

# **IGENOMIX ARABIA MEDICAL LLC LABORATORY**

## **Part of Vitrolife Group**

### **DIRECTORY OF TEST SERVICES**

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# 1. IGENOMIX LABORATORY

## 1.1 INTRODUCTION

**IGENOMIX ARABIA MEDICAL LLC** is a multinational private medical testing laboratory, (under Commercial Registration No. **1010739879**), specializing in reproductive genetic services. We are now part of Vitrolife Group, a global leader in reproductive health. Igenomix currently performs tests in-house that can summarize as the following:, Preimplantation Genetic Testing for Aneuploidy (PGT-A) and Baby Gender (21, 18, 13, X and Y Chr).

Additionally, Igenomix offers different tests that are currently outsourced including: Endometrial Receptivity Analysis (ERA), Endometrial Microbiome Metagenomic Analysis (EMMA), Analysis of Infectious Chronic Endometritis (ALICE); Non-invasive prenatal tests (NACE@5/NACE@24); Genomic Precision Diagnostic (Whole Exome sequencing, Precision Panels, Single Gene testing by NGS); Preimplantation Genetic Testing for monogenic Diseases (PGT-M); Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR); EMBRACE: Embryo Analysis of Culture Environment; Chromosomal Microarray (CMA 750 K & HD); Maternal Cell Contamination (MCC); Target Mutation Analysis; Karyotyping; QF-PCR; Products of Conception (POC); Non-invasive genetic testing for Products Of Conception (niPOC), Whole genome sequencing (WGS), MLPA (Multiplex Ligation-dependent Probe Amplification); and Expansion repeat analysis.

## OPENING TIMES

The laboratory facilities for sample reception are open:

+ from **Sunday to Thursday 9:00am to 6:00 pm**

Customer Support service is available:

+ From **Sunday to Thursday 9:00am – 6:00pm**

## 1.2 CONTACT DETAILS

General Enquiries contact details:

+ by **Email** to [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

+ by **Tel:** **+966 549109444**

## 1.3 LAB ADDRESS

**IGENOMIX ARABIA MEDICAL LLC LABORATORY,**

Second floor , Building No 6641-C12&C13,

Secondary No 4724, West Oraiya District. EXT 28, Western Ring Road,

cross with Almadina Al munawwarah service road Riyadh 14291 Saudi Arabia.

[labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

+966 549109444

Website: [www.igenomix.net](http://www.igenomix.net)

## 2. MAIN ACTIVITIES

### 2.1 GENERAL INFORMATION

All genetic tests are carried out as clinically appropriate. Additional information regarding the different tests offered is available to users on the [www.igenomix.net](http://www.igenomix.net) and can also be requested by email to: [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com) , for all the products, you can contact us directly on mobile **+966 549109444**.

Further interpretation of reports is available to users by calling the laboratory **(+971 4 551 9465)** and requesting to speak with our Genetic counsellor/General Supervisor by sending an email to [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

#### COMPLAINT PROCEDURE

The laboratory is committed to delivering service of the highest quality at all times to ensure patient safety and customer satisfaction. For your convenience, any complaints about the service can be addressed through different channels. After receipt, complaints will be passed to the relevant members of staff.

+ by Email write us to [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

+ by phone: call us in **+966 549109444**

+ through our “request information” section or

+ through the complaint form included in the Quality section, both accessible on our website <https://www.igenomix.net/quality/#suggestion-complaint>.

All complaints will be acknowledged in less than 2 business days.

### 2.2 LABORATORY POLICY ON PROTECTION OF PERSONAL INFORMATION

The laboratory follows strict policies on Information Governance and maintains a data protection infrastructure in line with Local REGULATION

Further information about Igenomix Privacy Policy can be found on the [www.igenomix.net](http://www.igenomix.net)

#### REQUIREMENTS PRIOR TO SENDING A SAMPLE

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

Before referrals can be made, users need to complete the “Clinic Enrolment Form” which can be requested by email from [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com) and [finance.me@igenomix.com](mailto:finance.me@igenomix.com). Once the form is completed it should be returned by email to [finance.me@igenomix.com](mailto:finance.me@igenomix.com). This process is required to register the User under Igenomix LIS system.

The Test Requisition Form and the Informed Consent Form need to be completed, placed into the provided return courier envelope, and included in the kit box along with the sample to be sent to the laboratory. The soft copies of completed TRF and consent form can also be sent over email to [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

Any Test Requisition Form or Test Informed Consent can be requested by email from [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

Igenomix highly recommends that the test instructions, which can be found on the Igenomix webpage or requested from our Customer Support Service by email or phone (see section 1.3), are carefully read prior to sending samples. These documents provide relevant information about sample requirements, patient preparation, test documentation, sample collection and sample shipping for the different offered tests.

## 2.3 LABORATORY CRITERIA FOR ACCEPTING AND REJECTING SAMPLES

The following cases may lead to sample rejection:

- Incorrectly labelled, unlabelled or damaged sample containers (usually tubes)
- Expired sample collection material
- Failure to meet the specific test requirements indicated in the test instructions (for example, specific timings of sample collection, minimum amounts of sample (sufficient quality and quantity), specific biological status of patient, etc.)
- Sample received under primary container or tube that is not validated by Igenomix.
- No dry ice or ice packs present with sample where required/necessary.
- Questionable appearance of sample(s).
- The secondary container is damaged.

### ➤ **Sample are not rejected but on standby status (not able to enter into the lab) if:**

- Sample documentation (Test Requisition Form and Informed Consent) has not been correctly completed
- Samples not accompanied with their documentation (Test Requisition Form and Informed Consent)
- Mandatory fields in sample documentation, identified on the forms with an asterisk (\*), have not been completed.
- Document received are not approved by Igenomix (other languages, other logo, outdated version).
- Missing patient and/or physician signature on the Test Requisition and Informed Consent.
- Samples on standby or rejected are registered as incidence and only upon resolution of incidence with appropriate corrective action the sample will be further proceeded for examination.

## 2.4 INSTRUCTIONS FOR COMPLETION OF REQUEST DOCUMENTATION

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

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

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

## 3. TESTS OFFERED

### 3.1 IGENOMIX ARABIA

The laboratory currently performs the following major tests in-house: -, Preimplantation Genetic Testing for Aneuploidy (PGT-A), Baby Gender (21, 18,13, X and Y Chr),

If you require additional information about our test portfolio, please contact our Customer Support service at [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

#### 3.1.1. Preimplantation Genetic Testing for Aneuploidy (PGT-A)

##### PGT-A test description:

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

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

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

##### PGT-A sample requirements:

For PGT-A, one cell from day 3 of embryonic development (blastomere biopsy) or 4-8 cells from day 5, 6 or 7 of embryonic development (trophectoderm biopsy) are required. The biopsied cell/s must be cleaned using the “washing/loading buffer” supplied by the laboratory to eliminate any potential source of contamination and transferred to a small sterile 0.2ml tube supplied by the laboratory. The lids of these tubes must be labelled with the female patient initials followed by the embryo number. The 0.2ml tubes must be placed in the “plate/rack” provided by the laboratory, the “plate/rack” placed between the cooler shipping box with the “ice packs” also provided by the laboratory. Further information on how to prepare a sample is found in the “Tubing Instructions” [SAU\\_L\\_I\\_PGT\\_002\\_EN: Instructions - PGT Tubing Loading protocol](#) that can be requested by email to Customer Support (see section 1.3).

The “Embryo Biopsy Worksheet” and the “Test Requisition Form and Consent” (included within the provided kit or can be requested from Customer support at [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)) must be completed and sent with the samples inside the shipping box or by e-mail to the laboratory.

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

Following the enrolment of a new clinic (see section 2.4), we recommend performing a “validation” or “dry run” for every embryologist involved in the embryo biopsy/tubing for PGT-A. This process aims to provide reduce the likelihood of difficulties with clinical cases that could lead to a failure to determine a result(s) for the sampled embryo(s). Instructions on how to complete a “validation run” can be requested by email to Customer support (see section 1.3). A validation/dry run report is issued after the analysis and signed by a senior member of laboratory staff or the Laboratory Director.

##### PGT-A sample transportation to the laboratory:

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

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

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

#### **PGT-A test turnaround time:**

**The Physician that has requested** the test will receive the results.

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

For PGT-A samples with **fresh transfer** results will be available **on the morning of the next day following the receipt of samples or 2 Calendar days** by Igenomix.

#### **PGT-A reporting:**

- **Normal/Euploid:** An embryo is considered as Normal/euploid when the graph shows no “Threshold” deviations from the reference bioinformatics baseline for any of the 24 chromosomes assessed. “Threshold” - Embryos with less than 50% chromosome aneuploidy in the biopsy will be reported as euploid.
- **Abnormal/Aneuploid:** An embryo is considered as "Aneuploid" when an aneuploidy [gain or loss of a chromosome (1-22, X, Y)] or partial aneuploidy [gain or loss of a piece of a chromosome arm (p, q)] is detected as a result of a “Threshold” deviation from the reference bioinformatics baseline with points shifting upwards for a gain (Trisomy) and downwards for a loss (Monosomy). Partial aneuploidies are specified with chromosome number, arm (p, q), cytoband and fragment size in megabases (Mb). “Threshold” - Embryos with more than 50% chromosome aneuploidy in the biopsy will be reported as aneuploid.
- **Complex abnormal:** An embryo is considered to be Complex aneuploid when 2-5 aneuploidies are detected in the provided sample.
- **Chaotic:** An embryo is considered to be Chaotic when 6 or more aneuploidies are detected in the provided sample. The predictive value of the chaotic result may be reduced. Re-biopsy and retesting can be considered depending on blastocyst quality.
- **No DNA detected:** Usually, if no DNA is detected, the cell did not have an intact nucleus. Other potential issues leading to No DNA Detected include poor embryo quality and degraded DNA. In some cases, cells may get lost during the washing procedure or may not be successfully transferred into a tube. Re-biopsy and retesting can be considered in these cases according to blastocyst quality.
- **Non-informative:** When a reliable result cannot be achieved with high confidence, the embryo result will be reported as non-informative. Re-biopsy and retesting can be considered in these cases according to blastocyst quality.

## **3.2 IGENOMIX AFFILIATE**

Igenomix Arabia Medical LLC offers different tests that are currently referred to IGENOMIX affiliated laboratory including: Endometrial Receptivity Analysis (ERA), Endometrial Microbiome Metagenomic Analysis (EMMA), Analysis of Infectious Chronic Endometritis (ALICE); Non-invasive prenatal tests (NACE@5/NACE@24); Genomic Precision Diagnostic (Whole Exome sequencing, Precision Panels, Single Gene testing by NGS); Preimplantation Genetic Testing for monogenic Diseases (PGT-M); Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR); EMBRACE: Embryo Analysis of Culture Environment; Chromosomal Microarray (CMA 750 K & HD); Maternal Cell Contamination (MCC); Target Mutation Analysis; QF-PCR; Products of Conception (POC); Non-invasive genetic testing for Products Of Conception (niPOC); MLPA (Multiplex Ligation-dependent Probe Amplification); and Expansion repeat analysis.

### 3.2.1 ERA

#### **Description:**

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

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

#### **Pre-requirements for accepting an ERA case:**

No specific pre-requirements are needed in order to accept an ERA case. We strongly encourage you to carefully read the "ERA-EMMA-ALICE Manual" for further information in addition to the specific ERA-EMMA-ALICE test instructions. You can download these documents from the Igenomix website and from the specific website [How to send a sample - Middle East \(igenomix.net\)](https://www.igenomix.net/en/how-to-send-a-sample-middle-east)

#### **ERA test sample requirements:**

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

In order to obtain a fully confident test result, the ERA-EMMA-ALICE Manual details must be strictly followed. This document can be downloaded either from the ERA-EMMA-ALICE website ([How to send a sample - Middle East \(igenomix.net\)](https://www.igenomix.net/en/how-to-send-a-sample-middle-east)), the Igenomix website or requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

The "Test Requisition Form" (provided within the provided kit and additionally available either from the ERA-EMMA-ALICE website [How to send a sample - Middle East \(igenomix.net\)](https://www.igenomix.net/en/how-to-send-a-sample-middle-east)) or requested by email) must be completed and sent with the sample inside the shipping box.

#### **ERA sample transportation to the laboratory:**

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

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



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

### ERA test turnaround time:

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

### ERA test reporting:

The result of the test can be:

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

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

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

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

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

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

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

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

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

## 3.2.2 Endometrial Microbiome Metagenomic Analysis (EMMA)

### EMMA test description:

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

EMMA can be beneficial for any woman wishing to conceive, especially those with recurrent implantation failure and recurrent pregnancy loss, by analyzing the microbial environment of the uterine cavity including the most frequently bacterial pathogens that cause Chronic Endometritis (CE). The EMMA test always includes the ALICE test.

### Pre-requirements for accepting an EMMA case:

No specific pre-requirements are needed in order to accept an EMMA case. We strongly encourage you to carefully read the “ERA-EMMA-ALICE Manual” for further information in addition to the specific ERA-EMMA-ALICE test instructions. You can download these documents from the Igenomix website [How to send a sample - Middle East \(igenomix.net\)](https://igenomix.net)

### **EMMA test sample requirements:**

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

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

The endometrial biopsy must be taken from the uterine fundus. Sample size should be approx. 70 mg and not exceed the white line marked on the Igenomix cryotube. Larger samples may still be evaluated to determine if the genetic material has been properly preserved. If this is not the case, a new sample will be requested. Ensure that the sample is made up of endometrial tissue and not solely blood or mucus. Label the cryotube with the patient's full name, DOB, and date of biopsy. As the microbiome can fluctuate over time, the samples should be sent as soon as possible. The cryotube containing the sample must be adequately closed, shaken and refrigerated (4-8° C) for a minimum of 4 hours before shipment. For shipping, the cryotube containing the endometrial biopsy must be placed in a blister pack as a secondary container. In order to obtain a fully confident test result, the ERA-EMMA-ALICE Manual details must be strictly followed. This document can be downloaded either from the ERA-EMMA-ALICE website ([How to send a sample - Middle East \(igenomix.net\)](https://igenomix.net)), the Igenomix website or requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

The “Test Requisition Form” for ERA-EMMA-ALICE are requested by email and must be completed and sent with the sample inside the shipping box.

### **EMMA sample transportation to the laboratory:**

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

The sample should be sent to the laboratory by either first class mail or a similar secure service (DHL, SMSA etc.) and must be packed according to a set IATA guideline for “Packaging Instructions and clearly labelled 'Exempt Human Specimen UN3373 when the sample is not delivered from Saudi (this courier service is not offered by the laboratory but outsourced to a third-party logistics company).

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

### **EMMA test turnaround time:**

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

### EMMA test reporting:

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

1. One table showing the reference ranges for 4 species of *Lactobacilli* (*L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*) and the values obtained in the endometrial sample.
2. One table showing the reference ranges for 17 species of common reproductive tract pathogens (*Actinomyces israelii*, *Atopobium vaginae*, *Bacteroides fragilis*, *Bifidobacterium spp*, *Clostridium sordelii*, *Dialister spp*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Mobiluncus spp*, *Mycobacterium tuberculosis*, *Peptostreptococcus anaerobius*, *Porphyromonas asaccharolytica*, *Prevotella bivia*, *Prevotella disiens*, *Sneathia spp* and *Treponema pallidum*) and the values obtained in the endometrial sample.
3. One table with ALICE results, showing the reference ranges for 10 species of pathogens causing chronic endometritis (CE) (*Streptococcus agalactiae* (group B) and *Streptococcus viridans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and the values obtained in the endometrial sample.
4. *Lactobacillus* is the predominant bacteria in the reproductive tract of women at reproductive age. If at least one of the *Lactobacillus* species is within the reference range, this is considered a normal result. *Lactobacillus* levels will be considered out of the reference range when all the targeted species are not detected or present values below the established reference range.
5. In case that DNA from *Haemophilus ducreyi*, *Mycobacterium tuberculosis*, *Treponema pallidum*, *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* is detected in the endometrial sample, an additional confirmatory test will be recommended. Infections caused by these bacteria are mandatory notification to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.
6. In case that DNA from *Actinomyces israelii*, *Clostridium sordelii* and/or *Fusobacterium nucleatum* is detected in the endometrial sample, an additional confirmatory test and follow-up by a physician will be recommended.
7. Values of pathogens out of the reference range are identified with an asterisk and highlighted in bold.
8. EMMA report includes a suggested therapy (if needed) considering each specific bacterium detected out of the reference range, to achieve a *Lactobacillus*-dominated reproductive tract, increasing the chances of achieving pregnancy, as is describes in the scientific literature. However, is the medical professional who must consider the possible prescription of an antibiotic and/or probiotic treatment in conjunction with the available clinical findings of each patient. In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

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

### 3.2.3 Analysis of Infectious Chronic Endometritis (ALICE)

#### ALICE test description:

ALICE is a molecular test performed using RT-PCR, which detects the presence of DNA from potentially pathogenic bacteria that most frequently cause chronic inflammation of the endometrium, known as Chronic Endometritis (CE). This disease has been linked to infertility and obstetric complications. ALICE can be helpful in determining which pathogenic bacteria are present in the uterine cavity and which may be the cause of chronic endometritis. These results may help determine the most appropriate treatment to eliminate the potential pathogens causing the disease.

### **Pre-requirements for accepting an ALICE case:**

No specific pre-requirements are needed in order to accept an ALICE case. We strongly encourage you to carefully read the “ERA-EMMA-ALICE Manual” for further information in addition to the specific ERA-EMMA-ALICE test instructions. You can download these documents from the Igenomix website [How to send a sample - Middle East \(igenomix.net\)](https://www.igenomix.net/middle-east/how-to-send-a-sample)

### **ALICE sample requirements:**

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

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

The endometrial biopsy must be taken from the uterine fundus. Sample size should be approx. 70 mg and not exceed the white line marked on the Igenomix cryotube. Larger samples may still be evaluated to determine if the genetic material has been properly preserved. If this is not the case, a new sample will be requested. Ensure that the sample is made up of endometrial tissue and not solely blood or mucus. Label the cryotube with the patient's full name, DOB, and date of biopsy. As the microbiome can fluctuate over time, the samples should be sent as soon as possible. The cryotube containing the sample must be adequately closed, shaken and refrigerated (4-8° C) for a minimum of 4 hours before shipment. For shipping, the cryotube containing the endometrial biopsy must be placed in a blister pack as a secondary container.

In order to obtain a fully confident test result, the ERA-EMMA-ALICE Manual details must be strictly followed. This document can be downloaded either from the ERA-EMMA-ALICE website ([How to send a sample - Middle East \(igenomix.net\)](https://www.igenomix.net/middle-east/how-to-send-a-sample)), the Igenomix website or requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)

The “Test Requisition Form” for ERA-EMMA-ALICE are requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com) and must be completed and sent with the sample inside the shipping box. If the mandatory fields in the ERA-EMMA-ALICE Test Requisition Form are not properly completed, samples may be rejected.

### **ALICE sample transportation to the laboratory:**

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

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

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

### ALICE test turnaround time:

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

### ALICE test reporting:

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

This includes:

- 1) The ALICE report shows a table with the reference ranges for 10 species of reproductive tract pathogens most often related to chronic endometritis (*Streptococcus agalactiae* (group B) and *Streptococcus viridans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and the values obtained in the endometrial sample. Values of pathogens out of the reference range are identified with an asterisk and highlighted in bold.
- 2) In case *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* are out of the normal range, an additional confirmatory test will be recommended. Infections caused by these bacteria are mandatory notifications to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.
- 3) ALICE report includes suggested therapy (if needed) taking into account each specific bacterium detected out of the reference range, to increase the chances of achieving a healthy pregnancy as described in the scientific literature. However, is the medical professional who must consider the possible prescription of an antibiotic and/or probiotic treatment in conjunction with the available clinical findings of each patient. In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

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

## 3.2.4 Preimplantation Genetic Testing for Monogenic Diseases (PGT-M)

### PGT-M test description:

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

### Pre-requirements for accepting a PGT-M case:

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

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

### PGT-M test sample requirements:

For pre-PGT-M, a minimum of 1x3 ml of peripheral blood (in EDTA tubes) and/or a buccal swab (less recommended) from the prospective parents and other relevant family members is needed. Based on the outcome of pre-PGT-M, the laboratory will inform



the IVF clinic by email whether PGT-M can be offered or not. The patients can then start their treatment towards PGT-M or seek alternative treatment which can be further discussed with a senior member of laboratory staff

For PGT-M, **1 embryo cell** is required for **day three biopsy**. **5-6 cells** are required for a **day five biopsy**.

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

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

#### **Professional user validation for PGT tests (‘DRY RUN’):**

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

#### **PGT-M sample transportation to the laboratory:**

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

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

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

#### **PGT-M test turnaround time (TAT):**

**The Physician that has requested** the test will receive the results.

Pre-PGT-M results will be available **within 20 business days** for common mutations and **45 business days** for the non-frequent mutations, from receipt of samples by Igenomix.

PGT-M results will be available **within 12 business days** from receipt of samples by Igenomix.

#### **PGT-M Reporting:**

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

- **Fully Informative (FI):** Each of the wild-type and mutant alleles in both members of the couple are unique.

- **Semi Informative (SI):** The wild-type and mutant alleles have unique polymorphic marker, but one of the values is equivalent between both members of the reproductive couple.
- **Non-Informative (NI):** The wild-type and mutant alleles have the same polymorphic marker in the individual carrying the mutation.
- **Not Applicable (NA):** The individual does not carry a mutation or is carrying a mutation/variant in homozygous state and so informativity is not applicable.

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

- **Normal:** Embryo found not to inherit the "at risk haplotype". This embryo is expected to be unaffected by the indicated genetic mutation.
- **Carrier:** Embryo found to inherit one parental "at risk haplotype". This embryo is expected to be a carrier for the tested genetic mutation, in the same way as the carrier parent(s).
- **Abnormal:** Embryo found to inherit the parental "at risk haplotype". This embryo is expected to be affected by the indicated disorder.
- **At risk:** This embryo has inherited the haplotype linked to the tested indication and is at risk of being affected.
- **Seek genetic counselling:** Genetic counselling is recommended to discuss the risks of transferring this embryo.
- **No DNA detected:** DNA was not detected, due to the absence of, or degraded DNA.
- **Non-informative:** A reliable result could not be achieved due to factors such as Allele Drop Out (ADO), parental/external contamination, recombination, and others.

### 3.2.5 Preimplantation Genetic Testing for structural rearrangements (PGT-SR)

#### PGT-SR test description:

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

#### Pre-requirements for accepting a PGT-SR case:

Before planning a PGT-SR cycle, the couple must provide the karyotype report of the structural anomaly to their prescribing physician for Igenomix staff review, who will request, if required, a pre-PGT-SR study. Pre-PGT-SR consists of a genetic study prior to the commencement of a PGT-SR cycle. This study is performed on a DNA sample of the carrier of a structural chromosomal abnormality, to confirm whether it is possible to address the case through PGT-SR and establish the diagnostic strategy to be applied in the PGT-SR cycle.

#### PGT-SR test sample requirements:

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

**For PGT-SR**, 4-8 cells from day 5, 6 or 7 of embryonic development (trophoctoderm biopsy) are required. The biopsied cell/s must be cleaned using the "washing/loading buffer" supplied by the laboratory to eliminate any potential source of contamination and, transferred to a small sterile 0.2ml tube supplied by the laboratory. The lid of these tubes must be labelled with the female patient

initials followed by the embryo number. The 0.2ml tubes must be placed in the “plate/rack” provided by Igenomix, the “plate/rack” placed inside the cooler shipping box with the “ice packs” also provided by the Igenomix.

Further information on how to prepare a sample is found in the “Tubing Instructions” **SAU\_L\_I\_PGT\_002\_EN: Instructions - PGT Tubing Loading Protocol** that can be requested by email to Customer Support at [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com). The “Embryo Biopsy Worksheet” and the “Test Requisition Form & Consent” (included within the provided kit and additionally can be requested by email to Customer support at [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com) ) must be completed and sent with the samples inside the shipping box or by e-mail to Igenomix.

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

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

#### **PGT-SR sample transportation to the laboratory:**

**For pre-PGT-SR**, blood samples should be sent to the laboratory by either first class mail or a similar secure service (DHL, SMSA etc.) and must be packed according to a set of IATA guidelines for “Packaging Instructions and clearly labelled “Exempt Human Specimen UN3373” when the sample is not delivered from Saudi (this courier service is not offered by the laboratory but outsourced to a third-party logistics company). Carriage is at Room Temperature. We recommend shipping the samples with a cold gel pack if outside temperatures exceed 35°C. Avoid freezing the sample when introducing the cold gel pack.

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

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

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

#### **PGT-SR test turnaround time:**

**The Physician that has requested** the test will receive the results.

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

**For PGT-SR** samples with **deferred transfer** results will be available **within 10 business days** from receipt of samples by Igenomix.

#### **PGT-SR reporting:**

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

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

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



- **Normal-euploid/balanced:** An embryo is considered as Normal/balanced when the graph shows no “Threshold” deviations from the reference bioinformatics baseline for any of the 24 chromosomes assessed. “Threshold” - Embryos with less than 30% full chromosome aneuploidy and less than 50% segmental and sex chromosome aneuploidy in the biopsy will be reported as normal/balanced. The test cannot detect structural abnormalities unless there is an imbalance in genetic material; therefore, PGT-SR cannot distinguish between an embryo that did not inherit a chromosomal rearrangement from an embryo that inherited the balanced chromosomal rearrangement.

**Abnormal-aneuploid/unbalanced:** An embryo is considered as “Abnormal/aneuploid” when an aneuploidy [gain or loss of a chromosome (1-22, X, Y)] or partial aneuploidy [gain or loss of a piece of a chromosome arm (p, q)] is detected as a result of a “Threshold” deviation from the reference bioinformatics baseline with points shifting upwards for a gain (Trisomy) and downwards for a loss (Monosomy). Partial aneuploidies are specified with chromosome number, arm (p, q), cytoband and fragment size in megabases (Mb). “Threshold” - Embryos with more than 70% full chromosome and/or segmental chromosome aneuploidy in the biopsy will be reported as aneuploid. An embryo is considered as “Unbalanced” when specific imbalances arising from the parental chromosomal rearrangement are detected as a result of a deviation from the reference bioinformatics baseline with points shifting upwards for a gain (trisomy) and downwards for a loss (monosomy).

- **No DNA detected:** Usually, if no DNA is detected, the cell did not have an intact nucleus. Other potential issues leading to No DNA Detected include poor embryo quality and degraded DNA. In some cases, cells may get lost during the washing procedure or may not be successfully transferred into a tube. Rebiopsy and retesting can be considered in these cases according to blastocyst quality.
- **Non informative:** When a reliable result cannot be achieved with high confidence, the embryo result will be reported as Non-informative. Re-biopsy and retesting can be considered in these cases according to blastocyst quality.

### 3.2.6 Embryo priority test (EMBRACE)

#### EMBRACE test description:

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

#### Pre-requirements for accepting an EMBRACE case:

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

#### EMBRACE sample requirements:

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

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

#### Professional user validation for EMBRACE tests (DRY RUN):

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

#### **EMBRACE sample transportation to the laboratory:**

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

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

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

#### **EMBRACE test turnaround time:**

**EMBRACE reporting:** The Physician that has requested the test will receive the results.

For EMBRACE samples all cases will be with **deferred transfer** and results will be available **within 10 business days** from receipt of samples by Igenomix.

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

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

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

### **3.2.7 NACE<sup>®</sup> & NACE<sup>®</sup>24**

#### **NACE<sup>®</sup> and NACE<sup>®</sup>24 test description:**

Unlike invasive prenatal diagnosis, which can pose a risk to an ongoing pregnancy, NACE<sup>®</sup> is a non-invasive prenatal genetic screening test. NACE<sup>®</sup> uses the latest sequencing technology (NGS) to analyze placental DNA compared to maternal DNA in order to detect certain fetal anomalies with high precision and reliability. Two in-house versions of the test exist: NACE<sup>®</sup> and NACE<sup>®</sup>24.

NACE<sup>®</sup> is designed to detect fetal Trisomy 21, 18, 13 and sex chromosome aneuploidies and NACE<sup>®</sup> 24 is designed to detect fetal chromosome aneuploidies in all 24 chromosomes.

#### **NACE<sup>®</sup> and NACE<sup>®</sup>24 Pre-requirements for accepting a case:**

Specific pre-requirements are needed in order to accept a case.

- This test is recommended for cases from week 10 of pregnancy onwards. Any case that does not fulfil this requirement will be rejected.

Other specific test indications and relevant clinical information can be reported in the test requisition form.

#### **NACE<sup>®</sup> and NACE<sup>®</sup>24 Sample requirements:**

Collect between 1x7ml (minimum) and 1x10 ml (maximum) of maternal peripheral blood in a Streck tube, using only the collection materials provided by the laboratory in the provided NACE kit.

Instructions on how to prepare a sample are available and can be downloaded from the Igenomix website or requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com). The "Test Requisition and Consent Form" (provided within the NACE kit and additionally can be requested by email [labsaudi@igenomix.com](mailto:labsaudi@igenomix.com)) must be completed and placed in the NACE kit.

#### **NACE<sup>®</sup> and NACE<sup>®</sup>24 sample transportation to the laboratory:**

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

We do not recommend storage of samples, after collection, for more than 5 days at room temperature or 7 days when refrigerated. Samples that exceeded these times when they reach Igenomix may be rejected.

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

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

#### **NACE<sup>®</sup> tests turnaround time:**

The Physician that has requested the test will receive the results within **10 business days** for NACE<sup>®</sup> and NACE24<sup>®</sup> of sample reception at Igenomix.

#### **NACE<sup>®</sup> test reporting:**

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

**+ No alteration detected:** The patient is considered to be at low risk for the studied condition(s).

**+ Alteration detected:** The patient is considered to be at high risk for the reported condition(s) with a very high Positive Predictive Value (PPV).

**+ Suspected alteration detected:** The patient is considered to be at high risk for the reported condition (s) with a low PPV.

**+ Non-informative:** It is not possible to offer information on the chromosomal state of the pregnancy from maternal blood due to inadequate quality and/or quantity of derived foetal DNA.

#### **+ Sex of the foetus (sexual chromosomes)**

- In single pregnancies, male or female sex is reported
- In the case of twin pregnancies, the presence or absence of Y chromosome is reported. This option is not available for NACE® 24

## **3.2.8 GENOMIC PRECISION DIAGNOSTIC:**

### **3.2.8.1 Single gene testing with NGS**

#### **Description**

Single gene testing with Igenomix includes the possibility to individually analyse single genes that are that are associated with a clinical phenotype using next generation sequencing. Our test menu represents all the clinically relevant genes that have been selected based on curated gene reviews, OMIM, variant databases (HGMD and ClinVar), most recent literature, and customer requests. Testing for single gene disorders can be done using several different technologies depending on the gene and disease in question

#### **Indication**

Single gene testing can be performed at all life stages and is indicated in patients with:

- Distinctive clinical features
- Family history of a specific disorder
- Family testing confirmation

#### **Applications**

Single gene sequencing by NGS can be applied:

- If the clinical indications are distinctive enough to diagnose a single gene disorder
- To sequence full coding regions of a gene in a single reaction
- For carrier testing of known familial genetic conditions
- To confirm biochemical / premarital tests with an indication of a single gene disorder

#### **Limitations**

The main limitation of single gene testing is the highly specific nature of the test. If the diagnosis of the patient is unknown or the patient has an unclear phenotype, we recommend visiting our precision panels or Whole Exome Sequencing page.

#### **Reporting and results**

There are three possible results that can be obtained from single gene sequencing by NGS

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that one or more variations have been identified in association with the disease phenotype under study. This scenario will allow to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members

**Negative:** A negative result indicates that no disease-causing genetic variant was identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology.

**Inconclusive/Variant of Uncertain Significance (VUS):** A finding of a variant of uncertain significance indicates that a change in a gene was detected, but it is currently unknown whether that change is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk to develop a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

### Sample requirements and Logistic

For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	22 Business days	EDTA tube	3mL	Room temperature
CVS	22 Business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	22 Business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	22 Business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	22 Business days	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a	Room temperature

			concentration of 50- 100 ng/microliters	
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\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples.

### **Single gene testing with NGS sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The 'informed consent' form and the 'test requisition form' (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

## **3.2.8.2 Precision Panels**

### **Description**

Our NGS precision panels are flexible, accurate and cost-effective alternatives to whole exome or whole genome sequencing. The precision panels presented test for a wide selection of hereditary genetic conditions. These panels allow us to utilize the Physician's diagnostic efforts to best benefit the patient with a genetic test that will analyze all the known genes associated with the phenotype in question. The gene composition of our panels is carefully selected by our specialists based on the latest publicly available information and our in-house expertise and research.

### **Indication**

The indication of choosing a specific panel is dependent on the clinical symptoms available, the patient's phenotype and the family history. Request can be made for the list of panels offered by Igenomix by email to [me-gc@igenomix.com](mailto:me-gc@igenomix.com) and [diagnostic-me@igenomix.com](mailto:diagnostic-me@igenomix.com). Prior discussion with the genetic counsellor is strongly recommended before choosing the panel.

### **Applications**

The use of precision panels is dependent on the clinical symptoms in question. Each panel is carefully curated to include the appropriate genes that relate to the condition in question. Our precision panels will be regularly updated based on new research, recommendations and feedback from our affiliated Physicians, researchers and genetic health care professionals.

### **Limitations**

The probes used for this test are designed to detect known genes in the curated panel. Therefore, this test is unable to detect genes not defined by the NCBI reference genome GRCh37 or non-human genome sequences including viral sequences or non-nuclear DNA that are designated in the specific panel.

In addition, due to the limitations of NGS technologies, the following variants cannot be readily detected: large deletions/duplications greater than 40 base pairs, copy number variations, homopolymer stretches, variants in pseudogene regions, gene fusions, balanced translocations, inversions, ploidy changes, uniparental disomy, and repeat expansion regions.

Furthermore, variants present outside the exons (non-coding region) could be missed; these variants can affect gene activity and protein production which may lead to genetic disorders. This technique does not cover the entire exome, (the % of bases with coverage above 20x is approximately 97%). It may not be possible to resolve certain details about variants such as mosaicism, phasing, or mapping ambiguity.

Analytical limitations may also occur due to the provided Physician information. Accurate and thorough clinical information of the patient(s) and family members is required as incomplete information may lead to false positive or negative results.

### **Reporting and results**

Igenomix uses an internally validated algorithm for precision panel analysis and interpretation. Genetic test results are classified and reported based on the recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015). According to the guidelines of the ACMG 2015, a genetic variant is classified as either Pathogenic, Likely pathogenic or Benign, Likely benign; any genetic variant which does not fulfill the criteria of pathogenic or benign is classified as a 'variant of uncertain significance'.

This test aims to identify the molecular cause of the genetic disease in question. A normal genetic result may significantly reduce, but cannot eliminate, the likelihood that the condition is genetic or that a genetic disorder will develop in the future. If any genetic condition is known in the family and molecular testing was already performed, then the specific gene/chromosome variation(s) present in the family should be disclosed at the time of testing.

There are three possible results that can be obtained from any precision panel:

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that one or more gene or chromosome variation has been identified in association with the disease phenotype. This scenario will allow healthcare professionals to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members.

**Negative:** A negative result indicates that no disease-causing genetic variant was identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology.

**Inconclusive/Variant of Uncertain Significance (VUS):** A finding of a variant of uncertain significance indicates that a change in a gene was detected, but it is currently unknown whether that change is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk of developing a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

### **Sample requirements and logistics for Precision Panel Sample**



For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	22 Business days	EDTA tube	3mL	Room temperature
CVS	22 Business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	22 Business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	22 Business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	22 Business days	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples.

Further information on how to send a sample can be found and downloaded from the website or requested by email to the Igenomix Customer Support service.

The 'informed consent' form and the 'test requisition from' (included within the provided kit) must be properly filled-in and signed by the patient and sent with the samples inside the shipping box or by e-mail to the laboratory. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

#### **Precision Panel sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.



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

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

The ‘informed consent’ form and the ‘test requisition form’ (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory

### 3.2.8.3 Whole exome sequencing

#### Description

The human genome is the complete set of genetic material of an individual. The exome is composed of all the protein coding exons within the genome and comprises about 2% of the human genome. Whole exome sequencing (WES) is a technique for sequencing all the protein-coding genes in a genome. The goal of this approach is to accurately identify genetic variants in the target regions, and to do this at a much lower cost than whole-genome sequencing (WGS). Although the exome is a small part of the genome about 85% of all known disease-causing variants are located in the exome. WES has proven to be an efficient method to determine the genetic basis of many Mendelian or single gene disorders and common polygenic diseases, as well as more complicated diseases such as cancer.

Sequencing is the process of determining the order of nucleotides in our DNA, the nucleotides are the building blocks of our DNA and are the set of four letters that make up the genetic code. Next-generation sequencing (NGS) is a sequencing technique that can allow rapid sequencing of large amounts of DNA at the same time.

WHOLE EXOME SEQUENCING analyses all genes associated with a phenotype based on the clinical and molecular evidence according to several reference databases (i.e.: OMIM database: <https://omim.org/>). A team of geneticists and specialized Physicians interpret the results by utilizing information from the latest publications and databases to produce a comprehensive clinical statement. Through our online portal, Physicians have full transparent access to their individual patients’ performance and quality data. Data return in various formats (BAM and VCF) is available for a fee upon request.

Due to the technological advances in genetic testing, WES can now be considered as a first-line genetic test in complex genetic cases. WES is increasingly used in healthcare and research to identify genetic variants that cause disease and to confirm diagnoses at a molecular level. Variants in the DNA that are not located in the exons but affect gene activity and protein production can be detected using whole genome sequencing (WGS), which pans the entire genome of an individual.

#### Indication

**WES Diagnostic:** Whole exome sequencing can be used as a diagnostic tool for patients with complex genetic disorders, where the correct diagnosis is difficult to establish due to overlapping symptoms, complicated medical histories or in cases where previous genetic testing has not yielded conclusive results.

WES Diagnostic Solo is a comprehensive genetic test that helps identify the disease-causing variant in an individual affected with a disease/condition. The protein-coding region of DNA (exons) are sequenced. Since most of the disease-causing variants are present in the exon, WES is an efficient technique to determine disease-causing variants that may lead to a disease.

WES Diagnostic Couple is also performed on the parents of an affected individual or a couple with a family history of a known condition to identify and report any variants relating to the phenotype in question. In addition, a screening test is offered to help identify common recessive variants between couples to attempt and prevent any further genetic disorders. This WES is recommended for affected individuals who have a suspected genetic diagnosis, to help diagnose affected individuals who have multiple differential diagnoses, if targeted or panel testing was negative and for couples who have a family history of a genetic disorder.

WES Diagnostic Trio is a comprehensive genetic test that is offered to the affected individual and their parents in order to identify and report any variants relating to the affected individuals disease/condition, additionally this test can help determine whether the disease-causing variant is inherited. . In addition, a screening test is offered to the couple to help identify common recessive variants between couples to attempt and prevent any further genetic disorders. This WES is recommended for affected individuals who have a suspected genetic diagnosis, to help diagnose affected individuals who have multiple differential diagnoses, if targeted or panel testing was negative and for couples who have a family history of a genetic disorder.

**WES Planning a healthy Family:** WES Screening is also an important genetic test that is recommended before planning a family. This test helps determine whether a couple is at risk of having a child with a genetic disorder. If the couple has one or more variants in common, preventative measures can be taken in order to have a healthy child. Carriers of genetic variants are usually healthy individuals. However, when both parents carry a variant in the same gene, they are at risk of having an affected child. This whole exome test is recommended for consanguineous couples and any couples who would like to rule out the risk of having an affected child.

### Applications

WES is used in diagnosing or evaluating a genetic disorder where the results are expected to influence medical management and clinical outcomes of a patient or a family directly or indirectly. With the advent of technology, sequencing has become a routine process in clinical diagnosis. In situations where the clinical presentation is unclear and the condition in question is unknown, sequencing and analysing a small number of genes at a time is costly and time-consuming process. This may further delay the diagnosis, which could have an impact on patient's quality of life.

WES is a cost-effective diagnostic solution which permits sequencing data from ~24,000 genes from a simple blood draw. WES examines a wider range of genes and variants, which is especially worthwhile for couples looking to know if they are carriers of common recessive disorders and to diagnose genetic disease.

### Limitations

The probes used for this test are designed to detect known genes in the human genome. Therefore, this test is unable to detect genes not defined by the NCBI reference genome GRCh37 or non-human genome sequences including viral sequences or non-nuclear DNA.

In addition, due to the limitations of NGS technologies, the following variants cannot be readily detected: large deletions/duplications greater than 40 base pairs, copy number variations, homopolymer stretches, variants in pseudogene regions, gene fusions, balanced translocations, inversions, ploidy changes, uniparental disomy, and repeat expansion regions.

Furthermore, variants present outside the exon (non-coding region) could be missed; these variants can affect gene activity and protein production that may lead to genetic disorders. This technique does not cover the entire exome, (the % of bases with coverage above 20x is approximately 97%). It may not be possible to resolve certain details about variants such as mosaicism, phasing, or mapping ambiguity.

Analytical limitations may also occur due to the provided Physician information. Accurate and thorough clinical information of the patient(s) and family members is required as incomplete information may lead to false positive or negative results.

### Reporting and results

Igenomix uses an internally validated algorithm for precision panel analysis and interpretation. Genetic test results are classified and reported based on the recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015). According to the guidelines of the ACMG 2015, a genetic variant is classified as either Pathogenic, Likely pathogenic or Benign, Likely benign; any genetic variant which does not fulfill the criteria of pathogenic or benign is classified as a 'variant of uncertain significance'.

This test aims to identify the molecular cause of the genetic disease in question. A normal genetic result may significantly reduce, but cannot eliminate, the likelihood that the condition is genetic or that a genetic disorder will develop in the future. If any genetic condition is known in the family and molecular testing was already performed, then the specific gene/chromosome variation(s) present in the family should be disclosed at the time of testing.

There are four possible results that can be obtained from any precision panel:

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that one or more gene or chromosome variation has been identified in association with the disease phenotype. This scenario will allow healthcare professionals to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members.

**Negative:** A negative result indicates that no disease-causing genetic variant was identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology.

**Inconclusive/Variant of Uncertain Significance (VUS):** A finding of a variant of uncertain significance indicates that a change in a gene was detected, but it is currently unknown whether that change is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk of developing a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

**Unexpected/Incidental/secondary results:** In rare instances, this test may reveal an important genetic change that is not directly related to the reason for ordering this test. For example, this test may provide information about an individual's risk for other genetic conditions. This information is likely to impact the individual's treatment options and is disclosed based on the informed consent provided by the patient.

Additionally, in accordance to the ACMG guidelines for reporting secondary findings in clinical exome sequencing (PMID: 27854360), pathogenic and likely pathogenic variants in the following genes are reported if consent is indicated on the Test Request Form: ACTA2, ACTC1, APC, APOB, ATP7B, BMPR1A, BRCA1, BRCA2, CACNA1S, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RET, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFB1, TGFB2, TMEM43, TNNI3, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, and WT1. It is encouraged to further ascertain the genotype-phenotype correlation and research to establish the efficacy of intervention in asymptomatic patients with a reported variant in any of the associated genes. This information is only applicable for the whole exome sequencing test and consent must be provided by the patient to obtain this information.

Result interpretation is based on currently available information in the medical literature, research, and scientific databases. Because the literature, medical and scientific knowledge are constantly changing, new information that becomes available in the

future may replace or add to the information that Igenomix used to interpret the results. Re-analysis of variants in previously issued reports considering new evidence is not routinely performed but is available upon request.

### Sample requirements and logistics

For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	22 business days	EDTA tube	3mL	Room temperature
CVS	22 business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	22 business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	22 business days	Tissue in sterile container in saline  Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	22 business days	In a sealed Eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples

### **WES sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The ‘informed consent’ form and the ‘test requisition form’ (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

### **3.2.8.4 Maternal Cell Contamination**

#### **Description:**

Prenatal testing on samples obtained from invasive procedures like chorionic villus sampling and amniocentesis, is associated with high risk of contamination with maternal cells/tissue, which can occur when a fetal specimen encounters maternal blood or tissue. The terminated prenatal product of conception can also be contaminated with the maternal cells/tissues while extracting.

If maternal cell contamination (MCC) is present, the maternal DNA may mask the results of any genetic testing performed on the fetal DNA. Therefore, the results of prenatal testing may be compromised. For the accurate prenatal diagnosis of inherited molecular, cytogenetic, or metabolic disorders, MCC analysis should be performed to rule out the presence of contaminating maternal or co-fetal material in case of multiple gestation pregnancies. To rule out the presence of MCC, a maternal blood specimen is necessary for comparison of maternal and fetal chromosomal markers. The presence of both maternal and non-maternal alleles for each fetal marker indicates the fetal specimen is not contaminated. MCC is confirmed when both alleles in the fetus are maternal.

#### **Utility of MCC Test:**

MCC test is mandatory for any prenatal molecular tests (whole exome sequencing, target gene sequencing, target mutation analysis, chromosomal analysis, etc.). Using the mother blood sample, the prenatal sample is compared for maternal and fetal chromosomal markers to make sure the sample in question is not contaminated with the mother cell/tissues. By this means, we can assure the results obtained in the required test are from the prenatal sample provided and are not influenced by the maternal cell/tissue contamination.

#### **MCC sample requirements:**

For the MCC test along with the prenatal test sample type mother’s peripheral blood sample is required.

Sample type	Container	Volume	Transportation temperature
Peripheral blood	EDTA vacutainer	3 - 4 ml	20-25°C
Product of Conception	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	3-4 mm POC specimen or 50-100 mg of each tissue	20-25°C
Amniotic Fluid	Sterile container	10-15ml	20-25°C
Chorionic villi Sample	Sterile container with culture medium or saline solution with 1% antibiotic	300-500mg	2-8°C

Further information on how to prepare a sample can be found and downloaded from the website or requested by email to the Igenomix Customer Support service (see section 1.3).

The 'informed consent' form and the 'test requisition form' (included within the provided kit) must be properly filled-in and signed by the patient and sent with the samples inside the shipping box or by e-mail to the laboratory.

#### **MCC sample transportation to the laboratory:**

The MCC test is associated with any other mainstream molecular tests like, whole exome sequencing, target gene sequencing, target mutation analysis, chromosomal analysis, etc. The sample collection will happen as per the requirements of the mainstream test.

Igenomix will send you all the docs needed for the pick-up and transportation of the required kits to our laboratory. Igenomix will pick up the samples with Test Requisition form filled in and signed, Test informed Consent form filled in and signed, EDTA tube of 4 ml with the blood collected and labelled, and included into the Rigid plastic blister (secondary container).

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

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

#### **MCC Test (TAT):**

The Physician that has requested the test will receive the results in a TAT of **7 Business Days**.

#### **MCC Test reporting:**

The sample and positive control DNA are subjected to multiplex STR Typing by AmpFISTR® Identifier in which different alleles present in 16 STR loci from chromosomes 2, 3, 4, 5, 8, 7, 11, 12, 13, 16, 18, 19, 21, X and Y are amplified in a single reaction, followed by capillary electrophoresis on SeqStudio Genetic Analyzer. After confirming the presence of expected STR allele peaks in the positive control DNA. The fetal STR profiles are compared to maternal profiles for the presence of maternal second allele in the fetal sample. The final results are printed in the form of 'positive' or 'negative' in the report.

The prenatal samples which are negative for MCC will be taken further for mainstream test validation and the results of the mainstream test will be provided along with the MCC test results. If the sample is positive for MCC, it may not be continued processing for the mainstream test as the test results will be compromised with the contamination leading to false positive or false negative results.

#### **Limitations of MCC Test:**

- Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

- Absence or presence of MCC does not rule in/out presence or absence of contamination by any another specimen or sub specimen obtained from fetus under consideration.
- This assay detects up to 1% MCC in the fetal sample.

### 3.2.8.5 Target mutation Analysis

#### Test Description

Target mutation analysis with Igenomix includes the possibility to individually analyses single nucleotide polymorphisms using Mini sequencing or fragment analysis technique that are associated with a clinical phenotype. The test can be offered to any individual or in pre-natal sample where mutation is defined in one of the family members. Before offering this test Igenomix ask for genetic report from the client and after reviewing the report test can be offered to the family members or the current fetus.

For performing the test, DNA is extracted from the sample (pre/post-natal sample) and the extracted DNA is used to detect single nucleotide polymorphisms. The protocol first involves amplifying a genomic DNA fragment that contains these nucleotide positions by means of specific primers. Single nucleotide variants (SNVs) are analyzed by the minisequencing or primer extension technique (3500XL, Applied Biosystem) which involves the use of a single base extension primer whose 3' end is located next to the base preceding the SNP. Fluorescent DNA fragments corresponding to small insertions, deletions and duplications are separated by fluorescent capillary electrophoresis (3500XL, Applied Biosystem) by size difference at a resolution of one nucleotide difference. . The detection limit is determined by GeneMapper software, which optimum range of peak height is 1,000-31,000, the minimal is 100 and the maximal is  $\geq 40,000$ .

Sensitivity: the estimated sensitivity per allele is 100 %.

Specificity: the estimated specificity per allele is 100 %.

Overall accuracy: the estimated overall accuracy per allele is 100 %.

#### Indication

Target mutation testing can be performed at all life stages and is indicated in patients with:

- Family history of a specific disorder
- Family testing confirmation
- Variant confirmation in current fetus

#### Test turnaround time (TAT):

**The Physician that has requested** the test will receive the results within **17 business days** once sample arrives in our laboratory

#### Reporting:

**For** Target mutation testing the following results can be obtained:

- **Homozygous:** The presence of two identical disease-causing alleles at a particular gene locus.
- **Heterozygous:** The presence of two different allele inherited from both the parents, one that is disease causing and another Normal allele at a particular gene locus.
- **Hemizygous:** This term is often used to describe X-linked genes in males who have only one X chromosome.
- **Absent:** The presence of two identical Normal alleles at a particular gene locus.

#### Limitations:

This assay is targeted to the specific genetic variant investigated and cannot detect other variants that could be present in this gene



or other of the patient.

Therefore, absence of a detectable variant does not rule out the possibility that a patient has an altered variant that cannot be detected with this method. Furthermore, when 2 or more variants are identified, the cis-/trans- status (whether the variants are on the same or opposite chromosomes) is not always known, and assumptions about phase and content are made to assign alleles.

This method can't rule out totally that the presence or absence of the variant to detect was or not located in a pseudogene which can interfere with the appropriate interpretation. In addition, the presence of polymorphisms in the hybridisation site of the PCR primers cannot be ruled out and may interfere with the interpretation of the result.

### Sample requirements and Logistic

For target mutation testing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	17 business days	EDTA tube	3mL	Room temperature
CVS	17 business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	17 business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	17 business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	17 business days	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature



\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples.

### 3.2.8.6 QF PCR

#### **TEST DESCRIPTION:**

The method employed by Elucigene QST<sup>®</sup>R plus v2 kits uses the QF-PCR (Quantitative Fluorescence polymerase chain reaction) technique. Using PCR amplification, fluorescent dye labelled primers target highly polymorphic regions of DNA sequence called short tandem repeats (STRs) that are located on chromosomes of interest. Each targeted STR marker is specific to the chromosomes on which it is located, thus the copy number of STR marker can be diagnostic of the copy number of the chromosome. Informative STR markers have been selected that exhibit a high heterogeneity so that copy number can be easily determined. A normal diploid sample has the normal complement of two of each of somatic chromosomes, thus two alleles of a chromosome specific STR are determined by the QF-PCR technique as two peaks in a 1:1 ratio. The observation of an extra STR allele as either a three peak pattern in a 1:1:1 ratio or two peak pattern in a 2:1 or 1:2 peak ratio in diagnostic of the presence of an additional sequence which in turn may represent an additional chromosome, as in the case of trisomy.

Amplified products of QF-PCR technique are analysed quantitatively on a capillary electrophoresis Genetic Analyzer to determine the copy number of the analysed STR markers.

The test determine diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). The analysis includes some markers for the determination of sex status as well.

#### **TEST METHODOLOGY:**

DNA extraction from a prenatal sample. QF-PCR amplification with Elucigene QST<sup>®</sup>R plus v2 kit. Fragment analysis of PCR products by capillary electrophoresis.

#### **SAMPLE REQUIREMENT:**

Sample type	Container	Volume	Transportation temperature
Amniotic Fluid	Sterile container	10-15ml	20-25°C
Chorionic villi Sample	Sterile container with culture medium or saline solution with 1% antibiotic	300-500mg	2-8°C

Further information on how to prepare a sample can be found and downloaded from the website or requested by email to the Igenomix Customer Support service (see section 1.3).

The 'informed consent' form and the 'test requisition form' (included within the provided kit) must be properly filled-in and signed by the patient and sent with the samples inside the shipping box or by e-mail to the laboratory.

#### **QF PCR sample transportation to the laboratory:**

Igenomix will send you all the docs needed for the pick-up and transportation of the required kits to our laboratory Igenomix will pick up the samples with Test Requisition form filled in and signed, Test informed Consent form filled in and signed, EDTA tube of 4 ml with the blood collected and labelled, and included into the Rigid plastic blister (secondary container)

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

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

**QF-PCR Test (TAT):** The Physician that has requested the test will receive the results in a TAT of **10 Business Days**.

### **Test reporting:**

**Normal:** To interpret a result as normal, at least two informative markers consistent with a di-allelic genotype are required with all other markers being uninformative. A normal result indicates the normal complement of two for the chromosomes tested.

**Abnormal:** To interpret a result as abnormal (i.e. trisomy present), at least two informative markers consistent with a tri-allelic genotype are required with all other markers being uninformative. It is not recommended to interpret a result as abnormal based on information from only one marker.

**Non-Informative:** when the quality of the sample is suboptimal and leads to result below the required quality thresholds

### **TEST LIMITATIONS:**

A normal result does not eliminate the possibility that the pregnancy is associated with other chromosomal or sub-chromosomal abnormalities (structural rearrangements, polyploidies, or abnormalities in any other chromosomes), birth defects, genetic conditions, or other conditions. The result can only be directly applied to the tissue tested and may not represent the fetal karyotype.

This prenatal test is validated for aneuploidy of any chromosome, including 21, 13, 18, X, and Y. A negative test result does not eliminate the possibility of chromosomal abnormalities for the tested chromosomes due to I) partial abnormalities II) Mosaicism III) maternal cell contamination.

## **3.2.8.7 MLPA**

### **Description:**

Multiplex Ligation dependent Probe Amplification (MLPA) is a multiplex PCR method used to detect abnormal copy numbers of up to 50 different genomic DNA or RNA sequences. Furthermore, MS-MLPA can detect DNA methylation changes. Is the most reliable and cost-effective method of detecting known deletion, duplications, and specific copy number variations (CNVs). Like array CGH, MLPA detects copy number changes and the interpretation of the results can be complicated by naturally occurring copy number variations. The probes or probe kits used have been selected and validated to reduce the likelihood of false positive or negative results.

### **Indication**

MLPA can be indicated across all life stages and is dependent on the condition in question and the clinical symptoms present. MLPA can be requested if:

- a) The patient is suspected to have a genetic disorder that could be caused by a deletion or duplication
- b) Previous genetic tests were negative or only identified a single variant in a gene or condition that is associated with autosomal recessive inheritance

- c) MLPA can be used to diagnose specific disorders associated to:
- d) deletions or duplications of specific regions, genes or exons
- e) imprinting alterations
- f) uniparental disomy (UPD)
- g) small rearrangements (small intragenic deletions)

#### **Application:**

MLPA can detect:

- a) small deletions and rearrangements associated to specific regions, genes or exons
- b) Specific microdeletion syndromes
- c) Diseases caused by methylation defects (MS-MLPA)
- d) Specific Uniparental disomy
- e) The most common genetic disorders detected by MLPA include:
- f) TSC1 deletion/duplication
- g) TSC2 deletion/duplication
- h) Congenital adrenal hyperplasia CYP21A2 (21-OH) deletion/duplication analysis
- i) ATP7B deletion/duplication
- j) HBB deletion/duplication
- k) HBA1 & HBA2 deletion/duplication
- l) IKBKG deletion/duplication analysis
- m) Ornithine transcarbamylase deficiency (OTC) deletion/duplication analysis
- n) Ataxia-telangiectasia (ATM) deletion/duplication
- o) Neurofibromatosis type 1 (NF1) deletion/duplication
- p) Neurofibromatosis type 2 (NF2) deletion/duplication
- q) Neurodegeneration with brain iron accumulation 2B (PLA2G6) deletion/duplication analysis
- r) Pantothenate kinase-associated degeneration (PANK2) deletion/duplication analysis
- s) Duchenne Muscular Dystrophy (DMD) deletion/duplication
- t) PMP22 deletion/duplication analysis
- u) Spinal Muscular Atrophy (SMN1/SMN2) deletion/duplication
- v) DiGeorge syndrome deletion/duplication analysis
- w) Cystic fibrosis (CFTR) gene deletion/duplication
- x) Prader-Willi/Angelman syndrome deletion/duplication
- y) Ad-hoc deletion/duplication analysis

#### **Limitations:**

MLPA cannot detect:

- a) Balanced chromosomal rearrangements
- b) Telomeric deletions and duplications
- c) Deletions and duplications that are not identified by the MLPA probes used
- d) Point mutations, small insertions and deletions
- e) Sequence repeats or disorders caused by mutations in mitochondrial DNA

Multiplex Ligation-dependent Probe Amplification. MLPA<sup>®</sup> probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%. MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected. The MLPA test will not detect the point mutations in the DMD genes. A point mutation or polymorphism in the sequence detected by a probe, which results in reduced probe binding efficiency, can also cause a reduction in relative peak area. Therefore, single exon deletions detected by MLPA should always be confirmed by other methods like multiplex PCR or sequencing.

#### **Reporting and results:**

There are two possible results that can be obtained from an MLPA test

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that a gene deletion or duplication has been identified in association with the disease phenotype under study. This scenario will allow to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members

**Negative:** A negative result indicates that no disease-causing deletions or duplications were identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology

#### **Sample requirements and logistics**

For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	22 Business days	EDTA tube	3mL	Room temperature
CVS	22 Business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	22 Business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	22 Business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not	Room temperature

Sample type	TAT	Container	Volume	Temperature
			available placental villi can be utilized	
Extracted DNA	22 Business days	In a sealed Eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples

#### **MLPA sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The 'informed consent' form and the 'test requisition from' (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

### **3.2.8.8 Expansion repeat Analysis:**

#### **Description:**

Repeat expansions are common genetic variations that are usually associated to neurogenetic disorders. These expansions are a different class of genetic disease that occur due to dynamic mutations that can change from generation to generation. Conditions that are caused by repeat expansions are characterized by unstable expansions of gene segments that consist of repeating unites of three or more nucleotides. PCR-based screening of repeat lengths is the gold standard assessment of diseases associated with expansion repeats. The Repeat Expansion Detection test is usually done by PCR or Southern Blot.

#### **Indication:**

Expansion repeats testing is indicated when individuals present with diagnostic or clinical symptoms relating to diseases caused by expansion repeats. This test can be offered in all life stages depending on clinical presentation.

The most common genetic disorders detected by Expansion repeat testing include:

- CAG/polyQ diseases
- Spinocerebellar ataxia types SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA31, SCA10

- C9ORF72 frontotemporal dementia/ amyotrophic lateral sclerosis
- Huntington disease
- Myotonic dystrophy types 1 and 2
- Oculopharyngeal muscular dystrophy
- Fragile X tremor ataxia syndrome
- Friedreich Ataxia
- Fragile X syndrome
- Myoclonic epilepsy

#### **Application:**

Depending on the type of suspected disorder there are different types of PCR tests that can be offered. When disorder is caused by a small number of repeats (< 50 repeats), amplification primers encompass the repeat. When a disorder is caused by a large number of repeats (>100 repeats), that conventional PCR cannot amplify. Amplification primers encompass the repeat and include the repeated sequence.

#### **Limitations:**

The scope of this assay is limited to the repeat expansion analysis of the targeted gene and may not reveal the exact number of repeats present in large expansions.

Gene sequencing and deletion/duplication analysis are not included in this assay but can be ordered separately.

This analysis does not include methylation studies

#### **Reporting and results:**

**Negative Result:** A negative results indicates that the individual has the normal alleles repeats in the target gene and is not at an increased risk of developing the disorder.

**Premutation Alleles:** A pre-mutation is a change that is of intermediate risk or in a “grey area”. Individuals with pre-mutations have a higher than normal number of repeat expansion but less than the number expected of the full mutation. The risk of an individual with a pre-mutation is variable and these pre-mutations are identified in such a manner because there is a small chance that they are unstable and may expand to a full mutation in future generations. There is no reported risk for an individual with an intermediate sized allele to have a child with a full mutation. Traditionally, a carrier of a genetic mutation is defined as a person who inherits an altered form of a gene but shows no effects of that mutation. However, this does not necessarily apply in cases with expansion repeats as depending on the condition, individuals with a pre-mutation might present with symptoms.

**Full mutation/ Disease-Causing Alleles:** A result of a full mutation indicates that the individual has the expansion repeats that would cause genetic disease and this individual is likely to have symptoms of the disorder. Reduce penetrance plays a role in some expansion repeat diseases and so individuals with the full mutation may have variable symptoms depending on the condition in question.

#### **Sample requirements and Logistic**

For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is – Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	Regular 22 Business days Custom 45 Business days	EDTA tube	3mL	Room temperature
CVS	Regular 22 Business days Custom 45 Business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	Regular 22 Business days Custom 45 Business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	Regular 22 Business days Custom 45 Business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	Regular 22 Business days Custom 45 Business days	In a sealed Eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples

### **Expansion Repeat sample transportation to the laboratory:**



The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The ‘informed consent’ form and the ‘test requisition form’ (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

### 3.2.9 Chromosomal Microarray (CMA)

#### Description:

Chromosomal microarray analysis (CMA), also known as array CGH is a diagnostic test that can detect clinically significant large (whole chromosome) and sub microscopic (microdeletion/microduplication) copy number changes throughout the genome. Chromosomal microarray analysis is the gold standard for the detection of large deletions and duplications along the whole genome. The higher the resolution and the K value of the test, the higher the sensitivity. This test allows the detection of microdeletions and microduplications of chromosome segments, which are too small to see under a microscope but may contain multiple genes.

#### Indication:

The Chromosomal Microarray analysis test can be indicated across all life stages:

##### **Pre-conception:**

CMA can be offered in the pre-conception phase if one or more of the below indications are present:

- If an affected child presents with a normal karyotype, but a genetic condition is suspected
- If a child presents with an undiagnosed condition and the Whole Exome sequencing is negative
- If an individual presents with undiagnosed intellectual or developmental delay that does not fit a specific syndrome (including Fragile X)
- To check whether a copy number variation that was detected in the affected child is de novo or inherited from their parents

**Pre-natal:** CMA is offered prenatally if one or more of the below indications are present:

- Abnormal fetal ultrasound
- Abnormal NIPT results indicated an increased risk of a chromosomally abnormal fetus
- Abnormal high-risk maternal serum screen
- The parents have a known chromosomal rearrangement, mosaicism or previous aneuploidies
- The parents have had previous livebirths or stillbirths with chromosomal abnormalities
- Fetal congenital abnormalities detected with ultrasound or MRI that indicate a significant risk of an unbalanced chromosomal abnormality

- High risk pregnancies

### **Post-natal:**

CMA is offered in childhood or adulthood if one or more of the below indications are present – the chromosomal microarray can be considered as a first line test in the following situations:

- If an individual or fetus presents with multiple congenital anomalies that are not specific to a well identified genetic syndrome
- If a karyotype is negative and the individual's phenotype is indicative of chromosomal aneuploidy.
- If an individual or fetus present with apparently nonsyndromic developmental delay or intellectual disabilities
- If an individual presents with autism spectrum disorders
- If a fetus is malformed or a still birth of unknown etiology occurs
- The CMA can be used in cases where other tests have failed to yield a diagnosis specifically if one or more of the below symptoms or conditions are present
- Unexplained seizure disorders
- Growth delay
- Psychiatric illness
- Neuromuscular conditions
- Skeletal dysplasia
- Short stature
- Excessive growth
- Microcephaly
- Macrocephaly

**Product of conception:** CMA can be offered on a product of conception if there was a case of spontaneous abortions.

### **Applications**

CMA can detect:

1. Small chromosomal microdeletions and duplications
2. Copy number variations
3. Numerical chromosomal aneuploidy
4. Unbalanced rearrangements
5. Excessive homozygosity – platform dependent
6. Suggestive risk of inheriting recessive disease or imprinting disorders – platform dependent
7. Triploid and tetraploid – platform dependent
8. Mosaicism greater than 20-30%

### **Limitations:**

CMA cannot detect:

1. Balanced chromosomal rearrangements (balanced translocations, inversions)
2. Small changes in the sequence of single genes (point mutations)
3. Tiny duplications and deletions of DNA segments within a single gene (Fragile X syndrome, for example)
4. Uniparental disomy (UPD)
5. Methylation alterations
6. Mosaicism less than 30%
7. Complete ploidy

### **Reporting and results**

Two different types of CMA are available in Igenomix – CystoScan HD and CytoScan 750K. These two tests are similar but have different indications, with CMA HD being the more sensitive test and CMA750K being the most cost effective.

CytoScan HD array	CytoScan 750K
Copy number probes (1.9 million) + SNP (750 K)	Copy number probes only (550K) + SNP (200 K)
Whole genome coverage	Emphasis on clinically relevant regions
Can detect regions of low heterozygosity, uniparental disomy (UPD), low level mosaicism and sample heterogeneity	Identification of regions of excessive homozygosity indicating UPD, may suggest consanguinity and determine candidate genes for further testing
Highest probe density	

Deletions smaller than 50 kb and duplications smaller than 400 kb may not be reviewed. Detected copy number variations (CNVs) are reported when found to have clear or suspected clinical relevance; CNVs devoid of relevant gene content or reported as common findings in the general population may not be reported. Regions of homozygosity are reported when a single long contiguous stretches of homozygosity (LCSH) is greater than 8-15 Mb (dependent upon chromosomal location and likelihood of imprinting disorder), or when the total autosomal LCSH proportion is greater than 3% (only autosomal LCSH greater than 3 Mb are considered for this estimate). Genomic linear positions are given relative to NCBI build 37 (hg19).

Test results are interpreted based on the recommendations and guidelines of International Standard of Cytogenomics Arrays (ISCA) as described below:

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that a copy number variant has been identified in association with the disease phenotype under study. This scenario will allow to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members

**Negative:** A negative result indicates that no disease-causing copy number variation was identified in the test performed. This does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology

**Inconclusive/Variant of Uncertain Significance (VUS):** A finding of a variant of uncertain significance indicates that a copy number variation was detected, but it is currently unknown whether that CNV is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk to develop a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

Result interpretation is based on currently available information in the medical literature, research, and scientific databases. Because the literature, medical and scientific knowledge are constantly changing, new information that becomes available in the future may replace or add to the information that Igenomix used to interpret the results. Re-analysis of the results in previously issued reports considering new evidence is not routinely performed but is available upon request

### Sample requirements and logistics:

The following sample types are accepted for Igenomix genetic tests. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	17 business days	EDTA tube	3mL	Room temperature
CVS	17 business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	17 business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	17 business days	Tissue in sterile container in saline  Cardiac or cord blood in Vacutainer	1 cm <sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	17 business days	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

\*Precedence will be given to all prenatal samples

### **CMA sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Conical tube for (CVS, Amnio), Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The ‘informed consent’ form and the ‘test requisition form’ (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

## **3.2.10 Testing for Products of Conception (POC)**

### **POC test description:**

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

### **Pre-requirements for accepting a POC case:**

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

### **POC test sample requirements:**

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

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

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

### **POC sample transportation to the laboratory:**

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

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

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

#### **POC tests turnaround time:**

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

#### **POC test reporting:**

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

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

### **3.2.11 Non-invasive Products of Conception Test (niPOC)**

#### **niPOC test description:**

Unlike tests classically used for the study of aneuploidy in foetal remains, in which it is necessary to have them available through medical or surgical intervention (curettage), niPOC is a non-invasive test. niPOC uses the latest massive sequencing technology (NGS) to analyse placental DNA in comparison with maternal DNA in order to detect certain foetal abnormalities with high accuracy and reliability.

#### **niPOC prerequisites:**

Specific prerequisites are needed to accept a case.

- This test is recommended for cases as early as 5 weeks of pregnancy. Any case that does not meet this requirement may be rejected.
- The blood sample should ALWAYS be taken prior to uterine evacuation, and drug treatment (if applicable).
- This test is recommended for cases in which the parents either do not know their karyotype, or the karyotype is normal. For those cases in which it is known and communicated to our laboratory that the karyotype of one of the parents is abnormal, the case must be evaluated individually before being accepted.

Other specific test indications and relevant clinical information can be communicated on the test request form.

#### **niPOC samples requirements:**

Collect between 1x7 ml (minimum) and 1x10 ml (maximum) of maternal peripheral blood in a Streck tube, using only the collection materials provided by the Igenomix in the niPOC kit supplied.

Instructions on how to prepare a sample are available and can be downloaded from the Igenomix website, or requested by e-mail. The "Test Requisition & Consent Form" (included in the supplied kit or requested by email) must be completed and inserted into the niPOC kit.

#### **Transport of the niPOC sample to the laboratory:**

The clinic must inform Igenomix when a sample is ready and Igenomix will offer to arrange the sample collection. Transport will be in customized kits provided by the laboratory. Transport will be at room temperature. We recommend sending the samples with cold packs if the outside temperature exceeds 35 °C or if they are sent outside Spain (international shipments). Avoid freezing the sample when inserting the cold packs.

We do not recommend the storage, after extraction, of samples for more than 5 days at room temperature or 10 days when refrigerated. Samples that have exceeded these time periods may be rejected if they reach Igenomix.

The sample must be sent to the laboratory by priority mail or similar courier service (DHL, SMSA, etc.) and must be packed according to ADR guidelines known as P650, or "P650 Packing Instructions" and clearly labelled as "Exempt Human Specimen UN3373" when the sample is not delivered from Spain (this courier service is not performed directly by Igenomix but is subcontracted to an external logistics company).

For more information on how to send samples, please review the test instructions included in the Igenomix website or contact Igenomix Customer Service (see section 1.3).

### **Turnaround time for niPOC test results:**

The ordering physician will receive the niPOC results within **10 Business days** from the sample receipt day by Igenomix.

Results will also be sent to patients if the email address was provided on the Test Order Form.

### **niPOC test results:**

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

+ Normal: when no aneuploidy or partial deletion/duplication has been detected.

+ Abnormal: when aneuploidy or partial deletion/duplication  $\geq 7$  Mb in size has been detected. Information on the detected abnormality is provided.

+ Non-informative: It is not possible to provide information on the chromosomal status of the pregnancy from maternal blood due to inadequate quality or quantity of the derived foetal DNA.

## **3.3 OTHER REFERRED TEST**

### **3.3.1. Karyotyping**

#### **Description**

To perform the karyotype, the sample is cultured, dividing cells are harvested, cells arrested in metaphase stage (the time of greatest chromosome condensation) by disruption of the spindle using drugs such as colchicine. Chromosomes are visualized by staining, including the use of special stains, GTL (Giemsa-Trypsin-Leisman) to elicit banding patterns, where the G-bands are acquired through treatment with Trypsin followed by staining with Leishman Giemsa (500 bands resolution). Karyotyping is able to detect polyploidy, aneuploidy, translocations, inversions, rings, and copy number changes in the size range of 4–6 Mb; smaller copy number changes require the use of molecular cytogenetic techniques. Karyotyping of CVS or amniocentesis specimens typically takes 7–14 days since cells need to be cultured prior to analysis. Only dividing cells may be used for karyotyping since chromosomes are not visible in nondividing cells. For patients who require faster results, or for testing of deletions of small areas of the chromosome below the level of resolution of a karyotype, fluorescent in situ hybridization, (FISH) may be offered. There are FISH probes for chromosomes 13, 18, 21, X, and Y which may be used for rapid detection of aneuploidy prior to formal karyotyping.



## Indication

### **Post-natal :**

- a. Ambiguous genitalia /Pseudohermaphroditism and Gonadal dysgenesis
- b. Psycho motor retardation ( fragile X, Klinefelter)
- c. Dysmorphism with visceral anomalies undetected during infancy
- d. Unexplained short stature (Turner)

**Product of conception / Stillbirth :**First or second trimester pregnancy loss/miscarriage

### **Pre-conception**

- a. Individuals with reproductive problems (Infertility)
- b. Parents of a child with a chromosomal disorder
- c. Sterility workup once gynecological or endocrine causes have been ruled out (Klinefelter, Turner syndrome)
- d. If more than two spontaneous miscarriages cytogenetic studies are indicated on the parents to rule out a translocation carrier

### **Pre-natal**

- a. Abnormal ultrasound high risk pregnancies
- b. Amniotic fluid cells obtained by amniocentesis, chorionic villi, lymphocytes obtained by cordocentesis in
- c. Advanced maternal age
- d. Previous pregnancy with an abnormal chromosomal complement
- e. Previous stillbirth with an abnormal chromosomal complement
- f. Balanced chromosomal rearrangement (translocation, inversion...) or not (marker, mosaicism, sexual aneuploidy) in one of the parents
- g. Abnormal maternal serological screening test
- h. Abnormal fetal ultrasound
- i. Confirming the NIPT test by diagnostic test

## Applications

What will the karyotype detect?

- Numerical Chromosomal Abnormality (Aneuploidies)
  - o Monosomy, trisomy
  - o Partial monosomy, partial trisomy
  - o Polyploidy
- Structural Chromosomal abnormalities
  - o Inversions
  - o Translocations

- Rings
- Marker chromosome

### **Limitations**

This study cannot detect: (i) low levels of mosaicism; (ii) cryptic chromosomal alterations; (iii) uniparental disomy (UPD) in which both copies of the chromosomes are inherited from a single parent DNA; (iv) single gene mutations that cause diseases such as sickle cell anemia, cystic fibrosis or Tay-Sachs disease, other genetic disorder caused by single gene.

Karyotyping relies on G-band quality and resolution. In general, blood samples give the best quality chromosomes and therefore provide the best chance of detecting small subtle chromosome abnormalities. Chromosomes from other tissues (e.g. amniotic fluid, chorionic villus and products of conception) give poorer quality chromosomes, hence the risk of missing a subtle abnormality is increased. • It should be noted that on rare occasions a subtle abnormality may be missed at prenatal diagnosis, only to be diagnosed later using a postnatal blood sample. A policy statement to this effect has been issued by the Association of Clinical Cytogeneticists (ACC). It should also be understood that even a G-band blood karyotype can never exclude extremely subtle chromosome abnormalities that are at the limit of resolution of light microscopy. Microarray testing should be performed in these cases where the patient meets the appropriate criteria. • The Laboratory adheres to national professional standards for the minimum acceptable banding resolution for specified types of clinical referral. If a repeat sample is required due to analysis failing to meet the minimum standard, this will be stated on the report.

The efficiency and reliability of the result can be affected by several reasons: (i) unsuitable, scarce and/or poor-quality samples; (ii) unsuitable sample collection tube; (iii) time elapsed from the sample collection until its processing; (iv) inappropriate storage and transportation of sample and at extreme temperatures.

### **Reporting and results**

<b>Regular Karyotype</b>
~400-550 bands
Useful for identifying large structural rearrangements
Gain or loss in large chunks of chromosomal materials

### **SAMPLE REQUIREMENT AND LOGISTIC**

**SPECIMEN TYPE: Whole Blood / Chorionic Villi / Amniocentesis / POC**

**Preferred collection container for Blood:** 3ml blood in green top tube (Sodium heparin /Lithium heparin)

**Preferred collection container For Amniocentesis:** 15-30ml Amniotic Fluid can be transferred into a sterilized conical tube sealed with parafilm to avoid any leaks without adding any media

**Preferred collection container For Chorionic Villi sample:** 20-50mg is the amount of tissue obtained in routine chorionic villus sampling. Sample transferred into a sterilized conical tube that contains (RPMI) 1640 Media and sealed with parafilm to avoid any leaks.

**Products of conception (POC):** 1 cm<sup>3</sup> (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized. Separate villi from maternal blood and decidua's to reduce the chance for maternal cell contamination. For IUFD or therapeutic abortion cases, amniotic fluid can be accepted.

**Note:** Clinical history and reason for referral are required with test order

#### **SPECIMEN PROCESSING:**

<b>Instructions</b>	Do not centrifuge or refrigerate
<b>Transport temperature:</b>	Room temperature. All sample are shipped with gel bags
<b>Specimen stability:</b>	Transport immediately after collection. Viability decreases during transit. Send specimen to testing lab for viability determination. Do not freeze. Do not reject.
<b>Rejection criteria:</b>	Frozen specimens unacceptable. Hemolyzed sample Leaked sample CVS/AF samples that reach after 48 hours to the lab

**Turn Around Time (TAT):** TAT for **Karyotyping 20 business days** upon receive in the lab. Apriority will be given to any prenatal sample.

#### **Karyotype sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including Sodium/Lithium Heparin Tubes, biohazard plastic pack, cooling/gel pack.

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

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

The 'informed consent' form and the 'test requisition form' (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	20 business days	EDTA tube	3mL	Room temperature
CVS	20 business days	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	20 business days	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	20 business days	Tissue in sterile container in saline  Cardiac or cord blood in Vacutainer	1 cm3 (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	20 business days	In a sealed Eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

### 3.3.2. Whole Genome sequencing (WGS):

#### Description:

Whole genome sequencing (WGS) is a Next Generation Sequencing (NGS) based approach used to identify genetic variants linked to a disorder. This methodology analyzes the entire genome/genes. This test included whole genome sequencing followed by diagnostic interpretation of the variants detected to identify genetic causes of this individual's reported symptoms.

#### Indication:

**WGS Diagnostic:** WGS can be used as a diagnostic test for patients with complex genetic disorders, where the correct diagnosis is difficult to establish due to overlapping symptoms, complicated medical histories or in cases where previous genetic testing has not yielded conclusive results.

**WGS Diagnostic Solo** is a comprehensive genetic test that helps identify the disease-causing variant in an individual affected with a disease/condition. Whole genome including the coding and non-coding regions are sequenced.

**WGS Diagnostic Trio** is a comprehensive genetic test that is offered to the affected individual and their parents in order to identify and report any variants relating to the affected individuals disease/condition, additionally this test can help determine whether the disease-causing variant is inherited. TRIO testing helps determine the inheritance and de novo nature of the variants at the same time.

### Applications:

WGS is used in diagnosing or evaluating a genetic disorder where the results are expected to influence medical management and clinical outcomes of a patient or a family directly or indirectly. In situations where the clinical presentation is unclear and the condition in question is unknown, sequencing and analysing a small number of genes at a time is costly and time-consuming process. This may further delay the diagnosis, which could have an impact on patient's quality of life.

WGS is applicable where the previous genetic testing is negative or inconclusive. It helps in identifying all possible type of sequencing variants with accuracy.

### Objectives:

To identify and prevent, without therapy, serious genetic disorders, many of which are fatal. It is the only clinically validated genetic test based on next generation massive sequencing (NGS). It includes the complete sequencing whole genome (exonic, intronic and intergenic regions), including all clinically relevant genes.

### Reporting and results:

Result interpretation is based on currently available information in the medical literature, research, and scientific databases. Because the literature, medical and scientific knowledge are constantly changing, new information that becomes available in the future may replace or add to the information that Igenomix used to interpret the results. Re-analysis of variants in previously issued reports considering new evidence is not routinely performed but is available upon request.

Igenomix uses an internally validated algorithm for analysis and interpretation. American College of Medical Genetics (ACMG) (Richards et al., 2015), Association for Clinical Genomic Science (ACGS) (Durkie et al., 2024), and European Society of Human Genetics (ESHG) (Deans et al., 2022); the classification of CNVs has been carried out according to the recommendations of the ACMG and Clinical Genome Resource (ClinGen) (Riggs et al., 2020). The categorization of genetic variants established by the ACMG is the following: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign.

There are four possible results that can be obtained:

**Positive (Pathogenic and likely pathogenic):** A positive result indicates that one or more gene or chromosome variation has been identified in association with the disease phenotype. This scenario will allow healthcare professionals to provide genetic counselling or personal guidance regarding possible medical treatments, disease progression, reproductive-/prevention-strategies and potential implications for other family members.

**Negative:** A negative result indicates that no disease-causing genetic variant was identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. Additionally, a negative result does not rule out a genetic cause of the disease nor does it eliminate the risk for future offspring. However, if a negative test result is obtained and the variant in question is known to be present in affected family members, this then rules out a diagnosis of that genetic disorder in the proband. A negative result may be explained by several causes, including limited genetic knowledge and limitations associated to the used methodology.

**Inconclusive/Variant of Uncertain Significance (VUS):** A finding of a variant of uncertain significance indicates that a change in a gene was detected, but it is currently unknown whether that change is associated with a genetic disorder or disease. A variant of uncertain significance is not the same as a positive result and does not clarify whether the proband is at an increased risk of developing a genetic disorder or disease. The change could be a normal genetic variant, or it could be disease-causing. Further analysis may be recommended, including testing both parents as well as other affected and unaffected family members. Sometimes, performing ancillary tests is necessary to prove the phenotype that the proband presents with. Detailed medical records or information from other family members also may be needed to help clarify the result.

**Unexpected/Incidental/secondary results:** In rare instances, this test may reveal an important genetic change that is not directly related to the reason for ordering this test. For example, this test may provide information about an individual's risk for other genetic conditions. This information is likely to impact the individual's treatment options and is disclosed based on the informed consent provided by the patient.

Incidental findings are not included in the report unless it is specifically requested and in accordance with international recommendations (Miller et al., 2023).

### **Sample requirements and logistics:**

For genetic testing through next generation sequencing, the following sample types are accepted. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is - Patient's full name, Date of birth, Gender and Medical Record Number.

\*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	45 business days	EDTA tube	3mL	Room temperature
Products of Conception	45 business days	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm3 (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	45 business days	In a sealed Eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

### **WGS sample transportation to the laboratory:**

The clinic must notify the laboratory before a sample is ready and the laboratory will offer to arrange for sample collection. The Igenomix kit provided by Igenomix must be used for the shipment, including EDTA tubes for blood, Sterile Container for POC, Eppendorf tube for DNA, biohazard plastic pack, cooling/gel pack.

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

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

The 'informed consent' form and the 'test requisition form' (included within the provided kit) must be properly filled-in and signed. Igenomix will send you all the documents needed for the pick-up and transportation of the appropriate kit to our laboratory.

## 4.0 CERTIFICATION, ACCREDITATION AND EXTERNAL ASSESSMENT SCHEMES

**IGENOMIX ARABIA MEDICAL LLC** is Commercially (Registration: No. **1010739879**), licensed by Ministry of Health as a Medical Laboratory specialized in Molecular Genetics and following international & local regulation standards.

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

All tests performed are included in an assessment program (either PT or AA) that may further assist in the continued assessment of the reliability of the tests offered by Igenomix.

IGENOMIX ARABIA MEDICAL LLC works with referral labs that meet certain criteria as per local and international quality standards to ensure the maximum assurance in the services provided.