Igenomix UK Ltd, Surrey Technology Centre, 40 Occam Road, Guildford, GU2 7YG. Email: info.uk@igenomix.com. Tel: +44(0)2080688176. www.igenomix.co.uk

User Manual

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1 IGENOMIX UK

1.1 INTRODUCTION

Igenomix UK Ltd is a private medical testing laboratory (Company No.10675550) specialising in reproductive genetic services and is part of a multinational company ('Igenomix Global') with headquarters in Valencia, Spain. Igenomix is now part of the Vitrolife Group AB.

The laboratory currently performs five tests in-house: Preimplantation Genetic Testing for Monogenic Disorders (PGT-M), Preimplantation Genetic Testing for Aneuploidy (PGT-A), Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR), MitoScore and EMBRACE.

The laboratory offers additional services that are currently outsourced to the headquarters in Spain, including Endometrial Receptivity Analysis (ERA); Endometrial Microbiome Metagenomic Analysis (EMMA); Analysis of Infectious Chronic Endometritis (ALICE); a combined ERA+ALICE+EMMA test (EndomeTRIO), non-invasive prenatal testing (NACE & NACE 24), Carrier Genetic Testing (multiple tests which are branded under 'CGT' and an associated family mutation test), Genomic Precision Diagnostics (GPDx), Sperm Aneuploidy Testing (SAT), and testing for Products of Conception (POC).

There are additional services that are currently outsourced to Igenomix Italy: CGT Essential (a variant of the Carrier Genetic Test), Cystic Fibrosis Screening, Karyotyping, and testing for Y Chromosome Microdeletions.

1.2 LABORATORY OPENING TIMES

The laboratory is open Monday – Friday 9:00am to 5:00pm

1.3 CONTACT DETAILS

1.3.1 KEY MEMBERS OF STAFF:

Seema Dhanjal, MSc, MPhil. State Registered Clinical Scientist (Genetics, HCPC- CS19543). Laboratory Director UK, Quality Manager, Health and Safety Manager, CQC Registered Manager.

Xuhui Sun, MSc., Laboratory Supervisor, Training Officer

Kate Hall. Office Manager.

Sati Jandu, PhD, Sales and Marketing Director - UK & Ireland and CQC Nominated Individual.

Lauren Salgado, BSc., MSc, Account Manager for Genetic Services (UK)

Kamran Khalid, BSc., Quality Specialist

1.3.2 GENERAL ENQUIRIES:

Email: support.uk@igenomix.com Tel: +44(0)2080688176

1.3.3 LABORATORY ENQUIRIES:

Email: lab.uk@igenomix.com Tel: +44(0)2080689410

1.3.4 GENETIC COUNSELLING ENQUIRIES

Email: gc.uk@igenomix.com Tel: +44(0)2036080544

1.3.5 ADDRESS

Igenomix UK Ltd
Surrey Technology Centre
40 Occam Road
Guildford, GU2 7YG

2 GENERAL INFORMATION

Genetic tests are performed based on referrals from medical professionals and self-referred patients. Additional information regarding the different tests offered is available to users on the Igenomix UK website and can also be requested by email from support.uk@igenomix.com.

Test results are issued to the referring clinician or to Igenomix UK genetic counsellors attending the self-referred patients. Should a medical professional require assistance with the interpretation of Igenomix UK test reports, please contact us according to the 'laboratory enquiries' details in section 1.3.

The laboratory is committed to delivering services of the highest quality at all times to ensure patient safety and customer satisfaction. Any comments, suggestions or complaints about any service should be sent to support.uk@igenomix.com, after which they will be reviewed by the relevant members of staff in accordance with internal policies. A complaints form, which can be filled with the details of the complaint can be accessed from the Igenomix UK website.

Igenomix UK follows strict policies on Information Governance and maintains a data protection infrastructure in line with Data Protection Regulations, including the Data Protection Act 2018 and the UK GDPR.

2.1 IMPORTANT GUIDANCE FOR ALL SAMPLES

2.1.1 GENERAL REASONS FOR SAMPLE REJECTION

Samples may be rejected if they are:

- Unlabelled or damaged
- Received without the required test documentation
- Received with incomplete test documentation, or documentation missing patient or clinician signatures
- Received in expired containers, where applicable
- Not received in an Igenomix UK kit

3 REFERRALS

Before referrals can be made, clinics need to complete the "Clinic Enrolment Form (CEF)" which can be downloaded from the Igenomix UK website or requested by email from support.uk@igenomix.com The completed form should be returned by email to support.uk@igenomix.com .

All tests must be accompanied by their required documentation, which includes a "Test Requisition Form" and if applicable a "Consent Form", which may, for certain services, be a combined form. Some tests have additional documentation requirements.

For PGT-SR, PGT-M, and CGTSync a referral is required before sending samples. Please contact the Igenomix UK laboratory for further information about these requirements.

4 TESTS SUMMARY

Test	TAT (working days)	Sample Type	Storage Temp	Maximum Storage time before transport
PGT-A	10	Embryo biopsy	-20°C	2 weeks
PGT-SR	10	Embryo biopsy	-20°C	2 weeks
Pre-PGT-M	20-30	Blood (EDTA tube) /Saliva/Buccal/DNA	4-8°C	2 weeks
PGT-M	10	Embryo biopsy	-20°C	2 weeks
Mitoscore	10	Embryo biopsy	-20°C	2 weeks
Embrace	10	Embryo culture medium	-20°C	2 weeks
ERA	10	Endometrial Tissue	4-8°C	3 weeks
EMMA	10	Endometrial Tissue	4-8°C	3 weeks
ALICE	10	Endometrial Tissue	4-8°C	3 weeks
EndomeTRIO	10	Endometrial Tissue	4-8°C	3 weeks
NACE	10	Blood (7-10 ml Streck tube)	Room	4 days
CGT	25	Blood (3ml EDTA tube) or saliva	4-8°C	2 weeks
GPDx	25	Blood (EDTA tube), saliva or DNA	4-8°C	2 weeks
SAT	10	Semen in culture media	Room	48 hours
POC	15	Tissue sample and maternal saliva sample	4-8°C	6 days
niPOC	10	Blood (7-10 ml Streck tube)	Room	4 days
CF Screening	20	Blood (3ml EDTA tube)	4-8°C	2 weeks
Karyotyping	25	Blood (3ml Lithium Heparin tube)	4-8°C	2 weeks
Y Chromosome microdeletions	20	Blood (3ml EDTA tube)	4-8°C	2 weeks

5 TESTS PERFORMED IN-HOUSE

The laboratory currently performs five tests in-house: Preimplantation Genetic Testing for Monogenic Diseases (PGT-M), Preimplantation Genetic Testing for Aneuploidy (PGT-A), Preimplantation Genetic Testing for Chromosomal Rearrangements (PGT-SR), MitoScore and EMBRACE.

5.1 USER VALIDATION FOR PGT-M, PGT-A AND PGT-SR:

Following the enrolment of a new clinic, a "validation" or "dry" run is performed for every embryologist involved in embryo biopsy and tubing for PGT-M. Instructions on how to complete a "validation run" (Tubing Validation- Instructions and biopsy sheet and Washing Tubing Instructions) can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.

A "validation/dry run report" is issued after the results have been analysed by a senior member of laboratory staff or the Laboratory Director.

Clinical samples taken by an embryologist will only be processed after their successful completion of a "validation/dry run". In certain cases, and after discussion with the Laboratory Director, user validation is not needed for every embryologist performing biopsy. This includes embryologists that have been previously validated for biopsy by an ISO 15189 (or equivalent) accredited diagnostic laboratory.

5.2 SAMPLE REQUIREMENTS FOR PGT-M, PGT-A AND PGT-SR:

Embryonic biopsy samples are required for PGT-M, PGT-A and PGT-SR. Sample requirements for other tests are described in the relevant sections.

- 5-6 cells are required from a biopsy performed on a blastocyst-stage embryo (typically day 5 or 6).
- Igenomix UK provides a tube of washing solution and sterile 0.2ml microcentrifuge tubes that are prelabelled with unique codes. This washing solution must be used for the washing and tubing of the biopsied cells. The cells must be tubed into the provided 0.2ml tubes. The lid of these tubes must be labelled with the female patient initials followed by the embryo number.
- All 0.2ml tubes must be placed in a "tube rack" (provided by Igenomix UK) with the lid labelled with the patient's name, date of birth and the unique patient ID number.
- The "tube rack" must be placed into a sterile plastic bag and placed in a cooler with two cool packs, also provided by Igenomix UK, which the IVF clinic must freeze in advance.

Note: The ice packs must be stored at -20°C and the tube racks at 4°C when received in the IVF lab and until they are used to send samples back to Igenomix UK. Please refer to the "PGT-A, PGT-SR, PGT-M Instructions" for clear guidance.

Further information on how to prepare a sample is found in the "Washing Tubing Instructions" and "PGT-A, PGT-SR, PGT-M Instructions", both of which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.

- The "Embryo Biopsy Worksheet" and "Test Requisition Form", which can also be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email, must be completed and placed in the cooler prior to transport, as described in the "PGT-A, PGT-SR, PGT-M Instructions".

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

PGT-M can be performed on embryos produced by *in-vitro* fertilisation (IVF) treatment to test for a known single gene disorder. PGT-M, requiring only a small number of cells, identifies which embryos are, and which embryos are not, at an increased risk of developing the disorder. The aim of PGT-M is to help couples conceive a "healthy" child by preferentially selecting to transfer "normal" embryos and avoid the difficult choice of having to terminate a pregnancy if a "positive" result is obtained through prenatal diagnosis. PGT-M is performed using a genome-wide linkage-based technique known as Karyomapping.

5.3.1 PRE-REQUIREMENTS FOR ACCEPTING A PGT-M CASE:

Prior to accepting a PGT-M case, genetics reports (issued from an ISO 15189 accredited laboratory or equivalent) for the relevant individuals with known disorder status must be available and sent to the Igenomix UK laboratory for evaluation. The report must clearly identify the gene responsible for the

disorder to be tested by PGT-M. A case-discussion with a senior member of laboratory staff may be required in certain instances. The scenarios where Karyomapping can be considered as a suitable method for PGT-M include the following:

- **Autosomal dominant disorders** where a family member, known as a "reference", with tested genetic status (affected) is available to provide a sample.

  The reference is chosen in the order of preference as follows:

  1. Child of the couple
  2. Parent of the affected member of the couple. If the affected parent (genetically tested) is deceased, then a sample from the unaffected parent can be used
  3. Sibling of the affected member of the couple

- **Autosomal recessive disorder**s where a child of the couple with known genetic status (affected or normal) is available to provide a sample as a reference.

  The reference is chosen in the order of preference as follows:

  1. Child of the couple
  2. Parents of the couple
  3. Siblings of the couple

- **X-linked disorders** where a member of the family with tested genetic status is available to provide a sample. The reference is chosen according to the following order of preference:

  1. Child of the couple
  2. Parent of the affected member of the couple. If the affected parent (genetically tested) is deceased, then a sample from the unaffected parent can be used
  3. Sibling of the affected member of the couple

Once the required samples, as listed above, have been received, Igenomix UK will perform an informativity testing process called Pre-PGT-M, also known as a 'workup', as a precursor to testing of embryo samples.

In the cases where a reference is not available and/or where the informativity of the Karyomapping platform did not reach the accepted threshold (e.g. consanguineous couple) then a mutation detection system will be developed if possible and used in conjunction with Karyomapping.

For further information consult UK_L_I_011: Pre-PGT-M Instructions, which can be downloaded from (https://www.igenomix.co.uk/send-a-sample/) or requested by email.

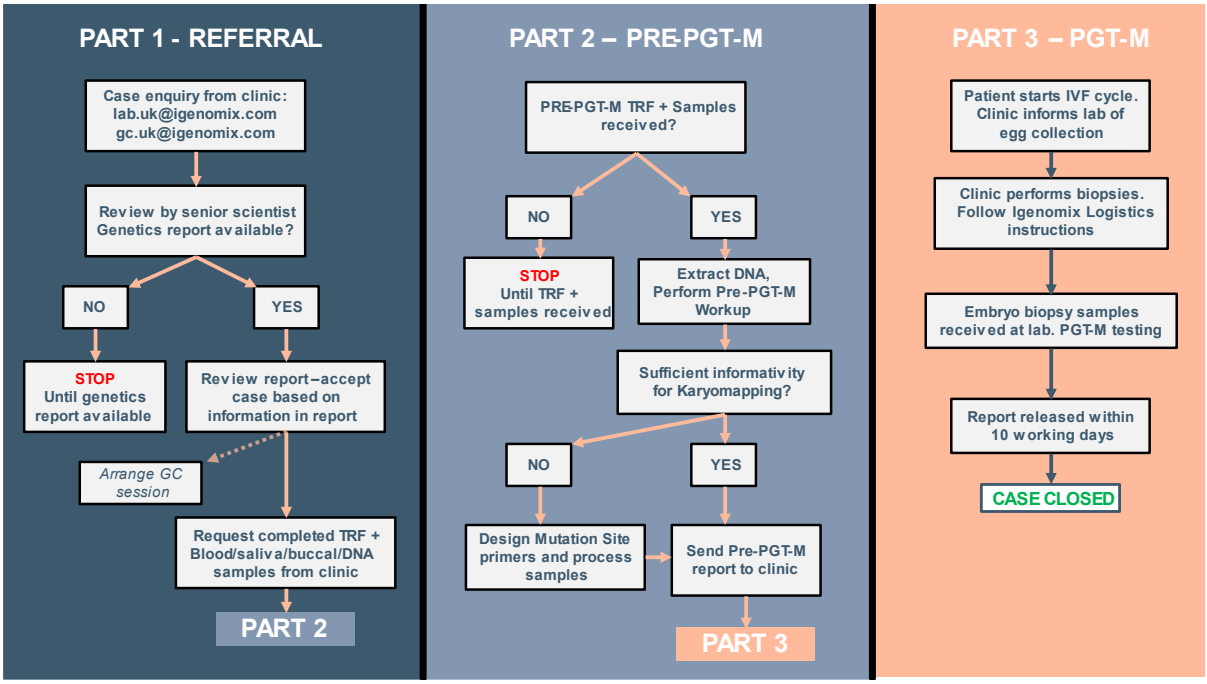
5.3.2 SAMPLE REQUIREMENTS FOR PRE-PGT-M:

- One of the following from the prospective parents and other relevant family members is required.
  - Saliva sample in Oragene 510 tube (Tubes provided by Igenomix UK)
  - Buccal swabs (Swabs provided by Igenomix UK)
  - Peripheral blood (in EDTA tubes)
  - Extracted DNA (10µl with a minimum concentration of 10 ng/µl)

- On the completion of the Pre-PGT-M a report will be sent to the IVF clinic indicating if the Karyomapping only or Karyomapping and mutation detection method is required for PGT-M. In rare cases if a Pre-PGT-M cannot be completed a case rejection report will be sent to the clinic. If feasibility of PGT-M is indicated in the Pre-PGT-M report, the couple can then start their treatment towards PGT-M or seek alternative treatment, which can be further discussed with a senior member of laboratory staff.

5.3.3 WORK FLOW OF PGT-M CASE

Below is a diagram describing the testing pathway that the laboratory follows for PGT-M:



Note: When PGT-M is performed for X-linked disorders, the sex of the embryo will be disclosed for embryos determined to be affected or carriers of the disorder.

Note: Karyomapping may detect certain chromosomal abnormalities, which will be reported. Karyomapping has been validated by Igenomix UK for the detection of meiotic chromosomal abnormalities. Karyomapping has **not** been validated for the detection of mitotic/mosaic chromosomal abnormalities.

Note: Direct mutation detection is currently not on the scope of accreditation, however the process has been verified in house.

5.4 PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES (PGT-A)

PGT-A is a genetic test that can be performed on embryos produced by IVF treatment to screen for numerical chromosomal abnormalities. Chromosomally normal (euploid) embryos are most likely to implant and develop to a healthy pregnancy and birth. PGT-A helps clinicians and patients undergoing IVF decide which embryos to transfer. The test, requiring only a small number of cells, analyses all 24 chromosomes for chromosomal copy number variations using Next Generation Sequencing (NGS). See sections 5.1 and 5.2 for user validation and sample requirements.

5.4.1 REPORTING MOSAICISM:

There are various different options available for the reporting of mosaicism varying from reporting all mosaicism to only reporting high mosaics. More information can be requested by contacting the laboratory at lab.uk@igenomix.com

If requested by the clinic/user, Igenomix UK will report mosaicism.

Although the specific criteria for reporting will depend on the clinic's choice, in general, the following methodology is applied for reporting mosaicism for whole chromosome abnormalities in all chromosomes except for 13, 18, 21, X, and Y:

- <30% ploidy deviation: Normal/euploid embryo.
- 30-50% ploidy deviation: Low mosaic embryo
- 50-70% ploidy deviation: High mosaic embryo
- >70% ploidy deviation: Abnormal embryo.

Mosaicism is not reported for chromosomes 13, 18, 21, X, and Y unless requested by the clinic. The methodology for reporting results for these chromosomes is:

- <30% ploidy deviation: Normal/euploid embryo.
- >30% ploidy deviation: Abnormal embryo.

5.4.2 REPORTING SEGMENTAL ABNORMALITIES:

Igenomix UK reports segmental abnormalities with a cut off of 10Mb.

Igenomix UK does not report mosaicism for segmental abnormalities, and segmental abnormality results will be reported as followed:

- <50% ploidy deviation: Normal embryo
- >50% ploidy deviation: Abnormal embryo

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

PGT-SR is a genetic test that can be performed on embryos produced by IVF treatments to detect specific chromosomal imbalances arising from parental chromosomal rearrangements. The test will also detect numerical chromosomal abnormalities not associated with the parental chromosomal rearrangement. This method, requiring only a small number of cells, uses NGS to analyse all 24 chromosomes. Currently, PGT-SR at Igenomix UK has been validated to detect chromosomal abnormalities that are ≥ 6Mb.

See sections 5.1 and 5.2 for user validation and sample requirements.

5.5.1 PRE-REQUIREMENTS FOR ACCEPTING A PGT-SR CASE:

Prior to offering PGT-SR, a "genetics report" (karyotype) (issued from an ISO 15189 accredited laboratory or equivalent) for the affected partner must be available for review by Igenomix UK. The report must clearly identify the chromosomal rearrangement to be tested for, and if appropriate, a case-discussion with a senior member of staff may be required.

5.5.1.1 GENERAL CASE ACCEPTANCE AND REJECTION CRITERIA

Cases are accepted if –

- all chromosome fragments involved in the rearrangement are ≥6Mb
- one fragment is <6Mb, however the remaining fragments must be ≥6Mb

Cases are **not** accepted if –

- "Complex" translocation cases where three or more chromosomes are involved in the rearrangement.

The probability of having a balanced gamete is very low (<5%) in these cases, and the risk of unpredicted recombination patterns is high.

- Cases with marker chromosomes (i.e. 47, XX, +mar).

These cases might involve heterochromatin or pericentromeric regions that cannot be detected with the current technology.

- Cases involving additional material (add) (i.e. 46, XY, add(12)(q13).

This additional material might be of unknown origin when inserted in a specific chromosome.

5.5.1.2 CASES INVOLVING SEX CHROMOSOMES:

Cases are accepted if-

- the translocation involves the Y chromosome
- the translocation involves the X chromosome of male carrier, but only balanced males are recommended for transfer

Cases are **not** accepted if-

- the translocation involves the X chromosome of a female carrier

After a review of the genetics report the clinic will be emailed to confirm if it is possible to acceptthe case or not.

Note: Igenomix UK has validated its limit of detection to 6Mb but is currently accredited to a 13Mb limit of detection for PGT_SR.

5.5.2 REPORTING MOSAICISM:

There are various different options available for the reporting of mosaicism varying from reporting all mosaicism to only reporting high mosaics. More information can be requested by contacting the laboratory at lab.uk@igenomix.com

If requested by the clinic/user, Igenomix UK will report mosaicism.

Although the specific criteria for reporting will depend on the clinic's choice, in general, the following methodology is applied for reporting mosaicism for whole chromosome abnormalities in all chromosomes except for 13, 18, 21, X, and Y:

- <30% ploidy deviation: Normal/euploid embryo.
- 30-50% ploidy deviation: Low mosaic embryo
- 50-70% ploidy deviation: High mosaic embryo
- >70% ploidy deviation: Abnormal embryo.

Mosaicism is not reported for chromosomes 13, 18, 21, X, and Y. The methodology for reporting results for these chromosomes is:

- <30% ploidy deviation: Normal/euploid embryo.
- >30% ploidy deviation: Abnormal embryo.

5.5.3 REPORTING SEGMENTAL ABNORMALITIES:

Igenomix UK reports segmental abnormalities with a cut off of 10Mb.

Igenomix UK does not report mosaicism for segmental abnormalities, and segmental abnormality results will be reported as followed:

- <50% ploidy deviation: Normal embryo

- >50% ploidy deviation: Abnormal embryo

5.6 MITOSCORE

The MitoScore test assesses embryos undergoing PGT-A or PGT-SR testing according to a mitochondrial biomarker developed by Igenomix, which provides an indicator of the energy status of an embryo. Embryos with MitoScore values associated with a normal energy reserve may be more likely to implant. MitoScore provides additional information to clinicians that may be useful in prioritising embryos for transfer.

Note- MitoScore is only available as complementary analysis when performing PGT-A or PGT-SR. No additional samples nor information are required.

Note- Mitoscore is not an accredited test.

5.7 EMBRACE

The EMBRACE test is an analysis of embryonic cell-free DNA released by a day-6/7 embryo (blastocyst) into the medium in which it has been cultured. The culture medium of each blastocyst is placed in a tube and shipped to the Igenomix laboratory, where the copy number for 24 chromosomes is analysed by Next Generation Sequencing (NGS). According to the NGS results for each sample, the corresponding embryo will be given an Embryo Priority Score for transfer.

Note- EMBRACE is not an accredited test.

5.7.1 SAMPLE REQUIREMENTS:

- 10µl of media collected on day 6 or 7 of embryo development
- The cells must be tubed into blue 0.2ml tubes provided by Igenomix UK. The lid of these tubes must be labelled with the female patient initials followed by the embryo number.
- All 0.2ml tubes must be placed in a "tube rack" (provided by Igenomix UK and labelled as EMBRACE) with the lid labelled with the patient's name, patient date of birth and the unique patient ID number.
- The "tube rack" must be placed into a sterile plastic bag and placed in -20°C for a minimum of 12 hours.
- To transport, place the tube rack in a cooler with two cool packs, also provided by Igenomix UK, which the IVF clinic must freeze in advance.

Note: The ice packs and the tubes must be stored at -20°C when received in the IVF lab and until they are used to send samples back to Igenomix UK. Please refer to the "EMBRACE Instructions" for clear guidance.

Further information on how to prepare a sample is found in the "EMBRACE IVF Lab Protocol" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.

- The "EMBRACE Test Requisition Form and Media Worksheet" which can also be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email, must be completed and placed in the cooler prior to transport, as described in the "EMBRACE Instructions".

5.7.2 USER VALIDATION:

Following the enrolment of a new clinic, a "validation" or "dry" run is performed before any clinical samples can be processed. Instructions on how to complete a "validation run" (EMBRACE IVF Lab Validation) requested by email.

A "validation/dry run report" is issued after the results have been analysed by a senior member of laboratory staff or the Laboratory Director.

Culture media samples taken by an embryologist will only be processed after the clinic's successful completion of a "validation/dry run".

6 OUTSOURCED TESTS

The laboratory offers additional services that are currently outsourced to the headquarters in Spain, including Endometrial Receptivity Analysis (ERA); Endometrial Microbiome Metagenomic Analysis (EMMA); Analysis of Infectious Chronic Endometritis (ALICE); a combined ERA+ALICE+EMMA test (EndomeTRIO), non-invasive prenatal testing (NACE & NACE 24), Carrier Genetic Testing (multiple tests which are branded under 'CGT' and an associated family mutation test), Sperm Aneuploidy Testing (SAT), and testing for Products of Conception (POC).

There are additional services that are also currently outsourced to Igenomix Italy: Pre-PGT-M, CGT Essential (a variant of the Carrier Genetic Test), Cystic Fibrosis Screening, Karyotyping, and testing for Y Chromosome Microdeletions.

6.1 ENDOMETRIAL RECEPTIVITY ANALYSIS (ERA)

The lack of synchronisation between an embryo transfer and endometrial receptivity is believed to be one of the causes of implantation failure. ERA is a test that has been developed and patented in 2009 by Igenomix after more than 10 years of research and development. The ERA test helps to evaluate a woman's endometrial receptivity and thus identify a 'window of implantation' based on molecular markers. The test uses RNA sequencing by NGS to analyse material biopsied from the endometrium. ERA assesses the expression levels of 248 genes linked to the status of endometrial receptivity. Following analysis, a specific computational predictor classifies samples, according to their expression profiles, as "Receptive" or "Non-Receptive", with further sub-categorisation of 12-hour receptivity shifts as "Pre-receptive", "Early receptive", "Late receptive", and "Post-receptive". This information enables a personalised embryo transfer (pET), synchronising endometrial receptivity with an embryo for transfer.

6.1.1 SAMPLE REQUIREMENTS:

- Endometrial tissue (~70mg by mass or ~10mm by size) placed in a cryotube containing RNA stabilizing solution (provided by Igenomix UK).
- The endometrial biopsy should be performed following the recommended protocol, as outlined in the "EndomeTRIO Manual" and "ERA, EMMA, ALICE, EndomeTRIO Instructions", both of which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "ERA – EMMA – ALICE – ENDOMETRIO Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) Transportation of Samples to Igenomix UK:

The clinic should notify Igenomix UK when a biopsy is ready, or up to 5 days in advance, and Igenomix UK will offer to arrange a courier for sample pickup. Shipment is at Room Temperature in kits provided by Igenomix UK.

6.2 ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

The "endometrial microbiome" is composed of various microorganisms co-existing within the endometrium/uterine cavity. Of these microorganisms, the bacterium *Lactobacillus* is a vital marker in predicting the "health" of the endometrial microbiome. Studies have shown that *Lactobacillus*-dominant

microbiomes are associated with better reproductive health than those where *Lactobacillus* abundance is lower; dysbiosis of the uterine cavity is associated with poor reproductive outcomes in assisted reproductive treatment patients. This suggests that altered endometrial *Lactobacillus* levels (and the presence of other bacteria) could play a role in infertility.

EMMA uses NGS to analyse the complete endometrial microbiome profile for an endometrial tissue sample. The test is based on DNA extraction followed by amplification and barcoded sequencing of the bacterial 16S ribosomal RNA gene. EMMA includes the ALICE test.

6.2.1 SAMPLE REQUIREMENTS:

- Endometrial tissue (~70mg by mass or ~10mm by size) placed in a cryotube containing RNA stabilizing solution (provided by Igenomix UK).
- The endometrial biopsy should be performed following the recommended protocol, as outlined in the "EndomeTRIO Manual" and "ERA, EMMA, ALICE, EndomeTRIO Instructions", both of which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.

6.3 ANALYSIS OF INFECTIOUS CHRONIC ENDOMETRITIS (ALICE)

A prime example of a pathology caused by an altered endometrial microbiome is chronic endometritis (CE). CE is a persistent, often asymptomatic, inflammation of the endometrial lining primarily caused by infection of the uterine cavity by bacterial pathogens.

ALICE uses NGS to analyse the endometrial microbiome profile for an endometrial tissue sample and detects the presence of bacteria most frequently associated with CE, including those that cannot be detected by methods such as bacterial culture. The test is based on DNA extraction followed by amplification and barcoded sequencing of the bacterial 16S ribosomal RNA gene.

ALICE is a subset test of EMMA that can be ordered as a stand-alone test.

6.3.1 SAMPLE REQUIREMENTS:

- Endometrial tissue (~70mg by mass or ~10mm by size) placed in a cryotube containing RNA stabilizing solution (provided by Igenomix UK).
- The endometrial biopsy should be performed following the recommended protocol, as outlined in the "EndomeTRIO Manual" and "ERA, EMMA, ALICE, EndomeTRIO Instructions", both of which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.

6.4 ENDOMETRIO

EndomeTRIO is a combined test including ERA, EMMA and ALICE.

6.4.1 SAMPLE REQUIREMENTS:

The combined test is available on a single sample. Samples must be collected according to the guidance for ERA, which has more stringent requirements than the EMMA or ALICE components of the test. Please refer to the requirements for ERA (section 5.1.1) for further information.

6.5 NACE BASIC & NACE 24

NACE is a non-invasive prenatal genetic screening test, which, unlike invasive prenatal diagnosis, does not pose a risk to an ongoing pregnancy. NACE uses NGS to analyse foetal DNA, which is found in maternal blood from 10 weeks of gestation. The foetal DNA is compared to maternal DNA in order to detect certain foetal abnormalities with high precision and reliability. Two versions of the test are available: NACE Basic and NACE 24. NACE Basic is designed to detect foetal Trisomy 21, 18, 13 and sex chromosome aneuploidies; NACE 24 is designed to detect foetal chromosome aneuploidies in all 24 chromosomes and detection of partial/segmental alterations greater than 7Mb in all chromosomes, depending on foetal fraction

6.5.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (7-10ml) in a Streck tube (provided by Igenomix UK).
- All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "NACE Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) Transportation of Samples to Igenomix UK:

The clinic must contact Igenomix UK in advance of blood sample collection, as, for reasons of sample integrity, there are limitations on the days of the week on which NACE samples can be taken. Igenomix UK will offer to arrange a courier for sample pickup. Shipment is at Room Temperature in kits provided by Igenomix UK.

6.6 CARRIER GENETIC TEST (CGT)

CGT is a genetic test which uses NGS to detect carriers of known pathogenic mutations that may pose a risk for future progeny of having a serious genetic disorder. A "positive" result indicates the presence of one or more mutations in the individual, in which case CGT testing of the individual's reproductive partner is strongly recommended if they intend to have a child. Alternatively, both partners can be tested simultaneously in a "couple" test, for which a combined result of risk to the potential child is issued. CGT may be used for gamete donors.

If both reproductive partners are carriers of a mutation in the same gene – a situation referred to as "co-carriage" – or if the female partner is a carrier of an X-linked disorder, there is a significant risk of having a child affected by the associated genetic disease. There are a range of options available for these reproductive couples to consider, including but not limited to performing PGT-M, using a gamete donor, adoption, or conceiving naturally and performing prenatal diagnosis.

A negative result indicates that the person does not carry any of the mutations included in the requested panel of mutations in specific genes. CGT is available as several different tests, which each have a different panel, the details of which are available on the website (https://www.igenomix.co.uk/genetic-solutions/carrier-genetic-test-clinics/cgt-list/) or may be requested by email.

6.6.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK), or
- Saliva sample in Oragene 510 tube (Tubes provided by Igenomix UK)

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "CGT Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) For CGTSync, a case discussion with the Igenomix UK laboratory and genetic counselling teams is required before sending samples.

6.7 GENOMIC PRECISION DIAGNOSTICS (GPDX)

Human Genome is composed of a protein codifying region made up of exons or Exome (1.35% of the genome) and a non-codifying region made up of introns (30% of the genome). 85% of all known disease-

causing variants are located within the Exome. There are over 7,000 genetic disorders, many of which are fatal or severely debilitating. Genetic disorders can be caused by a mutation in one gene (monogenic disorder), by mutations in multiple genes (multifactorial inheritance disorder), by a combination of gene mutations and environmental factors, or by damage to chromosomes.

The aim with the test performed by GPDx is to provide a genetic diagnosis to people suffering from a genetic condition or suspecting its presence to identify the cause.

6.7.1 SAMPLE REQUIREMENTS:

One of the following samples is required-

- Saliva sample in Oragene tube (Tubes provided by Igenomix UK)
- Peripheral blood (in EDTA tubes)
- Extracted DNA (10µl with a minimum concentration of 50 ng/µl)

All samples must be accompanied by the completed "Genomic Precision Diagnostic Test Requisition and Consent Form" which can be requested by email. For GPDx a case discussion with the Igenomix UK laboratory and genetic counselling teams is required before sending samples.

6.8 SPERM ANEUPLOIDY TEST (SAT)

The Sperm Aneuploidy Test (SAT) is a test that helps to assess male infertility by measuring the percentage of spermatozoa with chromosomal abnormalities in a semen sample. The test uses Fluorescence *in Situ* Hybridization (FISH) to specifically analyse the chromosomes most commonly observed in spontaneous miscarriages and affected offspring with chromosomal abnormalities (chromosomes 13, 18, 21, X and Y).

6.8.1 SAMPLE REQUIREMENTS:

- Semen sample suspended in 5ml of culture media in a 10 ml conical tube (provided by Igenomix UK).

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "SAT Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/) Alternative arrangements for sample types and processing are available. Please contact the Igenomix UK laboratory for further information.

6.9 TESTING FOR PRODUCTS OF CONCEPTION (POC)

POC is a genetic test that can provide information to help determine whether a miscarriage is associated with one or more chromosomal abnormalities. POC testing, performed on tissue retrieved from the lost pregnancy, uses NGS to analyse all 24 chromosomes to detect any gross chromosomal abnormalities. Additionally, the test uses STR analysis to compare foetal and maternal DNA, meaning that any maternal contamination is detected.

6.9.1 SAMPLE REQUIREMENTS:

Both of the following samples are required:

- Biopsied tissue from the lost pregnancy, placed in a specimen pot (provided by Igenomix UK) and covered with saline solution.
- Maternal saliva sample (collection tube provided by Igenomix UK).

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "POC Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/).

6.10 TESTING FOR NON-INVASIVE PRODUCTS OF CONCEPTION (NIPOC)

niPOC is an advanced non-invasive test which uses a blood sample to investigate whether a pregnancy loss may have been caused by a chromosome abnormality, by analysing circulating foetal DNA.

6.10.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (7-10ml) in a Streck tube (provided by Igenomix UK).

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "niPOC Instructions" which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/).

6.11 CYSTIC FIBROSIS SCREENING

Cystic fibrosis screening provides analysis of the CFTR gene to detect mutations that identify an individual as a carrier of cystic fibrosis.

6.11.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK).

All samples must be accompanied by the completed "Test Requisition & Consent Form" which can be requested by email and packaged in accordance with the "CGT Instructions" (which also apply for cystic fibrosis screening) which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/).

6.12 KARYOTYPING

Karyotyping analyses a DNA sample to establish the chromosomal complement of an individual, known as a karyotype. Karyotyping aims to detect certain abnormalities, such as structural translocations, which may be associated with infertility.

6.12.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (3ml) in a lithium heparin tube (provided by Igenomix UK).

For sample instructions and other guidance, please contact the laboratory. Please do NOT collect blood for karyotyping without prior consultation with Igenomix UK.

6.13 TESTING FOR Y CHROMOSOME MICRODELETIONS

Microdeletions in the Y chromosome in men may be associated with infertility. This service aims to detect these abnormalities.

6.13.1 SAMPLE REQUIREMENTS:

- Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK).

For sample instructions and other guidance, please contact the laboratory. Please do NOT collect blood for testing for Y chromosome microdeletions without prior consultation with Igenomix UK.

7 SAMPLE ACCEPTANCE CRITERIA

Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
PGT-A, PGT-M, PGT-SR, Mitoscore	Trophectoderm sample	Day 5, 6, or 7 trophectoderm biopsy with	Samples that have been stored at or below -20°C will be	The lot number of the wash/tubing buffer (which is the same lot

Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
		Igenomix wash buffer in 0.2ml tubes provided by Igenomix	accepted for up to 2 weeks from the biopsy date. Samples that have been in transit for longer than 48 hours may be rejected	number as the kit label) will be checked for the expiration date. If the expiration date has passed, Igenomix UK will contact the user/clinic to explain that tubing with expired wash/tubing buffer might lead to sub-optimal results. Igenomix UK will only accept case if the clinic provides, by email, consent for testing and confirms that they understand and accept responsibility for potential sub-optimal results.
EMBRACE	Blastocyst culture medium	10µl of media collected on day 6 or 7 of embryo development in 0.2ml tubes provided by Igenomix	Samples that have been stored at or below -20°C will be accepted for up to 3 weeks from the media collection date. Samples that have been in transit for longer than 48 hours may be rejected.	
Pre-PGT-M	Blood sample	Peripheral blood sample (3ml) in an EDTA tube. Multiple tubes will be accepted	Samples that have been stored between 4-8°C will be accepted for up to 3 weeks from the date of the draw. Samples that have been left at room temperature for longer than 3 days will be rejected.	
	Buccal swab	2 buccal swabs per patient, sealed inside the swab pouch (swabs provided by Igenomix UK)	Samples that have been stored at room temperature will be accepted for up to 3 weeks from the date	

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Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
			of the collection date.	
	Saliva sample	Saliva sample in Oragene 510 tube (Tubes provided by Igenomix UK)	Samples that have been stored at room temperature will be accepted for up to 3 weeks from the date of the collection date.	
	Extracted DNA	Extracted DNA (10µl with a minimum concentration of 10 ng/µl)		
ERA, EMMA, ALICE, EndomeTRIO	Endometrial biopsy	Sample size of ~70mg by mass or ~10mm by size; not more than 1/3 of the cryotube	Samples that have been stored between 4-8°C will be accepted for up to 3 weeks from the biopsy date. Samples that have been left at room temperature for longer than 5 days will be rejected	Sample must be in the supplied cryotube containing RNA stabilising solution
NACE, NACE24	Blood sample	Peripheral maternal blood sample (10ml) in a Streck tube (provided by Igenomix UK). 10ml is recommended with a minimum of 7ml.	Samples must arrive at the testing laboratory within 4 days of blood draw. Users/clinics will be given guidance by the customer support team, which may include instructions to take blood samples for NACE on specific days of the week.	Samples that have been stored between 4-8°C will be accepted for up to 10 days from the date of the draw.
CGT	Blood sample	Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK).	Samples that have been stored between 4-8°C will be accepted for up to 3 weeks from the date of the draw.	

Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
		Multiple tubes will be accepted.	Samples that have been left at room temperature for longer than 3 days will be rejected	
	Saliva sample	Saliva sample in Oragene 510 tube (Tubes provided by Igenomix UK)	Samples that have been left at room temperature for longer than 3 days will be rejected	
Genomic Precision Diagnostics (GPDX)	Blood sample	Peripheral blood sample (3ml) in an EDTA tube. Multiple tubes will be accepted	Samples that have been stored between 4-8°C will be accepted for up to 3 weeks from the date of the draw. Samples that have been left at room temperature for longer than 3 days will be rejected	
	Extracted DNA	Extracted DNA (10µl with a minimum concentration of 50 ng/µl)		
	Saliva sample	Saliva sample in Oragene 510 tube (Tubes provided by Igenomix UK)	Samples that have been stored at room temperature will be accepted for up to 3 weeks from the date of the collection date.	
SAT	Ejaculated sperm sample	Semen sample suspended in 5ml of culture media in a 10 ml conical tube	Samples that have been left at room temperature for longer than 48 hours will be rejected.	NOTE – different sample preparation methods are possible. Contact the laboratory team for more information
POC	Tissue sample	Sample of minimum size 3x3 mm in a sterile container,	Samples that have been stored between 4-8°C will be accepted for up to	

Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
		covered by saline solution.	6 days from the collection date. Samples that have been left at room temperature for longer than 36 hours will be rejected	
	Blood sample	Peripheral maternal blood sample (4ml) in an EDTA tube (provided by Igenomix UK).	Samples that have been stored between 4-8°C will be accepted for up to 5 days from the date of the draw. Samples that have been left at room temperature for longer than 3 days will be rejected	
niPOC	Blood sample	Peripheral maternal blood sample (10ml) in a Streck tube (provided by Igenomix UK). 10ml is recommended with a minimum of 7ml.	Samples must arrive at the testing laboratory within 4 days of blood draw. Users/clinics will be given guidance by the customer support team, which may include instructions to take blood samples for niPOC on specific days of the week.	Samples that have been stored between 4-8°C will be accepted for up to 10 days from the date of the draw.
Cystic Fibrosis Screening	Blood sample	Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK).	Samples that have been stored between 4-8°C will be accepted for up to 8 weeks from the date of the draw. Samples that have been left at room temperature for longer than 3 days will be rejected	
Karyotyping	Blood sample	Peripheral blood sample (3ml) in a		Please do NOT collect blood for testing without

Test(s)	Sample Type	Physical Criteria	Degradation criteria	Other criteria
		Lithium Heparin tube (provided by Igenomix Italy).		prior consultation with Igenomix UK.
Testing for Y Chromosome Microdeletions	Blood sample	Peripheral blood sample (3ml) in an EDTA tube (provided by Igenomix UK).		Please do NOT collect blood for testing without prior consultation with Igenomix UK.

8 TRANSPORTATION OF SAMPLES

8.1 IN-HOUSE TESTS

For the Pre-PGT-M workup, saliva, buccal, blood or extracted DNA samples, should be sent to the laboratory by either first class mail or a similar secure service and packaged according to UN packing requirement PI 650 and clearly labelled 'diagnostic specimen UN3373'.

For PGT-A, PGT-M, PGT-SR embryo testing & EMBRACE, the clinic should notify the Igenomix UK laboratory and support team before a sample is ready, and Igenomix UK will arrange for sample pickup. Shipment is at room temperature, including the pre-frozen “cool packs”.

8.2 OUTSOURCED TESTS

For all Outsourced tests, all samples must be accompanied by the completed “Test Requisition & Consent Form,” which can be requested by email, and packaged in accordance with the instructions which can be downloaded from the website (https://www.igenomix.co.uk/send-a-sample/).

The clinic should notify Igenomix UK when a biopsy is ready, or up to 5 days in advance, and Igenomix UK will offer to arrange a courier for sample pickup. Shipment is at Room Temperature in kits provided by Igenomix UK.

For Karyotyping and Testing for Y chromosome microdeletions please contact the laboratory. Please do NOT collect blood for testing without prior consultation with Igenomix UK.

Requests for alternative transportation arrangements must be discussed with the laboratory first.

9 GENETIC COUNSELLING

Igenomix UK offers consultations with our UK-based genetic counsellor. These consultations are held remotely using a digital videoconferencing platform. Genetic counselling is provided free of charge for certain tests and specific results.

Requesting genetic counselling:

For information about how to request genetic counselling, or for further information about the inclusion of genetic counselling with tests from Igenomix UK, please contact support.uk@igenomix.com.

10 ACCREDITATION AND ENROLMENT IN EXTERNAL ASSESSMENT SCHEMES

Igenomix UK is a UKAS accredited medical laboratory No. 10131.

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The scope of accreditation can be accessed by searching our name or laboratory number at:

https://www.ukas.com/find-an-organisation/

The laboratory is registered with the CQC under the Diagnostic and Screening criteria.

The laboratory participates annually in Genomics Quality Assessment (GenQA) schemes for PGT-M, PGT-A and PGT-SR. To date Igenomix UK has received satisfactory performance for all tests. Any recurring poor performance will be communicated to all users.

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