Patient:
Clinic:
Date:

# EMBRYOTEST PLUS™

**Result Report** - Preimplantation Genetic Testing for Aneuploidies (PGT-A).

## GENERAL DATA

| | | | |
|---|---|---|---|
| **Patient Name:** | | **Date:** | |
| **Medical Center:** | | **Batch ID:** | |
| **Doctor:** | | **Sample No.:** | |
| **Email:** | | **Phone(s):** | |

The following report lists the results of the patient's embryo biopsies in General Data hereunder. The herein does not represent any diagnosis or suggestion. It only informs the findings retrieved by processing using Next Generation Sequencing (NGS) of each sample.

## REQUESTED STUDY

There are normally 23 pairs of chromosomes in each cell of our body. An aneuploidy means a change in the number of chromosomes. EmbryoTest Plus™ (ETP) analyzes the 46 chromosomes of human embryos to detect possible gains or losses of genetic material (aneuploidies) during their cellular division. The Next Generation Sequencing technique is used to screen the chromosomes. It represents a very useful test as it supports Assisted Reproductive Technology (ART) and reproductive medicine.

## RATIONALE

Since alterations in the set of chromosomes can cause failed implantations in ART cycles, spontaneous abortion, and chromosomal abnormalities in newborn babies, EmbryoTest Plus™ allows the selection of those chromosomally normal embryos from all evolutionary embryos of a patient. This increases their reproductive possibilities and the chances of evolving correctly leading to a healthy child.

EmbryoTest Plus™ increases the chances of transferring embryos free of chromosomal abnormalities to the mother. Aneuploidies can cause spontaneous abortion leading to congenital defects and intellectual disability in newborn babies or they are not compatible with life. However, within the viable chromosomal aneuploidies that can be detected, there are the most common syndromes, non-sexual, such as Down Syndrome (trisomy 21), Edwards Syndrome (trisomy 18), and Patau Syndrome (trisomy 13).

## METHODOLOGY

EmbryoTest Plus™ consists of a series of steps with quality controls in each one of them.

1. Embryo Biopsy: It consists of extracting some embryonic stem cells for genetic study. They are evaluated according to internationally established criteria to determine embryo viability.
2. Whole Genome Amplification (WGA): It consists of making genetic material, or genome, copies of the embryonic stem cell to have a greater quantity of work material for the test.
3. WGA Quality Control: It is made through the electrophoresis technique, which allows visualizing the quality of amplified genetic material in the previous step. Likewise, a fluorometric method is used to quantify the amplified genome to continue the process.
4. Library Generation: DNA fragment synthesis compatible with the flow cell using WGA.
5. Cluster Generation: It is a "bridge amplification" process that generates copies of each one of the fragments attached to the flow cell, leading to clusters.
6. Next-Generation Sequencing: It is a sequencing process by synthesis.
7. File Generation: It is a process in which the sequencing generates database files (FASTQ) and genome assembly (BAM).
8. Result Analysis: FASTQ and BAM are analyzed through mathematical algorithms to generate graphs that allow the semiquantitative and qualitative analysis of aneuploidies. All the above is summarized in the below results table.
9. Results quality control: during the analysis, the number of readings and the sample noise are reviewed.

## TECHNIQUE LIMITATIONS

EmbryoTest Plus™ does not detect monogenic diseases or specific mutations and it is limited to those samples that meet the quality criteria necessary for an informative result. It is neither possible the detection of specific structural chromosomal rearrangements.

## SAMPLE EXCLUSION CRITERIA

The “Service Order and Client Information” document provided lists the reasons why a sample may be rejected upon arrival at the Semper Genomics service laboratory, such as:

1. Disagreement in the identification number and code of the samples with the recorded data in the Sample Collection, Transportation, and Handling Form for Embryo Test Plus ™.
2. Thawing of samples: it is requested that these remain frozen (-4°F) to keep the genetic material integrity before their collection. Centrifuge them before freezing if possible.
3. Sample absence: the microtube does not contain the sample.

In addition to these criteria and after performing the Whole Genome Amplification (WGA), the first point in quality control is to perform an agarose gel electrophoresis to verify whether the sample is amplified or not. If the sample is not visible on the gel, it is discarded, and the process is not continued.

Next, the samples with a positive gel are quantified by the fluorometric method to determine the resulting amount of amplified DNA. If the sample gets a very low result (less than 1ng/ul) it is also discarded, since the sequencing does not provide results for a sample of poor quality and consumes readings that could affect the result of the rest of the samples. Once they are sequenced for data quality control analysis, we verify that the Copy Number Variations (CNVs) are informative and interpretable with an emphasis on the number of reads obtained per sample (>250,000) and noise (<0.4) obtained in the run. The quality controls of the non-informative samples are summarized in the "Quality Values Table" in the QUALITY CONTROL annex.

**Note:** Samples will not be sequenced if they do not pass the WGA quality control filter and quantification. These exclusions are notified by email to the address registered in this form.

## RESULTS

Analysis software generates a graph that represents the number of copies of each analyzed embryo chromosome, and it compares it with a reference normal human genome. An embryo is considered normal when any chromosome has differences from the reference genome. An embryo is considered abnormal when there is the presence of one aneuploidy in any of the chromosomes, either a loss (-) or a gain (+) in the graph. The presence of more than five (5) aneuploidies is considered a complex aneuploidy. An embryo that was not detected does not provide information.

### Terms in the Results Table

- Normal or euploid: no chromosomal abnormalities were detected; result of autosomal chromosome copy number is 1.8-2.2x
- Abnormal or aneuploid: chromosomal abnormalities were detected, if the result of the autosomal chromosome number is 2.8x or greater, a gain is declared, and if it is 1.2x or less, loss of the whole chromosome is declared.
- Complex abnormal (AC): 5 or more chromosomal abnormalities were detected.
- Non-informative: no informative data was derived from this sample.

## RESULTS TABLE

| No. | PIN | Embryo | Quality Control | Sex | Result |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |

PIN = Personal Identification Number, AC = Complex Abnormal, NI = Non-informative, NA = Not Applicable


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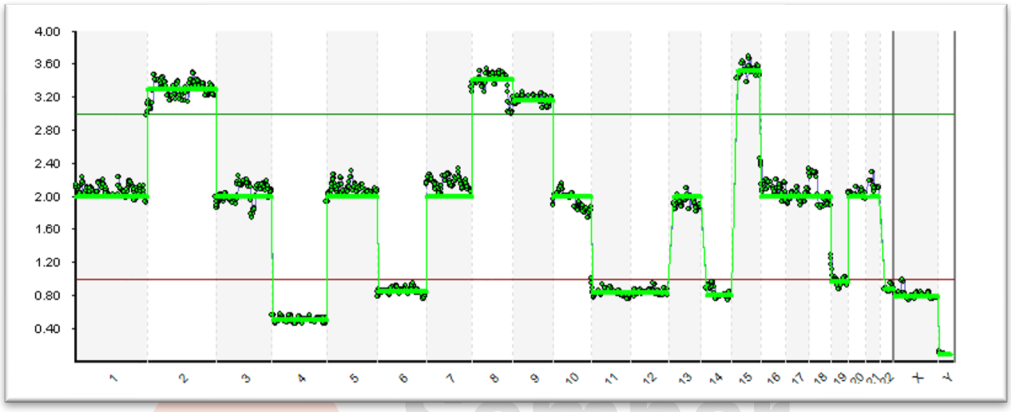
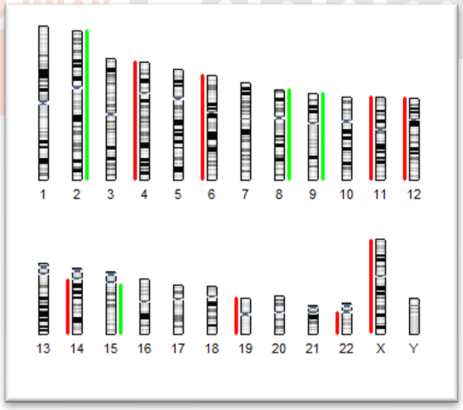
# EMBRYOTEST PLUS™

## GRAPHS

| No. | PIN | Embryo | Quality Control | Sex | Result |
|---|---|---|---|---|---|
| 1 | | | | | |




Observations:

Semper Genomics

**Patient:**
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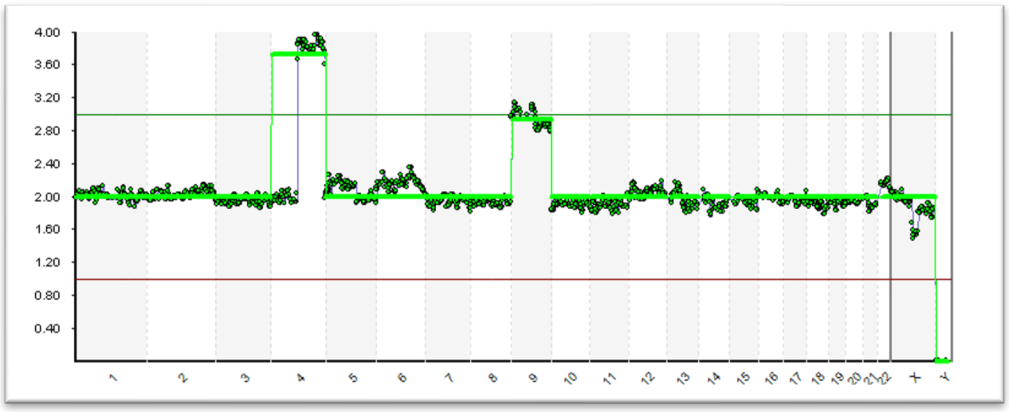
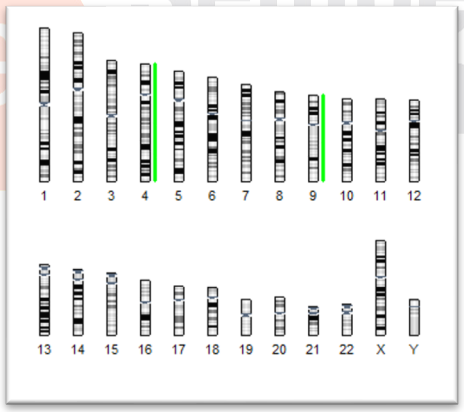
**Date:**

# EMBRYOTEST PLUS™

| No. | PIN | Embryo | Quality Control | Sex | Result |
|---|---|---|---|---|---|
| 2 | | | | | |




Observations:

## QUALITY CONTROL ANNEX

There are cases where the samples can be seen as "non-informative" and it may be for one of the following reasons: high amount of noise present when performing the sequencing data analysis, mainly due to low quality of the sample or PCR artifacts generated during whole genome amplification. Likewise, there are several factors that can generate artifacts during the PCR-based WGA, including a suboptimal biopsy, DNA degradation, incomplete cell lysis, cells in the apoptosis process, and the presence of PCR inhibitors in the culture medium. During the analysis, if there is noise present for reasons or a low number of readings, this impacts the interpretability and confidence of the results observed in the graphs. Therefore, that sample is "non-informative", and IT IS NOT RECOMMENDED TO TRANSFER THAT EMBRYO. It is suggested to repeat the analysis with a new biopsy.

This kind of test poses a risk of false positive/negative results. Such results commonly occur by biological reasons called "mosaicisms", present in approximately 5% of embryo biopsies. Mosaicism means that the embryo can have cells with different numbers of chromosomes (ploidy) both in the normal number (euploidy) and abnormal (aneuploidy). Other important factors that lead to false results are sample contamination, codification problems, rare genetics that interferes with the analysis, technical issues, and human error. Among all these factors, the chance of a false result that is not associated with mosaicism is about 2%.

## QUALITY VALUES (ONLY FOR NON-INFORMATIVE SAMPLES)

| No. | PIN | Embryo | WGA band in Gel | Quantification ng/ul | Readings (PDF) | Noise | Graph Informative |
|---|---|---|---|---|---|---|---|
| REF | Reference values | - | Yes | Higher than 1 | Higher than 250,000 | Lower than 0.40 | Yes |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |

PIN = Personal Identification Number, WGA = Whole Genome Amplification, ng/ul = Nanograms per microliter, PF = Passing Filter, REF = Internal quality control reference values for sample exclusion criteria.

## DATA PRIVACY

The test results will be protected under strict privacy, and they can be provided by email only to the assigned people herein. The data and records privacy will be 90 calendar days from the sending of results to the client.

## TERMINATION OF SERVICE

Semper Genomics will terminate the service in accordance with the results 72 hours after the file sending if no notification is received from the client.

## REFERENCES


- Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29(12):2802–2813.
- Yang Z, Liu J, Collins GS, Salem SA, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis patients: results from a randomized pilot study. Mol Cytogenetic. 2012;5: 24.
- ESHRE PGT-SR/PGT-A Working Group; Coonen E, Rubio C, Christopikou D, Dimitriadou E, Gontar J, Goossens V, Maurer M, Spinella F, Vermeulen N, De Rycke M. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. Hum Reprod Open. 2020 May 29;2020(3):hoaa017. DOI: 10.1093/hropen/hoaa017. PMID: 32500102; PMCID: PMC7257111.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|---|---|---|
| Signature | Signature | Signature |
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***This document DOES NOT represent a diagnosis. For decision-making based on your medical history, you must reach out to your doctor.**


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