Assay Summary

Factor IX Gene Mutation Analysis

Hemophilia B

Synopsis

Alterations of the \( F9 \) gene on the X chromosome, resulting in deficiency of factor IX, are causative for hemophilia B. Sequence analysis for point mutations and small insertion/deletions detects the vast majority of causative \( F9 \) mutations, while large deletion/duplication analysis accounts for most of the sequence negative cases, with nearly 100% sensitivity in affected males when both are performed. In cases where a male proband is not available for testing, analysis may be performed on a sample from an obligate carrier or otherwise at-risk relative. Identification of underlying \( F9 \) gene mutations for families with hemophilia B can permit accurate determination of carrier status of at-risk individuals in these families and provides options for prenatal diagnosis.

Indications for testing

- Individuals with a diagnosis of hemophilia B
- Obligate carriers or appropriate at-risk relatives of hemophilia B patients
- Known carriers of familial \( F9 \) mutations desiring prenatal diagnosis (Please contact the laboratory in advance of sending prenatal orders).

Methodology

Factor IX sequencing: Coding exons, associated intron junctions, and \( \sim100 \) bp of 5’UTR sequence (covering \( F9 \) Leyden region) are captured and enriched using custom Agilent SureSelect technology. Next-generation sequencing is performed on Illumina MiSeq. Additional Sanger sequencing is performed for any regions with insufficient depth of coverage or for verification of suspect variant calls. Targeted testing for known familial mutation is performed by Sanger sequencing.

MLPA analysis for known large deletions and duplications: SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit is used, which includes probes targeting all 8 exons of the \( F9 \) gene. The MLPA products are analyzed by DNA fragment analysis on an automated fluorescent sequencer. The absence or presence of deletions of one or more exons is confirmed by MLPA analysis using an independently amplified segment.

Limitations

Factor IX sequencing: The sequence analysis will not detect mutations located in regions of the Factor IX gene that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The sequencing method also will not detect gross genetic alterations including most duplications, inversions, or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

MLPA analysis for known large deletions and duplications: MLPA is designed to detect deletions/duplications of one or more exons of the gene, but does not determine precise breakpoints of
alterations detected. MLPA analysis will not detect certain genetic alterations, such as point mutations or small deletions/insertions and inversions. Additionally, MLPA analysis may be sensitive to DNA sample purity and other experimental conditions. Probe signals may also be adversely affected by sequence variants situated in the vicinity of, or at the probe ligation site; therefore, apparent deletions/duplications detected by a single probe should be confirmed by other methods. Partial exonic deletions/duplications outside of the probe target sequence may not be detected.

**Specimen Requirements**

(a) **Blood samples**: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

**Note:**
- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

(b) **Prenatal samples**: 2 T25 flasks of confluent amniocytes or CVS sent padded to arrive on M/Tu/W. **Maternal cell contamination studies** (required and performed concurrently for prenatal): If not previously tested in our lab, a blood sample from the mother is required, as described in (a).

**Test Request Form (TRF)**

(a) A completed CMDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

(b) Pedigree and [Hemophilia Patient Information Form](#) (recommended)

<table>
<thead>
<tr>
<th><strong>Order Codes</strong></th>
<th><strong>CPT Codes</strong></th>
<th><strong>TAT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>F9-SEQ (Factor IX gene, full gene sequencing by NGS)</td>
<td>81405, G0452</td>
<td>3 wks</td>
</tr>
<tr>
<td>F9-CAS (Factor IX gene, targeted mutation analysis)</td>
<td>81403, G0452</td>
<td>2 wks</td>
</tr>
<tr>
<td>F9-PD (Factor IX gene, prenatal sequence analysis for known mutation detection, with maternal cell contamination studies)</td>
<td>81403, 81265, G0452</td>
<td>2 wks</td>
</tr>
<tr>
<td>F9-DEL (Factor IX gene, MLPA analysis)</td>
<td>81479, G0452</td>
<td>3 wks</td>
</tr>
<tr>
<td>F9-DEL-CAS (Factor IX gene, MLPA analysis, known deletions/duplications)</td>
<td>81479, G0452</td>
<td>3 wks</td>
</tr>
<tr>
<td>F9-DEL-PD (Factor IX gene, prenatal MLPA analysis for known deletion/duplication, with maternal cell contamination studies)</td>
<td>81479, 81265, G0452</td>
<td>2 wks</td>
</tr>
</tbody>
</table>

**References**