalities. Chromosomally normal embryos are most likely to implant and develop to term. Smart PGT-A helps clinicians and patients undergoing IVF decide which embryos to transfer. The method, requiring only a small number of cells, is comprehensive as it analyses all 24 chromosomes for chromosomal copy number using Next Generation Sequencing (NGS).

Pre-requirements for accepting a Smart PGT-A case:

No specific pre-requirements are needed in order to accept a case. Specific test indications and relevant clinical information can be reported in the test requisition form.

Pre-requirements for accepting a Smart PGT-A Plus case:

No specific pre-requirements are needed in order to accept a case. Specific test indications and relevant clinical information can be reported in the test requisition form.

Smart PGT-A sample requirements:

For Smart PGT-A, one cell from day 3 of embryonic development (blastomere biopsy) or 4-8 cells from day 5, 6 or 7 of embryonic development (trophectoderm biopsy) are required. The biopsied cell/s must be cleaned using the “washing/loading buffer” supplied by the laboratory to eliminate any potential source of contamination and transferred to a small 0.2ml tube supplied by the laboratory. The lids of these tubes must be labelled with the female patient initials followed by the embryo number. The 0.2ml tubes must be placed in the “plate/rack” provided by the laboratory, the “plate/rack” placed in a plastic bag and inside the cooler shipping box with the “ice packs” also provided by the laboratory. Further information on how to prepare a sample can be found and downloaded from the website or requested by email to the Igenomix Customer Support service (see section 1.3).

The “Embryo Biopsy Worksheet” and the “Test Requisition Form” (included within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and sent with the samples inside the shipping box or by e-mail to the laboratory.

Professional user validation for PGT tests (DRY RUN):

Following the enrolment of a new clinic (see section 2.4), we recommend performing a "validation" or "dry run" for every embryologist involved in the embryo biopsy/tubing for Smart PGT-A. This process aims to reduce the likelihood of difficulties with clinical cases that could lead to a failure to determine a result(s) for the sampled embryo(s). Instructions on how to complete a "validation run" can be requested by email. A validation/dry run report is issued after the analysis and signed by a senior member of laboratory staff or the Laboratory Director.

Smart PGT-A sample transportation to the laboratory:

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. Igenomix provides a PGT Kit and a thermal box with a cool-rack for the shipment of biopsies. **Freeze the ice packs, and cool-rack before the shipment.** The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled as 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

Smart PGT-A test turnaround time:

The clinician that has requested the test will receive the results.

For Smart PGT-A samples with **deferred transfer** results will be available **within 7 working days** from receipt of samples by Igenomix.

For Smart PGT-A samples with **fresh transfer** results will be available **on the morning of the day following the receipt of samples** by Igenomix.

Smart PGT-A reporting:

Igenomix uses an internal validated algorithm for whole chromosome aneuploidies, segmental gains/losses and mosaicism calling. The following results can be obtained, for each embryo, as a result of performing this test:

- **Euploid:** when mosaic aneuploidy levels are <30%, and no segmental gains/losses ≥10Mb in size are detected.
- **Mosaic:** mosaicism is defined when there is a combination of chromosomally normal cells and others with aneuploidy. An embryo is considered mosaic when the level of aneuploidies detected in the biopsy is greater than 30% and less than 70%. Mosaic samples are reported as either "low mosaic" when having 30%-<50% aneuploid cells, or "High mosaic" when having ≥50%-<70% aneuploid cells. Only high mosaicism for segmental gains/losses and sex chromosome aneuploidies are reported. Embryos that have another uniform

aneuploid chromosome are never reported as mosaic but as abnormal/aneuploid.

- **Aneuploid:** when mosaic aneuploid levels are ≥70% and/or segmental gains/losses ≥10Mb in size are detected.
    - Complex aneuploid: when 2-5 aneuploidies are detected in the provided sample.
    - Chaotic: when six or more aneuploidies are detected in the provided sample.
- **No DNA detected:** when insufficient DNA is detected in the sample.
- **Non informative:** when a reliable result cannot be achieved with high confidence.

If no mosaicism information is requested by the clinic/user, the threshold for euploid and aneuploid is considered 50%. Therefore, embryos with mosaic levels below 50% are reported as euploid, and embryos with mosaic levels ≥50% are reported as aneuploid.

MitoScore: The MitoScore test can also be performed on the DNA obtained from the same biopsy to identify which embryos could have a greater capacity and suitability for achieving pregnancy. The level of mitochondrial DNA (MitoScore) is obtained using NGS, which offers information on both nuclear and mitochondrial DNA.

Smart PGT-A Plus test description: Smart PGT-A Plus is an optional analysis that includes Smart PGT-A for 24 chromosome aneuploidy screening and adds ploidy assessment, cohort check and contamination testing. Triploidy (when there is an additional set of all chromosomes) and haploidy (when there is only a single set of all chromosomes) are the most common ploidy abnormalities and are incompatible with normal growth and development. Smart PGT-A Plus includes a QC analysis for embryos, confirming that embryos within a cohort demonstrate the expected genetic relatedness and assessing for DNA contamination. This analysis can reduce the risk of misdiagnosis and provide additional reassurance about the IVF process.

Smart PGT-A Plus test turnaround time:

The clinician that has requested the test will receive the results.

The **turn-around time** for Smart PGT-A Plus is up to 21 working days.

Smart PGT-A Plus reporting:

Additional to the Smart PGT-A results, the following results can be obtained for each embryo when Smart PGT-A Plus (ploidy, contamination, cohort check) is requested:

Ploidy results:

- **Diploid:** Ploidy analysis is consistent with 2 sets of chromosomes and a diploid state (2N).
- **Triploid:** Ploidy analysis is consistent with 3 sets of chromosomes and a triploid state (3N).
- **Haploid:** Ploidy analysis is consistent with a single set of chromosomes and a haploid state (1N).
- **Ploidy non-informative:** Ploidy analysis is non-informative.

Contamination results:

- **Contamination detected:** Admixture of exogenous DNA is detected in the sample analyzed.

Cohort check results:

- **Consistent:** The sample demonstrates the expected genetic relatedness to other samples submitted for the patient, suggesting that the sample belongs to the patient's cohort.
- **Inconsistent:** The sample does not show the expected genetic relatedness to other samples submitted for the patient.
- **Cohort check non-informative:** genetic relatedness analysis between the sample and other samples in the patient's cohort is non-informative.
- **Not applicable:** The cohort check is not performed for embryos with haploid or triploid results, for contaminated samples, when there is only one embryo available for analysis, or when insufficient DNA is detected in the PGT-A analysis.

3.4 Preimplantation Genetic Testing for structural rearrangements (PGT-SR)

PGT-SR test description:

PGT-SR is a genetic test to detect specific chromosomal imbalances in embryos arising from parental chromosomal rearrangements. The test will also detect numerical chromosomal abnormalities not associated with parental chromosomal rearrangement. This method uses NGS to analyze all 24 chromosomes and requires multiple trophectoderm cells from a blastocyst biopsy. Currently, PGT-SR at Igenomix has been validated to detect chromosomal abnormalities that are ≥ 6Mb.

Pre-requirements for accepting a PGT-SR case:

Before planning a PGT-SR cycle, the couple must provide the karyotype report of the structural rearrangement to their prescribing physician for Igenomix staff review, who will request, if required, a pre-PGT-SR study. Pre-PGT-SR consists of a genetic study prior to the commencement of a PGT-SR cycle. This study is performed on a DNA sample of the carrier of

a structural chromosomal rearrangement, to confirm whether it is possible to address the case through PGT-SR and establish the diagnostic strategy to be applied in the PGT-SR cycle.

PGT-SR test sample requirements:

For pre-PGT-SR (if required), 4 mL of peripheral blood (in EDTA or Heparin-Lithium tubes, as requested by the Igenomix staff to the prescribing physician) from the carrier of the structural chromosomal rearrangement (and/or other family members if required) are needed. Based on the outcome of the pre-PGT-SR, the laboratory will inform the IVF clinic by email whether PGT-SR can be offered.

For PGT-SR, 4-8 cells from day 5, 6 or 7 of embryonic development (trophectoderm biopsy) are required. The biopsied cell/s must be cleaned using the “washing/loading buffer” supplied by the laboratory to eliminate any potential source of contamination and, transferred to a small 0.2ml tube supplied by the laboratory. The lid of these tubes must be labelled with the female patient initials followed by the embryo number. The 0.2ml tubes must be placed in the “plate/rack” provided by the laboratory, the “plate/rack” placed in a plastic bag and inside the cooler shipping box with the “ice packs” also provided by the laboratory.

Further information on how to prepare a sample is found in the “Washing Tubing Instructions” that can be downloaded from the Igenomix website or requested by email. The “Embryo Biopsy Worksheet” and the “Test Requisition Form” (included within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and sent with the samples inside the shipping box or by e-mail to the laboratory.

Professional user validation for PGT-SR tests (DRY RUN):

Following the enrolment of a new clinic (see section 2.4), we recommend performing a “validation” or “dry run” for every embryologist involved in the embryo biopsy/tubing for PGT-SR. This process aims to provide reduce the likelihood of difficulties with clinical cases that could lead to a failure to determine a result(s) for the sampled embryo(s). Instructions on how to complete a “validation run” can be requested by email. A validation/dry run report is issued after the analysis and signed by a senior member of laboratory staff or the Laboratory Director.

PGT-SR sample transportation to the laboratory:

For pre-PGT-SR, blood samples should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or “Packaging Instructions P650” and clearly labelled 'diagnostic specimen UN3373 when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company). The carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack.

For PGT-SR The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. Igenomix provides a PGT Kit and a thermal box with a cool-rack for the shipment of biopsies: **freeze the ice packs, cool-rack and biopsied samples before shipment.** The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or “Packaging Instructions P650” and clearly labelled 'Exempt

Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

PGT-SR test turnaround time:

The clinician that has requested the test will receive the results.

For pre-PGT-SR, results will be available **within 4 weeks** from receipt of samples by Igenomix.

For PGT-SR samples with **deferred transfer** results will be available **within 7 working days** from receipt of samples by Igenomix.

For PGT-SR (Robertsonian translocation only) samples with **fresh transfer** results will be available **on the morning of the day following the receipt of samples** by Igenomix.

PGT-SR reporting:

For **pre-PGT-SR** there are two possible results:

- The structural alteration that is the subject of study for pre-PGT-SR **can be detected;** therefore, PGT-SR can be offered.
- The structural alteration that is the subject of study for the pre-PGT-SR **cannot be detected**; therefore, The PGT-SR cannot be offered.

For PGT-SR, Igenomix uses an internal validated algorithm for whole chromosome aneuploidies, segmental gains/losses and mosaicism calling. The following results can be obtained as a result of performing this test:

- **Euploid/balanced**: when mosaic aneuploidy levels are <30%, and no segmental gains/losses ≥6Mb in size are detected.
- **Mosaic:** mosaicism is defined when there is a combination of chromosomally normal cells and others with aneuploidy. An embryo is considered mosaic when the level of aneuploidies detected in the biopsy is greater than 30% and less than 70%. Mosaic samples are reported as either "low mosaic" when having 30%-<50% aneuploid cells, or "High mosaic" when having ≥50%-<70% aneuploid cells. Only high mosaicism for segmental gains/losses and sex chromosome aneuploidies are reported. Embryos that have another uniform aneuploid chromosome are never reported as mosaic but as abnormal/aneuploid.

- **Aneuploid:** when mosaic aneuploid levels are ≥70% and/or segmental gains/losses ≥6Mb in size are detected.
    - Complex aneuploid: when 2-5 aneuploidies are detected in the provided sample.
    - Chaotic: when six or more aneuploidies are detected in the provided sample.
- **Aneuploid/Unbalanced:** when specific unbalances (gains or losses) arising from the parental chromosomal rearrangement are detected as a result of a deviation from the reference bioinformatics baseline.
- **No DNA detected:** when insufficient DNA is detected in the sample.
- **Non informative:** when a reliable result cannot be achieved with high confidence.

If no mosaicism information is requested by the clinic/user, the threshold for euploid and aneuploid is considered 50%. Therefore, embryos with mosaic levels below 50% are reported as euploid, and embryos with mosaic levels ≥50% are reported as aneuploid.

MitoScore: The MitoScore test can also be performed on the DNA obtained from the same biopsy to identify which embryos could have a greater capacity and suitability for achieving pregnancy. The level of mitochondrial DNA (MitoScore) is obtained using NGS, which offers information on both nuclear and mitochondrial DNA.

PGT-SR Plus test description:

PGT-SR Plus is an optional analysis that includes PGT-SR and adds ploidy assessment, cohort check and contamination testing. Triploidy (when there is an additional set of all chromosomes) and haploidy (when there is only a single set of all chromosomes) are the most common ploidy abnormalities and are incompatible with normal growth and development. PGT-SR Plus includes a QC analysis for embryos, confirming that embryos within a cohort demonstrate the expected genetic relatedness and assessing for DNA contamination. This analysis can reduce the risk of misdiagnosis and provide additional reassurance about the IVF process.

PGT-SR Plus test turnaround time:

The clinician that has requested the test will receive the results.

The **turn-around-time** for PGT-SR Plus is up to 21 working days.

PGT-SR Plus reporting:

Additional to the PGT-SR results, the following results can be obtained for each embryo when PGT-SR Plus (ploidy and embryo QC) is requested:

Ploidy results:

- **Diploid:** Ploidy analysis is consistent with 2 sets of chromosomes and a diploid state (2N).
- **Triploid:** Ploidy analysis is consistent with 3 sets of chromosomes and a triploid state (3N).
- **Haploid:** Ploidy analysis is consistent with a single set of chromosomes and a haploid state (1N).
- **Ploidy non-informative:** Ploidy analysis is non-informative.

Contamination results:

- **Contamination detected:** Admixture of exogenous DNA is detected in the sample analyzed.

Cohort check results:

- **Consistent:** The sample demonstrates the expected genetic relatedness to other samples submitted for the patient, suggesting that the sample belongs to the patient's cohort check.
- **Inconsistent:** The sample does not show the expected genetic relatedness to other samples submitted for the patient.
- **Cohort check non-informative:** genetic relatedness analysis between the sample and other samples in the patient's cohort is non-informative.
- **Not applicable:** The cohort check is not performed for embryos with haploid or triploid results, for contaminated samples, when there is only one embryo available for analysis, or when insufficient DNA is detected in the PGT-A analysis.

3.5 Endometrial Receptivity Analysis (ERA)

ERA test description:

The lack of synchronization between the embryo, which must be ready to be implanted, and endometrial receptivity is believed to be one of the causes of recurrent implantation failure. ERA is a test that was developed and patented in 2009 by Igenomix after more than 10 years of research and development.

The ERA test helps to evaluate the woman's endometrial receptivity and thus identify a 'window of implantation' from a molecular perspective. The test analyses the expression levels of 248 genes linked to the status of endometrial receptivity, using RNA sequencing (through NGS) on material biopsied from the endometrium. Following the analysis, a specific computational predictor classifies the samples according to their expression profile in the corresponding endometrial stage (proliferative, pre-receptive, receptive, late receptive or post-receptive). This data will enable a personalized embryo transfer (pET), synchronizing endometrial receptivity with an embryo prepared for implantation.

Pre-requirements for accepting an ERA case:

No specific pre-requirements are needed in order accept an ERA case. We strongly encourage you to carefully read the "ERA-EMMA-ALICE Manual" for further information in addition to the specific ERA-EMMA-ALICE test instructions. You can download these documents from the Igenomix website (www.igenomix.eu)

ERA test sample requirements:

Endometrial tissue (~70mg by mass or ~7mm by size) placed in a cryotube containing RNA stabilizing solution (1,5 ml) provided by the laboratory. The ERA test requires an endometrial biopsy that should be carried out on day LH+7/HCG+7 (natural cycle) or day P+5 (Hormone Replacement Therapy cycle) or following the usual clinical protocol for blastocyst transfer. The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours before shipping. For shipment, the cryotube containing the endometrial biopsy must be placed inside a blister as secondary container.

In order to obtain a fully confident test result, the ERA-EMMA-ALICE Manual details must be strictly followed. This document can be downloaded either from the Igenomix website (www.igenomix.eu) or requested by email.

The "Test Requisition Form" must be completed and sent with the sample inside the shipping box.

ERA sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack. To maintain sample stability, transit at room temperature should not exceed 5 days in order to ensure the preservative action of the liquid in the cryotube.

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

ERA test turnaround time:

The clinician that has requested the test will receive the results **within 15 natural days** from sample reception by Igenomix.

ERA test reporting:

The result of the test can be:

+ Receptive (R): This gene expression profile is compatible with a normal, receptive endometrium. In this case, we recommend performing a blastocyst(s) transfer following the same protocol utilized during this Endometrial Receptivity Analysis (ERA) test.

+ Late Receptive (eT): This gene expression profile means that the endometrium is at the end of the receptive stage. In this case, we recommend advancing the embryo transference 12 hours regarding the moment in which the biopsy was taken.

+ Proliferative (F): This gene expression profile is concordant with an endometrium at a proliferative stage. We recommend that you contact the ERA laboratory to evaluate the protocol in which this endometrial biopsy was performed.

+ Pre-receptive (PREd1/PREd2): This gene expression profile is concordant with an endometrium at a pre-receptive stage due to the potential displacement of the window of implantation. For some results, we may require analysis of a second biopsy on the recommended day to be able to provide a transfer timing recommendation.

+ Post-receptive (T): This gene expression profile is concordant with an endometrium at a post-receptive stage due to the potential displacement of the window of implantation. To confirm this result, analysis of a second biopsy on the recommended day is required.

+ Non-informative: The profile analyzed does not match the control gene expression profiles present in the ERA predictor. We recommend that you contact the ERA laboratory to evaluate the protocol in which this endometrial biopsy was performed.

+ Insufficient RNA: It was not possible to determine the gene expression profile of the sample because there was not enough genetic material. A new endometrial biopsy is required.

+ Invalid RNA: It was not possible to determine the gene expression profile of the sample due to the poor quality of genetic material obtained. A new endometrial biopsy is required.

The ERA report for most samples includes a recommendation for performing a personalized embryo transfer (pET). For some patients, as indicated above, another biopsy may be required.

3.6 Endometrial Microbiome Metagenomic Analysis (EMMA)

EMMA test description:

A molecular test that provides microbiota information in endometrial tissue by analyzing a customized panel of bacteria. It includes information about *Lactobacillus* and potentially pathogenic bacteria of the reproductive tract, some of them related to Chronic Endometritis. This method is based on detecting bacterial DNA through real-time polymerase chain reaction (RT-PCR) which translates into different profiles that have been linked to the success of pregnancy.

Igenomix reserves the right to analyse EMMA samples using NGS technology, subject to prior notification and information to the customer.

EMMA is indicated for patients with Recurrent Implantation Failure (RIF), Recurrent Pregnancy Loss (RPL), suspected Chronic Endometritis (CE) or a history of previous infections, by analyzing the microbial environment of the uterine cavity including the most frequently bacterial pathogens that cause Chronic Endometritis. The EMMA test always includes the ALICE test.

EMMA test sample requirements:

A single endometrial biopsy is sufficient for the EndomeTRIO test (includes ERA, EMMA, and ALICE). If the clinic's standard ERA protocol includes a double biopsy, please note that microbiome analysis will only be performed on the first biopsy. If an EndomeTRIO test is requested, the endometrial biopsy must be taken according to the ERA timing provided in the EndomeTRIO manual (120 hours of progesterone exposure in an HRT cycle or 168 hours after hCG administration in a natural cycle or following the routine protocol for blastocyst transfer). It is imperative to properly control endogenous progesterone by ensuring levels are <1ng/ml at hCG+0 (in natural cycles) or within the 24 hours prior to the first intake of exogenous progesterone (in HRT cycles).

If only an EMMA test is requested, the endometrial biopsy may be taken following the same protocol as for ERA or between days 15 and 25 of a natural cycle (only for patients with regular cycles between 26-32 days). If the patient does not have regular cycles, we recommend performing an HRT cycle and taking the sample during the progesterone intake days, preferably at day P+5. Alternatively, ovulation can be controlled, and the sample can be taken between LH/hCG+2 and LH/hCG+12, or between Ov+1 and Ov+11. Another option is to collect the sample while the patient is on Oral Contraceptive Pills (OCPs) between day 14-21 of active pills (if the patient takes placebo pills) or after day 14 and onwards if taking active pills continuously (note: not as all OCPs are valid for EMMA testing, we recommend checking it with our specialists before scheduling the biopsy).

The endometrial biopsy must be taken from the uterine fundus. Sample size should be approx. 70 mg and not exceed the white line marked on the Igenomix cryotube. Larger samples may still be evaluated to determine if the genetic material has been properly preserved. If this is not the case, a new sample will be requested. Ensure that the sample is made up of endometrial tissue and not solely blood or mucus. Label the cryotube with the patient's full name, DOB, and date of biopsy. As the microbiome can fluctuate over time, the samples should be sent as soon as possible, following the minimum 4-hour refrigeration period. The "Test Requisition Form" (that can be found within the kit or requested by email) must be completed and placed in the kit.

EMMA sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Shipment can be at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. To maintain sample stability, transit at room temperature should not exceed 5 days to ensure the preservative action of the liquid in the cryotube.

The sample must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company). Please inform us by email about each shipment, indicating the number of samples and their clinical or reference record number. You may use your usual courier or alternatively ask us about our pickup service.

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

EMMA test turnaround time:

The clinician that has requested the test will receive the results **within 15 natural days** from sample reception by Igenomix.

EMMA test reporting:

The EMMA report will provide information about the overall microbial health of the uterine cavity. This includes:

- One table shows information about detection of DNA from *Lactobacillus spp* as well as from the species *L. crispatus, L. gasseri, L. iners,* and *L. jensenii.*

- One table showing the reference ranges for 16 species of common reproductive tract pathogens (*Actinomyces israelii, Atopobium vaginae, Bacteroides fragilis, Bifidobacterium spp, Clostridium sordelii, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Mobiluncus spp, Mycobacterium tuberculosis, Peptostreptococcus anaerobius, Porphyromonas asaccharolytica, Prevotella bivia, Prevotella disiens, Sneathia spp* and *Treponema pallidum*) and the values obtained in the endometrial sample.

- One table with ALICE results, showing the reference ranges for 10 species of pathogens causing chronic endometritis (CE) (*Streptococcus agalactiae* (group B) and *Streptococcus viridans*, *Staphylococcus aureus, Enterococcus faecalis, Mycoplasma hominis, Mycoplasma genitalium, Escherichia coli, Klebsiella pneumoniae, Ureaplasma urealyticum, Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and the values obtained in the endometrial sample.

- In cases in which there are no pathogens detected out of their reference range, if at least one of the *Lactobacillus* species or *Lactobacillus spp* are detected, this is considered a normal result.

- In case that DNA from *Haemophilus ducreyi, Mycobacterium tuberculosis, Treponema pallidum, Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* is detected in the endometrial sample, an additional confirmatory test could be recommended according to endometrial profile. Infections caused by these bacteria are mandatory notifications to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.

- In case that DNA from *Actinomyces israelii, Clostridium sordelii* and/or *Fusobacterium nucleatum* is detected in the endometrial sample, an additional confirmatory test and follow-up by a physician will be recommended.

- Values of pathogens out of the reference range are identified with an asterisk and highlighted in bold.

- EMMA report includes a suggested therapy (if needed) considering each specific bacterium detected out of the reference range, to achieve pathogens-free endometrium, increasing the chances of achieving pregnancy, as is described in the scientific literature. However, is the medical professional who must consider the possible prescription of an antibiotic and/or probiotic treatment in conjunction with the available clinical findings of each patient. In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

In some other cases, for some patients, another biopsy may be suggested.

3.7 Analysis of Infectious Chromic Endometritis (ALICE)

ALICE test description:

ALICE is a molecular test performed using RT-PCR, which detects the presence of DNA from potentially pathogenic bacteria that most frequently cause chronic inflammation of the endometrium, known as Chronic Endometritis (CE). This disease has been linked to infertility and obstetric complications.

Igenomix reserves the right to analyse ALICE samples using NGS technology, subject to prior notification and information to the customer.

ALICE can be helpful in determining which pathogenic bacteria are present in the uterine cavity and which may be the cause of chronic endometritis. These results may help determine the most appropriate treatment to eliminate the potential pathogens causing the disease.

ALICE sample requirements:

A single endometrial biopsy is sufficient for the EndomeTRIO test (includes ERA, EMMA, and ALICE). If the clinic's standard ERA protocol includes a double biopsy, please note that microbiome analysis will only be performed on the first biopsy. If an EndomeTRIO test is requested, the endometrial biopsy must be taken according to the ERA timing provided in the EndomeTRIO manual (120 hours of progesterone exposure in an HRT cycle or 168 hours after hCG administration in a natural cycle or following the routine protocol for blastocyst transfer). It is imperative to properly control endogenous progesterone by ensuring levels are <1ng/ml within the 24 hours prior to the first intake of exogenous progesterone.

If only an ALICE test is requested, the endometrial biopsy may be taken following the same protocol as for ERA or between days 15 and 25 of a natural cycle (only for patients with regular cycles between 26-32 days). If the patient does not have regular cycles, we recommend performing an HRT cycle and taking the sample during the progesterone intake days, preferably on day P+5. Alternatively, ovulation can be controlled, and the sample can be taken between LH/hCG+2 and LH/hCG+12, or between Ov+1 and Ov+11. Another option is to collect the sample while the patient is on Oral Contraceptive Pills (OCPs) between day 14-21 of active pills (if the patient takes placebo pills) or after day 14 and onwards if taking active pills continuously (note: not as all OCPs are valid for ALICE testing, we recommend checking it with our specialists before scheduling the biopsy).

The endometrial biopsy must be taken from the uterine fundus. Sample size should be approx. 70 mg and not exceed the white line marked on the Igenomix cryotube. Larger samples may still be evaluated to determine if the genetic material has been properly preserved. If this is not the case, a new sample will be requested. Ensure that the sample is made up of endometrial tissue and not solely blood or mucus. Label the cryotube with the patient's full name, DOB, and date of biopsy. As the microbiome can fluctuate over time, the samples should be sent as soon as possible, following the minimum 4-hour refrigeration period. The "Test Requisition Form" (that can be found in the kit or requested by email) must be completed and placed in the kit.

ALICE sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Shipment can be at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. To maintain sample stability, transit at room temperature should not exceed 5 days to ensure the preservative action of the liquid in the cryotube.

The sample must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company). Please inform us by email about each shipment indicating the number of samples and their clinical or reference record number. You may use your usual courier, or alternatively ask us about our pickup service.

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

ALICE test turnaround time:

The clinician that has requested the test will receive the results **within 15 natural days** from sample reception by Igenomix.

ALICE test reporting:

The ALICE report will provide information about the bacteria that most frequently cause chronic inflammation of the endometrium, known as Chronic Endometritis (CE). This includes:

- The ALICE report shows a table with the reference ranges for 10 species of reproductive tract pathogens most often related to chronic endometritis (*Streptococcus agalactiae*

(group B) and *Streptococcus viridans, Staphylococcus aureus, Enterococcus faecalis, Mycoplasma hominis, Mycoplasma genitalium, Escherichia coli, Klebsiella pneumoniae, Ureaplasma urealyticum, Chlamydia trachomatis and Neisseria gonorrhoeae*) and the values obtained in the endometrial sample. Values of pathogens out of the reference range are identified with an asterisk and highlighted in bold.

- In case *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* are out of the normal range, an additional confirmatory test will be recommended. Infections caused by these bacteria are mandatory notifications to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.

- ALICE report includes suggested therapy (if needed) taking into account each specific bacterium detected out of the reference range, to increase the chances of achieving a healthy pregnancy as described in the scientific literature. However, is the medical professional who must consider the possible prescription of an antibiotic and/or probiotic treatment in conjunction with the available clinical findings of each patient. In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

In some other cases, for some patients, another biopsy may be suggested.

3.8 Sperm Aneuploidy Testing (SAT)

SAT test description:

The Sperm Aneuploidy Test (SAT) is a diagnostic test that helps to assess male infertility by measuring the percentage of spermatozoa with chromosomal abnormalities in a semen sample. The SAT result provides an estimation of the transmission risk of chromosomal abnormalities to the embryo and potential offspring. The test specifically analyses the chromosomes most commonly observed in spontaneous miscarriages and affected offspring with chromosomal abnormalities (chromosomes 13, 18, 21, X and Y). The test uses Fluorescence In Situ Hybridization (FISH).

Pre-requirements for accepting a SAT case:

Prior to offering a SAT analysis due to an abnormal karyotype in the patient, a "genetics report" that clearly identifies the karyotype is required, and if appropriate, a case-discussion with a senior member of staff.

Prior to offering a SAT analysis for ejaculate, testis and epididymis frozen samples, a case-discussion with a senior member of staff will be needed to clarify the sample pre-processing protocol at the referring lab.

SAT sample requirements:

Ejaculate, epididymis and testicle sperm samples washed and suspended in sperm culture medium inside a conical tube (the culture medium is not provided by Igenomix).

Instructions on how to prepare a sample are available and can be downloaded from the Igenomix website, kits.igenomix.com, or requested by email. The “Test Requisition Form” (provided within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and sent with the sample inside the shipping box.

SAT sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack.

To ensure sample quality, we strongly recommend sending SAT samples to Igenomix within 3 days of collection.

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or “Packaging Instructions P650” and clearly labelled 'Exempt Human Specimen UN3373’ when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

SAT test turnaround time:

The clinician that has requested the test will receive the results **within 10 working days** from sample reception by Igenomix.

SAT test reporting:

The following results can be obtained as a result of performing this test:

+ **Normal:** the sample shows a similar percentage of abnormal sperm compared to an internal control dataset.

+ **Abnormal:** the sample shows a statistically significant increase in the percentage of abnormal sperm compared to the internal control dataset.

3.9 Testing for Products of Conception (POC)

POC test description:

POC is a genetic test that can provide information to help determine if pregnancy loss was caused by a chromosomal abnormality. POC testing, performed on tissue retrieved from the lost pregnancy, is comprehensive as it analyses all 24 chromosomes for gross chromosomal abnormalities using NGS.

Pre-requirements for accepting a POC case:

No specific pre-requirements are needed in order accept a case. Specific test indications and relevant clinical information can be reported in the test requisition form.

POC test sample requirements:

Tissue from the lost pregnancy is required. A tissue sample with a minimum size of 3x3 mm, preferably without blood, must be placed in a specimen pot (usually provided by the laboratory) and covered with saline solution.

In addition, and as a control to test for maternal contamination and polyploidy (when appropriate) by STR analysis, 1x4ml of peripheral blood from the mother in EDTA tubes (provided by the laboratory) is required.

Instructions on how to prepare a sample are available (POC Instructions) and can be downloaded from the Igenomix website, kits.igenomix.com, or requested by email. The “Test Requisition Form” (provided within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and sent with the sample inside the provided shipping box.

POC sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack.

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or “Packaging Instructions P650” and clearly labelled 'Exempt Human Specimen UN3373’ when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included in the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

POC tests turnaround time:

The clinician that has requested the test will receive the results **within 12 working days** from sample reception by Igenomix.

POC test reporting:

The following results can be obtained as a result of performing this test:

- **Normal:** when no aneuploidy or partial deletion/duplication has been detected, and the additional STR analysis does not identify maternal cell contamination or polyploidy.
- **Abnormal:** when aneuploidy or partial deletion/duplication ≥10Mb in size has been detected. Information about the detected abnormality is provided.
- **Maternal cell contamination:** when a normal female result has been obtained but the additional STR analysis only detects maternal origin of the sample.
- **Non informative:** when the quality of the sample is suboptimal and leads to an NGS result below the required quality thresholds.

3.10 Carrier Genetic Test (CGT)

CGT test description:

CGT is a family of genetic tests designed to detect carriers of known pathogenic mutations that pose risks for future progeny of having a serious genetic disorder. A “positive” result indicates the presence of one or more mutations in the individual. In these cases, we strongly recommend similar testing of the individual’s partner if the couple wishes to have a child. Alternatively, both partners can be tested simultaneously.

If both reproductive partners are carriers of a mutation in the same single gene, there is high risk (25%) of having a child affected by a genetic disease. In these cases, there are options to significantly reduce the risk of having affected children, such as PGT-M, gamete donation, and other options. It is also possible to conceive naturally and resort to prenatal diagnosis. A negative result indicates that the person does not carry any of the mutations studied by the test. The test uses mainly NGS technology for detecting mutations, but additional studies to detect frequent mutations not detected through NGS are used for some genes.

Lists of genes and mutations analysed for each test are available on the webpage https://cgt.igenomix.com

CGT test pre-requirements for accepting a case:

No specific pre-requirements are needed in order accept a case. Specific test indications and relevant clinical information can be reported in the test requisition form.

CGT test sample requirements:

There are different sample collection options for this test: SALIVA, PERIPHERAL BLOOD or MOUTH CELLS.

The SALIVA sample should be collected using the ORAGENE-DNA tube provided, included in the ORAGENE-DNA box supplied by the laboratory. Alternatively, a minimum of 1x 3ml of peripheral BLOOD in an EDTA tube, normally provided by the laboratory, is required. The MOUTH CELL sample should be collected using the two buccal swabs provided by the laboratory.

Instructions on how to prepare a sample are available (see CGT Instructions) and can be downloaded from the Igenomix website, kits.igenomix.com, or requested by email. The "Test Requisition Form" (provided within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and placed in the CGT kit.

CGT sample transportation to the laboratory:

The clinic needs to notify Igenomix when a sample will be ready, and the laboratory will offer to arrange for sample collection. Transportation will be conducted in custom-made kits provided by the laboratory. Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35ºC. Avoid freezing the sample when introducing the cold gel pack.

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or "Packaging Instructions P650" and clearly labelled 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

CGT test turnaround time:

The clinician that has requested the test will receive the results within 20 working days from sample reception by Igenomix. For some CGT Matching cases 3 additional working days may be required.

CGT test reporting:

The following results can be obtained as a result of performing this test:

- **A positive test result** indicates the detection of a mutation(s) in a tested gene(s). If a patient and partner are both carriers of mutations in the same gene associated with AR inheritance, there is a 25% chance that any child they have together would be affected. If a woman is a carrier of a mutation in a gene associated with X-linked inheritance, there is a 50% chance that male children the patient has may be affected; any female children have a 50% chance of being a carrier.
- **A negative test result** indicates that mutations have not been detected in the analyzed genes. For genes with a negative test result, the risk of having affected children for the

corresponding disorders is decreased significantly compared to the general population.

3.11 Embryo priority test (EMBRACE)

EMBRACE test description:

EMBRACE is a genetic test that may be performed on the culture media in which the embryos grow during IVF treatment to screen for numerical chromosomal abnormalities. Chromosomally normal culture media are most likely to implant and develop to term. EMBRACE helps clinicians and patients undergoing IVF to prioritize which embryos to transfer first. The method, requiring only a small volume of culture medium, is comprehensive as it analyses all 24 chromosomes for chromosomal copy number using Next Generation Sequencing (NGS).

Pre-requirements for accepting an EMBRACE case:

No specific pre-requirements are needed in order accept a case. Specific test indications and relevant clinical information can be reported in the test requisition form.

EMBRACE sample requirements:

For EMBRACE, a small volume of 5-15 microliters of culture medium is required. The culture medium is transferred to a small 0.2ml tube supplied by the laboratory. The lids of these tubes must be labelled with the female patient initials followed by the embryo number. The 0.2ml tubes must be placed in the “plate/rack” provided by the laboratory, the “plate/rack” placed in a plastic bag and inside the cooler shipping box with the “ice packs” also provided by the laboratory. Further information on how to prepare a sample can be found and downloaded from the website or requested by email to the Igenomix Customer Support service (see section 1.3).

The “Media Collection Worksheet” and the “Test Requisition Form” (included within the provided kit and additionally available either from the Igenomix website or requested by email) must be completed and sent with the samples inside the shipping box or by e-mail to the laboratory.

Professional user validation for EMBRACE tests (DRY RUN):

Following the enrolment of a new clinic (see section 2.4), we recommend performing a “validation” or “dry run” for every IVF laboratory. This process aims to provide reduce the likelihood of difficulties with clinical cases that could lead to a failure to determine a result(s) for the culture media. Instructions on how to complete a “validation run” can be requested by email. A validation/dry run report is issued after the analysis and signed by a senior member of laboratory staff or the Laboratory Director.

EMBRACE sample transportation to the laboratory:

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The PGT kit provided by Igenomix must be used for the shipment, including the cooler box: freeze the ice packs, cool-rack and biopsied samples before the shipment.

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, UPS etc.) and must be packed according to a set of ADR guidelines known as P650, or

"Packaging Instructions P650" and clearly labelled as 'Exempt Human Specimen UN3373' when the sample is not delivered from Spain (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

For further details on how to send the samples please review the test instructions included on the Igenomix website or contact to Igenomix Customer Support service (see section 1.3).

EMBRACE test turnaround time:

The clinician that has requested the test will receive the results.

For EMBRACE samples all cases will be with **deferred transfer** and results will be available **within 7 working days** from receipt of samples by Igenomix.

EMBRACE reporting:

Igenomix uses an internal validated algorithm for whole chromosome aneuploidies and partial deletion/duplications. This algorithm estimates the euploidy score of each medium. As a result, a priority order is established for each medium according to the euploidy score based on the results that can be obtained, for each culture media:

- **Normal/euploid**: when there are two copies of each chromosome pair, and no partial deletion/duplications ≥10Mb in size are detected.
- **Abnormal/aneuploid:** when there is an abnormal copy number for one or more chromosomes and/or partial deletion/duplications ≥10Mb in size are detected. There are different combinations of chromosomal abnormalities and each of them is associated with a different euploidy score.
- **No DNA detected:** when insufficient DNA is detected in the sample.
- **Non informative:** when the quality of the sample is suboptimal and leads to an NGS result below the required quality thresholds.

In samples with no DNA-detected or non-informative a euploid score is given to each sample according to the aneuploidy risk associated to the corresponding female age.

4 CERTIFICATION, ACCREDITATION AND EXTERNAL ASSESSMENT SCHEMES

SPAIN IGENOMIX LABORATORY is CLIA certificated (Clinical Laboratory Improvement Amendment), since 2018 (CLIA ID NUMBER 99D2146167), for the CGT and ERA test, since 2021 for the test SAT and since 2022 for the test POC. In addition, ERA, SAT and POC test are under the scope of ISO 15189:2013 accreditation since 2020, PGT-M since 2024, PGT-A, SR & Ploidy since 2025 and EMMA-ALICE (OpenArray) since 2026.

The laboratory annually participates in External Quality Assessments (EQA) (also known as Proficiency Testing, PT) with internationally recognized schemes accredited to ISO 17025 or offered by CLIA or CAP organizations.

For some tests, no EQA scheme is available. For these tests, the lab performs an internal Alternative Assessment (AA) twice a year to provide objective evidence for the acceptability of examination results.

All tests that are included in certification/accreditation schemes participate in any assessment program (either PT or AA) that may further assist in the continued assessment of the reliability of the offered tests by Igenomix.