Male Infertility Panel: Sequencing and CNV Analysis

Test Code: XM050
Turnaround time: 3 weeks
CPT Codes: 81220 x1, 81224 x1, 81405 x1, 81406 x1, 81479 x1

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 genetic causes of male infertility due to azoospermia and oligospermia (mild, moderate or severe) including: mosaic chromosome analysis, Y chromosome microdeletion, CFTR mutation analysis, and pathogenic variants in other genes associated with male infertility.

Approximately 5-10% of males with non-obstructive azoospermia, oligospermia, or other abnormalities of sperm morphology or motility are caused by structural or numerical abnormalities, including Y chromosome microdeletions. 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 Klinefelter syndrome (47,XXY). Chromosomal mosaicism is defined as the presence of two or more cell populations with different chromosomes. Testing for chromosomal mosaicism provides a more thorough chromosome analysis by examining a total of 50 cells (30 additional cell counts). Chromosomal rearrangements that can cause male infertility, including large deletions (deletion of Yq12) or structural abnormalities (including pseudodicentric Y inversions or translocations) are detectable by this assay. Results from 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 translocation of SRY or SOX 9 duplication).

Approximately 5-10% of males with non-obstructive azoospermia or oligospermia have microdeletions in the AZFa, AZFb, AZFc or AZFd regions on the Y chromosome that are not detectable by standard cytogenetic methods. Each AZF region contains multiple genes that are involved in different stages of spermatogenesis. Most cases of Y chromosome microdeletions are new mutations. If assistive reproductive technologies are used, a male child receiving the Y chromosome will have the microdeletion, and therefore, all sons will be affected.

Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations are associated with obstructive azoospermia and oligospermia. Approximately two-thirds of males with congenital bilateral absence of the vas deferens (CBAVD) have mutations of the CFTR gene. Males with unilateral absence of the vas deferens or obstruction of the epididymides may also have mutations in CFTR. For males carrying CFTR gene mutations, cystic fibrosis (CF) screening is indicated for their partners to better estimate the chance of having a child with CF.

This panel tests for the 39 most common CFTR mutations (listed below), including the core panel of 23 mutations for cystic fibrosis as recommended by the American College of Medical Genetics in 2004.

<table>
<thead>
<tr>
<th>Mutation</th>
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<tbody>
<tr>
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<td>DeltaS57</td>
<td>R117H</td>
<td>W1282X</td>
<td>S120+1G&gt;A</td>
<td>G85E</td>
<td>Y122X</td>
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<td>R347H</td>
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<td>G553X</td>
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<td>R1162X</td>
<td>S1255X</td>
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<td>711+1G&gt;T</td>
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<td>1717-1G&gt;A</td>
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<td>3905insT</td>
<td>2307insA</td>
<td>2789+5G&gt;A</td>
<td>3659delC</td>
<td>3849+10kbC&gt;T</td>
</tr>
</tbody>
</table>

Genes

AR, CATSPER1, CFTR, FSHR, LHCGR

Indications

This test is indicated:

- Males with a clinical diagnosis of infertility.

Methodology

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

Y chromosome microdeletion: Y chromosome deletions are detected by multiplex PCR amplification.

CFTR mutation analysis: This test utilizes a PCR based oligoligation assay for 32 CFTR mutations, with positive and negative controls.

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.

**Y chromosome microdeletion:** Over 99% of AZFa, AZFb, AZFc and AZFd deletions will be detected by this assay.

*CFTR* mutation analysis identifies 85-90% of CF mutations in the Caucasian population, 97% in the Ashkenazi Jewish population, 72% in the Hispanic population, and 69% in the African American population.

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. *CFTR* mutation detection and Y chromosome microdeletion detection are both qualitative molecular assays.

## Specimen Requirements

**Submit only 1 of the following specimen types**

**Type: DNA, Isolated**

**Specimen Requirements:**

Microtainer 15μg

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 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.

## Related Tests

- The Female Infertility Panel (MG) is available to screen for common genetic causes of female infertility.
- Components of this test may be ordered separately if previous genetic testing was performed: *CFTR* mutation analysis (CF), mosaic chromosome analysis (MM), Y chromosome microdeletions (YD).