Premature Ovarian Failure Panel: Sequencing, CNV Analysis, and FMR1 CGG Repeat Analysis

**Condition Description**

*FMR1*-related disorders include fragile X syndrome, fragile X-associated tremor/ataxia syndrome (FXTAS), and *FMR1*-related premature ovarian insufficiency (POI). *FMR1*-related premature ovarian insufficiency (POI) is the onset of ovarian dysfunction or menopause before the age of 40 years. Women who are carriers of *FMR1* premutation range expansions are at increased risk for POI (estimated as high as 21%), though penetrance of POI is not complete. Recent reports indicate that women who carry a premutation size expansion may have a slight increased risk for developing fragile X-associated tremor/ataxia syndrome (FXTAS), a disorder that causes tremors, balance problems, difficulty walking, and memory difficulty.

The *FMR1* gene is located on the X chromosome. *FMR1*-related disorders are associated with the presence of a triplet (CGG) repeat expansion in the promoter of *FMR1* leading to methylation and subsequent inactivation of the *FMR1* gene. The normal range of CGG repeats is approximately 5-44. Repeats in this range are considered 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 a 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. Females with expansions in this range are at risk for POI. The number of repeats 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 a full size expansion are not at increased risk of POI. Mosaicism has also been reported in some individuals with *FMR1* mutations, indicating the presence of two different repeat sizes or variation in the extent of methylation.

All other genes on this panel have been associated with premature ovarian failure. Click here for the GeneReviews summary on this condition.

**Genes**

- BMP15, CYP17A1, CYP19A1, DIAPH2, EIF2B2, EIF2B3, EIF2B5, FIGLA, FMR1, FOXL2, FSHR, GALT, GDF9, HFM1, LHCGR, LMNA, NOBOX, NR5A1, POF1B, POR, PSMC3IP

**Indications**

This test is indicated for adult females with premature ovarian failure.

**Methodology**

The DNA surrounding the CGG repeat in the *FMR1* gene is amplified by PCR and the size of the repeat is determined by 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**

All cases of premutation expansion mutations for *FMR1* will be detected by this assay. 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**

Normal for FMR1: < 44 CGG repeats, Intermediate: 45-55 CGG repeats, Premutation: 55-~200 repeats, Full mutation: >200 CGG repeats

**Specimen Requirements**

*Submit only 1 of the following specimen types*

**Type: DNA, Isolated**

**Specimen Requirements:**
Microtainer 20µg
Isolation using the Perkin Elmer™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.

**Type: Whole Blood (EDTA)**

**Specimen Requirements:**
EDTA (Purple Top)
Infants and Young Children (2 years of age to 10 years old): 3-5 ml
Older Children & Adults: 5-10 ml
Autopsy: 2-3 ml unclotted cord or cardiac blood

**Specimen Collection and Shipping:**
Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

**Related Tests**

- **FXTAS (FJ)** is indicated for older men with late-onset, progressive ataxia and intention tremor or for fathers of women who are premutation range carriers of an FMR1 expansion.
- **Testing for fragile X syndrome (MFRAX)** is indicated for males and females with symptoms of Fragile X.
- The **female infertility panel (MFMR1)** is available for women experiencing infertility due to ovarian dysfunction or menopause.