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.

The laboratory currently performs four 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), and MitoScore.

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).

One variant of the Carrier Genetic Test, called CGT Essential, is outsourced to the Igenomix Italy laboratory for analysis.

An extended non-invasive prenatal test (NACE Extended 24) is outsourced to Illumina in the United States of America for analysis.

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:

Prof. Alan Thornhill, PhD. State Registered Clinical Scientist (Clinical Embryology). Country Manager UK.

Dr Roy Pascal Naja, MSc, PhD, DipRCPath. State Registered Clinical Scientist (Genetics). Laboratory Director UK.

Ms Seema Dhanjal, MSc, MPhil. State Registered Clinical Scientist (Genetics). Deputy Laboratory Director UK, Senior Laboratory Scientist, Quality Manager, Health and Safety Manager.

Ms Kate Hall. Office Manager.

1.3.2 GENERAL ENQUIRIES:

Email: info.uk@igenomix.com, 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 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. Additional information regarding the different tests offered is available to users on the Igenomix UK website and can also be requested by email from info.uk@igenomix.com or support.uk@igenomix.com.

Test results are issued to the referring clinician. 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 info.uk@igenomix.com & support.uk@igenomix.com, after which they will be reviewed by the relevant members of staff in accordance with internal policies.

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 Regulation (EU) 2016/679 of the European Parliament and of the Council ('GDPR').

2.1 IMPORTANT GUIDANCE FOR ALL SAMPLES

2.1.1 GENERAL REASONS FOR SAMPLE REJECTION

Any 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

2.1.2 GUIDANCE FOR TEMPERATURE CONTROL

- When the outside ambient temperature exceeds 35°C, please contact the laboratory for further instructions on how to send samples.

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 "Consent Form", which may, for certain services, be a combined form. Some tests have additional documentation requirements.

For PGT-SR, PGT-M, and CGTOne, a referral is required before sending samples, as described in section 2. Please contact the Igenomix UK laboratory for further information about these requirements.

Referrals will be accepted only from medical professionals.

4 TESTS PERFORMED IN-HOUSE

The laboratory currently performs four 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), and MitoScore.

4.1 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.

4.1.1 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 or normal) 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
  3. Sibling of the affected member of the couple

- Autosomal recessive disorder where a child of the couple with known genetic status (affected or normal) is available to provide a sample as a reference. If a child is not available, then samples are required from the parents of the couple or their siblings (least favourable scenario).

- X-linked disorder 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
  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 where the informativity of the Karyomapping platform did not reach the accepted threshold (e.g. because the couple is consanguineous) then a mutation detection system will be developed and coupled with Karyomapping.

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.

4.1.2 SAMPLE REQUIREMENTS:

For the Pre-PGT-M workup-

- peripheral blood (in EDTA tubes), extracted DNA, and/or a buccal swab from the prospective parents and other relevant family members is required.
- Based on the outcome of the Pre-PGT-M workup, the laboratory will inform the IVF clinic by email whether Karyomapping is suitable for PGT-M embryo testing 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.

For PGT-M-

- 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 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 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 or requested by email.

- The “Embryo Biopsy Worksheet” and “Test Requisition Form”, which can also be downloaded from the website 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”.

4.1.3 USER VALIDATION:

Following the enrolment of a new clinic, a “validation” or “dry” run is performed for every embryologist involved in embryo biopsy for PGT-M. Instructions on how to complete a “validation run” (Embryo Biopsy_Tubing Validation Instructions and Washing_Tubing Instructions) can be downloaded from the website 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.

4.1.4 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

For the Pre-PGT-M workup, blood samples, extracted DNA, and/or buccal swabs 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-M embryo testing, 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”.

4.1.5 TURNAROUND TIME (TAT):

Pre-PGT-M**:**

20-30 working days, dependent on the case, from the receipt of the required samples by Igenomix UK.

PGT-M:

10 working days from the receipt of embryo samples at Igenomix UK.

4.1.6 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “PGT-A, PGT-SR, PGT-M Instructions” document may be rejected.

Additional criteria that may lead to the rejection of embryo biopsy samples include:

- Samples that have been stored at -20ºC for longer than 2 weeks
- Samples that have been in transit for longer than 48 hours

Additional criteria that may lead to the rejection of blood samples (for Pre-PGT-M workup) include:

- Samples that have been stored between 4-8°C for longer than 3 weeks
- Samples that have been stored at room temperature/in transit for longer than 3 days

Additional criteria that may lead to the rejection of buccal swab samples (for Pre-PGT-M workup) include:

- Samples that are received more than 3 weeks from their collection date

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

4.2 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).

4.2.1 SAMPLE REQUIREMENTS:

- 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 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 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 or requested by email.

- The "Embryo Biopsy Worksheet" and "Test Requisition Form", which can also be downloaded from the website 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".

4.2.2 USER VALIDATION:

Following the enrolment of a new clinic, a "validation" or "dry" run is performed for every embryologist involved in embryo biopsy for PGT-A. Instructions on how to complete a "validation run" (Embryo Biopsy_Tubing Validation Instructions and Washing_Tubing Instructions) can be downloaded from the website 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.

4.2.3 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

For PGT-A embryo testing, 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".

4.2.4 TURNAROUND TIME:

10 working days from the receipt of embryo samples at Igenomix UK.

4.2.5 REPORTING MOSAICISM:

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

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.

4.2.6 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

4.2.7 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “PGT-A, PGT-SR, PGT-M Instructions” document may be rejected.

Additional criteria that may lead to the rejection of embryo biopsy samples include:

- Samples that have been stored at -20ºC for longer than 2 weeks
- Samples that have been in transit for longer than 48 hours

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

4.3 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.

4.3.1 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. The general criteria for considering a PGT-SR case are as follows:

- All chromosome fragments involved in the rearrangement are ≥6Mb
- Cases where one fragment is <6Mb, the remaining fragments must be ≥6Mb
- Cases involving three or more chromosomes, marker chromosomes or additional material are not accepted.

Special considerations for translocations involving sex chromosomes are as follows:

- Cases where the translocation involves the Y chromosome are accepted
- Cases where the translocation involves the X chromosome of female carrier are not accepted

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

4.3.2 SAMPLE REQUIREMENTS:

- 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 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 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 or requested by email.

- The "Embryo Biopsy Worksheet" and "Test Requisition Form", which can also be downloaded from the website 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".

4.3.3 USER VALIDATION:

Following the enrolment of a new clinic, a "validation" or "dry" run is performed for every embryologist involved in embryo biopsy for PGT-SR. Instructions on how to complete a "validation run" (Embryo Biopsy_Tubing Validation Instructions and Washing_Tubing Instructions) can be downloaded from the website 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.

4.3.4 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

For PGT-SR embryo testing, 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".

4.3.5 TURNAROUND TIME:

10 working days from the receipt of embryo samples at Igenomix UK.

4.3.6 REPORTING MOSAICISM:

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

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.

4.3.7 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

4.3.8 SAMPLE ACCEPTANCE/ REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “PGT-A, PGT-SR, PGT-M Instructions” document may be rejected.

Additional criteria that may lead to the rejection of embryo biopsy samples include:

- Samples that have been stored at -20^{o}C for longer than 2 weeks
- Samples that have been in transit for longer than 48 hours

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

4.4 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.

MitoScore is only available as complementary analysis when performing PGT-A or PGT-SR. No additional samples nor information are required. Please refer to sections 4.2 and 4.3 for further information.

5 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 branded under 'CGT' and an associated family mutation test), Sperm Aneuploidy Testing (SAT), and testing for Products of Conception (POC).

One variant of the Carrier Genetic Test, called CGT Essential, is outsourced to the Igenomix Italy laboratory for analysis.

An extended non-invasive prenatal test (NACE Extended 24) is outsourced to Illumina in the United States of America for analysis.

5.1 ENDOMETRIAL RECEPTIVITY ANALYSIS (ERA)

The lack of synchronisation between an embryo for 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.

5.1.1 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 or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.
- Samples must be accompanied by the "Test Requisition & Consent Form" and packaged in accordance with the "ERA, EMMA, ALICE, EndomeTRIO Instructions", both of which can be downloaded from the website or requested by email.

5.1.2 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.

5.1.3 TURNAROUND TIME:

10 working days from the receipt of samples at Igenomix UK.

5.1.4 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under "sample requirements", the protocols under "transportation to the laboratory", or the instructions in the "ERA, EMMA, ALICE, EndomeTRIO Instructions" document may be rejected.

Additional criteria that may lead to the rejection of endometrial samples for ERA include:

- Samples that have not been collected in accordance with the "EndomeTRIO Manual" and "ERA, EMMA, ALICE, EndomeTRIO Instructions", including samples

that are too small (<5 mm) or excessively large (>7 mm) or containing an excess of blood and/or mucous

- Samples that have been stored between 4-8°C for longer than 3 weeks
- Samples that have been stored at room temperature/in transit for longer than 5 days

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.2 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 a 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.

5.2.1 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 or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.
- Samples must be accompanied by the "Test Requisition & Consent Form" and packaged in accordance with the "ERA, EMMA, ALICE, EndomeTRIO Instructions", both of which can be downloaded from the website or requested by email.

5.2.2 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.

5.2.3 TURNAROUND TIME:

10 working days from the receipt of samples at Igenomix UK.

5.2.4 SAMPLE ACCEPTANCE/REJECTIONCRITERIA:

Samples that do not meet the requirements listed under "sample requirements", the protocols under "transportation to the laboratory", or the instructions in the "ERA, EMMA, ALICE, EndomeTRIO Instructions" document may be rejected.

Additional criteria that may lead to the rejection of endometrial samples for EMMA include:

- Samples that have not been collected in accordance with the “EndomeTRIO Manual” and “ERA, EMMA, ALICE, EndomeTRIO Instructions”, including samples that are too small (<5 mm) or excessively large (>7 mm) or containing an excess of blood and/or mucous
- Samples that have been stored between 4-8°C for longer than 3 weeks
- Samples that have been stored at room temperature/in transit for longer than 5 days

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.3 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.

5.3.1 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 or requested by email.
- The cryotube containing the sample must be refrigerated (4-8°C) for a minimum of 4 hours prior to shipping.
- Samples must be accompanied by the “Test Requisition & Consent Form” and packaged in accordance with the “ERA, EMMA, ALICE, EndomeTRIO Instructions”, both of which can be downloaded from the website or requested by email.

5.3.2 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.

5.3.3 TURNAROUND TIME:

10 working days from the receipt of samples at Igenomix UK.

5.3.4 SAMPLE ACCEPTANCE CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “ERA, EMMA, ALICE, EndomeTRIO Instructions” document may be rejected.

Additional criteria that may lead to the rejection of endometrial samples for ALICE include:

- Samples that have not been collected in accordance with the “EndomeTRIO Manual” and “ERA, EMMA, ALICE, EndomeTRIO Instructions”, including samples

that are too small (<5 mm) or excessively large (>7 mm) or containing an excess of blood and/or mucous,

- Samples that have been stored between 4-8°C for longer than 3 weeks
- Samples that have been stored at room temperature/in transit for longer than 5 days

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.4 ENDOMETRIO

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

5.4.1 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.

5.4.2 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.

5.4.3 TURNAROUND TIME:

10 working days from the receipt of samples at Igenomix UK. Note that the ERA and EMMA/ALICE components of the test may be reported separately within this timeframe.

5.4.4 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

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 5.1.4) for further information.

5.5 NACE BASIC, NACE 24 & NACE EXTENDED 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 fetal DNA, which is found in maternal blood from 10 weeks of gestation. The fetal DNA is compared to maternal DNA in order to detect certain fetal abnormalities with high precision and reliability. Three versions of the test are available: NACE Basic, NACE 24 and NACE Extended 24. NACE Basic is designed to detect fetal Trisomy 21, 18, 13 and sex chromosome aneuploidies; NACE 24 and NACE Extended 24 are designed to detect fetal chromosome aneuploidies in all 24 chromosomes; with NACE Extended 24 also designed to detect six additional microdeletions.

5.5.1 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” and packaged in accordance with the “NACE Instructions” both of which can be downloaded from the website or requested by email.

5.5.2 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.

5.5.3 TURNAROUND TIME:

10 working days from the receipt of samples at Igenomix UK.

5.5.4 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “NACE Instructions” document may be rejected.

Additional criteria that may lead to the rejection of blood samples for NACE tests include:

- Samples that arrive at Igenomix UK more than 4 days after the date of blood draw

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.6 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://cgt.igenomix.com/ or may be requested by email.

A variant of CGT, known as CGTOne, uses NGS to perform a comprehensive analysis of up to three specifically selected genes to identify the presence of possible mutations according to an extensive, predefined Igenomix database. Details of the mutations able to be detected for a specific gene and options for testing beyond three genes will be provided upon request.

5.6.1 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” and packaged in accordance with the “CGT Instructions” both of which can be downloaded from the website or requested by email.

For CGTOne, a prior case discussion with the Igenomix UK laboratory and genetic counselling teams is required before sending samples.

5.6.2 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

The clinic should notify Igenomix UK when a sample 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.

5.6.3 TURNAROUND TIME:

25 working days from the receipt of samples at Igenomix UK.

5.6.4 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “CGT Instructions” document may be rejected.

Additional criteria that may lead to the rejection of blood samples for CGT include:

- Samples that have been stored between 4-8°C for longer than 8 weeks
- Samples that have been stored at room temperature/in transit for longer than 3 days

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.7 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).

5.7.1 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” and packaged in accordance with the “CGT Instructions” both of which can be downloaded from the website or requested by email.

Alternative arrangements for sample types and processing are available. Please contact the Igenomix UK laboratory for further information.

5.7.2 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

The clinic should notify Igenomix UK when a sample 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.

Transportation for alternative arrangements for sample collection and processing should be discussed with the laboratory.

5.7.3 TURNAROUND TIME:

15 working days from the receipt of samples at Igenomix UK.

5.7.4 SAMPLE ACCEPTANCE/REJECTIONCRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “SAT Instructions” document may be rejected.

Additional criteria that may lead to the rejection of endometrial samples for SAT include:

- Samples that have been left at room temperature/in transit for longer than 48 hours

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

5.8 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 fetal and maternal DNA, meaning that any maternal contamination is detected.

5.8.1 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.
- Peripheral blood sample (4ml) in an EDTA tube (provided by Igenomix UK).

All samples must be accompanied by the completed “Test Requisition & Consent Form” and packaged in accordance with the “POC Instructions” both of which can be downloaded from the website or requested by email.

5.8.2 TRANSPORTATION OF SAMPLES TO IGENOMIX UK:

The clinic should notify Igenomix UK when a sample 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.

5.8.3 TURNAROUND TIME:

15 working days from the receipt of samples at Igenomix UK.

5.8.4 SAMPLE ACCEPTANCE/REJECTION CRITERIA:

Samples that do not meet the requirements listed under “sample requirements”, the protocols under “transportation to the laboratory”, or the instructions in the “POC Instructions” document may be rejected.

Additional criteria that may lead to the rejection of POC tissue samples include:

- Tissue samples that have not been submerged in saline solution soon after collection
- Samples that have been stored between 4-8°C for longer than 6 days
- Samples that have been in transit for longer than 36 hours

Additional criteria that may lead to the rejection of blood samples for POC testing include:

- Samples that have been stored between 4-8°C for longer than 5 days
- Samples that have been stored at room temperature/in transit for longer than 3 days

For additional information regarding sample acceptability, please contact the Igenomix UK laboratory team.

6 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.

7 ACCREDITATION AND ENROLMENT IN EXTERNAL ASSESSMENT SCHEMES

The laboratory is accredited for ISO 15189:2012 by UKAS and the scope of accreditation is detailed in the following link: https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/10131%20Medical%20Multiple.pdf 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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