Female Infertility Panel: Sequencing and CNV Analysis

Condition Description

Infertility is defined as the failure to conceive after a couple attempts to become pregnant for 12 months or more. This panel tests for common genetic causes of female infertility including: chromosomal abnormalities, \textit{FMR1}-related Premature Ovarian Failure (POF), as well as pathogenic variants in genes associated with POF.

Mosaic chromosome analysis is used to detect chromosomal abnormalities including sex chromosome aneuploidies, chromosomal mosaicism, and chromosomal rearrangements. Sex chromosome aneuploidies may include conditions such as Turner syndrome (45,X). Testing for chromosomal mosaicism provides a more thorough chromosome analysis by examining 20 cells plus scans of an additional 30 cells for sex chromosomes to detect the presence of mosaicism, meaning the presence of two or more cell populations with different chromosomes. Chromosomal rearrangements that can cause female infertility, including large deletions, duplications, or translocations (such as sex chromosome rearrangements seen in variant forms of Turner syndrome), are detectable by this assay. Results of chromosome analysis may suggest investigation of other single gene disorders of sex development when the karyotype results are discordant from the phenotypic gender of the patient (such as androgen insensitivity (Androgen Receptor (AR) gene)).

\textit{FMR1}-related Premature Ovarian Failure (POF) is the onset of menopause, or ovarian dysfunction, before the age of 40 years due to an alteration within the \textit{FMR1} gene. \textit{FMR1} is located on the X chromosome. \textit{FMR1}-related POF is caused by a triplet (CGG) repeat expansion in the promoter of the \textit{FMR1} gene. The normal range of CGG repeats is approximately 5-44. Repeats in this range are stable when passed from parent to child. Repeats in the 45-54 range are considered intermediate (or grey-zone), for which the risk of expansion to full mutation of 200 repeats or more when passed to children is low but not well defined at this time. Individuals with approximately 55-200 CGG repeats are premutation carriers. The CGG repeat number in this range is unstable and may expand when passed to children. Individuals with Fragile X have over 200 CGG repeats. Males with over 200 repeats are almost always affected, while females may be more mildly affected.

Women who are carriers of premutation sized \textit{FMR1} expansions are at increased risk for POF (estimated as high as 21%), however penetrance of POF is not complete. Recent reports indicate that women who carry a premutation size expansion may also have a slight increased risk for developing Fragile X Tremor/Ataxia Syndrome (FXTAS), a disorder that causes tremors, balance problems, difficulty walking, and memory difficulty.

Genes

\textit{BMP15}, \textit{FMR1}, \textit{FSHR}, \textit{LHB}, \textit{LHCGR}, \textit{ZP1}

Indications

This test is indicated for:

- Women with infertility, premature ovarian failure, or ovarian dysfunction.

Methodology

Mosaic chromosome analysis: PHA stimulated cultures are used for G-banded analysis. ISCN nomenclature is followed.

\textit{FMR1}-related POF: The CGG repeat in the \textit{FMR1} gene is analyzed by PCR amplification and capillary electrophoresis.

For all remaining genes:

Next Generation Sequencing: In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

Copy number analysis: Comparative analysis of the NGS read depth (coverage) of the targeted regions of genes on this panel was performed to detect copy number variants (CNV). The accuracy of the detected variants is highly dependent on the size of the event, the sequence context and the coverage obtained for the targeted region. Due to these variables and limitations a minimum validated CNV size cannot be determined; however, single exon deletions and duplications are expected to be below the detection limit of this analysis.

Detection

Mosaic chromosome analysis:
**FMR1 repeat and methylation analysis:** All cases of premutation expansion mutations will be detected by this assay. This analysis will not detect mutations in the **FMR1** gene.

For all remaining genes:

**Next Generation Sequencing:** Clinical Sensitivity: Unknown. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical/biochemical phenotype.

Analytical sensitivity for sequence variant detection is ~99%.

**Copy number analysis:** The sensitivity and specificity of this method for CNV detection is highly dependent on the size of the event, sequence context and depth of coverage for the region involved. The assay is highly sensitive for CNVs of 500 base pairs or larger and those containing at least 3 exons. Smaller (< 500 base pairs) CNVs and those that involving only 1 or 2 exons may or may not be detected depending on the sequence context, size of exon(s) involved and depth of coverage.

**Reference Range**

Chromosome analysis is by ISCN and ACMG guidelines with a minimum band resolution of 500-550.

**FMR1 repeat and methylation analysis:** Normal: Approximately 5-44 CGG repeats; Intermediate: Approximately 45-54 unmethylated CGG repeats; Premutation: Approximately 55-200 CGG repeats and methylation of expanded allele.

**Specimen Requirements**

*Submit only 1 of the following specimen types*

**Type: Whole Blood (EDTA and Sodium Heparin)**

**Specimen Requirements:**
- Sodium Heparin and EDTA
- Infants (Children (>2 years): 3-5 ml in both tubes
- Older Children & Adults: 7-10 ml in both tubes

**Specimen Collection and Shipping:**
Ship sample at room temperature for receipt at EGL within 24 hours of collection. Do not refrigerate or freeze.

**Type: DNA, Isolated**

**Specimen Requirements:**
- Microtainer
- 20µg
- Isolation using the Perkin Elmer™Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

**Specimen Collection and Shipping:**
Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

**Related Tests**

- The Male Infertility Panel (MH) is available to screen for common genetic causes of male infertility.
- Fragile X (**FMR1**) Methylation and Expansion Analysis is indicated for males or females with a clinical diagnosis of Fragile X; unexplained mental retardation, developmental delay or autism; or children a person who carries a **FMR1** expansion.
- Fragile X Tremor/Ataxia Syndrome or FXTAS, (FJ) is indicated for older men with late-onset, progressive cerebellar ataxia and intention tremor or for men with daughters who are carriers for Fragile X.
- Components of this infertility panel may be ordered separately if previous genetic testing was performed: Mosaic Chromosome Analysis (MM), **FMR1**-related Premature Ovarian Failure (FK).