HEMOPHILIA A (FACTOR VIII) AND HEMOPHILIA B (FACTOR IX)

**Background:**
Hemophilia A (Factor VIII deficiency; OMIM 306700) is the most common severe bleeding disorder that is inherited as a classic X-linked recessive disease. Mutations occur in the F8 gene with a birth prevalence of ~1:4,000. Hemophilia B (Factor IX deficiency, OMIM 306900) is also inherited as an X-linked recessive disease. Mutations occur in the F9 gene with a birth prevalence of ~1:20,000. Both diseases have varied clinical presentations from mild to severe disease, and severity of disease depends on the mutation type. Factor IX Leyden is caused by mutations in the promoter of F9 gene, and severity of disease decreases after an affected male reaches puberty. Clinically, Hemophilia A and Hemophilia B are indistinguishable in the manifestations of bleeding episodes and can be differentiated by Factor VIII and Factor IX clotting assays, respectively, prior to initiating molecular analysis.

**Assay:**
For Hemophilia A (F8), tests available are inversion analysis for intron 22 and intron 1, sequencing of all 26 exons and exon-intron borders, and deletion/duplication analysis. PCR analysis for inversion. Direct mutation analysis by full sequencing. Real-time PCR for deletion/duplication analysis.

For Hemophilia B (F9), tests available are sequencing of all 8 exons and exon-intron borders and ~50 bases of the 5' promoter region.

**Utility:**
Diagnostic confirmation, carrier detection in at-risk females, and prenatal diagnosis. Identification of the specific mutation might predict risk for development of alloimmune inhibitors in affected males.

**Sensitivity:**
In ~48% of all cases of severe Hemophilia A, a characteristic inversion in intron 22 is present, and in 2-3% of cases an inversion in intron 1 is present. If the patient has severe Hemophilia A (<1% Factor VIII), the combination of inversion PCR, full sequencing, and deletion/duplication analysis will identify the disease causing mutation ~99% of the time in the F8 gene. A deletion or duplication is estimated to account for ~2% of mutations in moderate to severe Hemophilia A. Mild to moderate Hemophilia A will have a sequencing mutation in 76-98% of cases.

In Hemophilia B, sequencing mutations are identified in 97-100% of cases and are associated with mild to severe disease. A deletion or duplication is estimated to account for ~3% of cases. Factor IX Leyden has been reported to only be associated with mutations in the promoter region of the F9 gene.

Mutations in non-coding sequences, insertions, or novel rearrangements will not be detected in our current assays. Linkage analysis is available for families in which an F8 gene mutation has not been identified; however, linkage will only be performed if all other analysis is negative.

**Turnaround:**
Hemophilia A (Factor VIII)
- 3-4 weeks for inversion
- 2-3 weeks for the deletion/duplication analysis
- 2-3 weeks for a known familial mutation

Hemophilia B (Factor IX)
- 4-6 weeks for full sequencing
- 3-4 weeks for linkage analysis
- 7-10 days for prenatal diagnosis on known mutation

**Fees:**
Hemophilia A (Factor VIII)
- $360 for inversion testing to include intron 22 and intron 1 analysis (as needed)
- $1,350 for direct mutation screening by full sequencing
- $560 for deletion/duplication analysis
- $740 for linkage analysis (please call the laboratory before ordering this test)
- $360 for known familial inversion or point mutation
- $460 for prenatal diagnosis for inversion or point mutation (cost includes MCC studies )
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Fees:
- Hemophilia B (Factor IX)
  - $840-direct mutation screening by full sequencing to include F9 promoter region
  - $360-analysis for F9 promoter region – ONLY for known Factor IX Leyden diagnosis
  - $360-known familial point mutation
  - $460-prenatal diagnosis for point mutation or F9 promoter analysis (cost includes MCC studies)

CPT codes:
- Hemophilia A (Factor VIII)
  - Inversion analysis: 81403
  - Full sequencing: 81407
  - Deletion/Duplication Analysis: 81406
  - Linkage analysis: 81479
  - Known familial mutation: 81403
  - Prenatal diagnosis: 81403, 81265 for inversion or point mutation

- Hemophilia B (Factor IX)
  - Full sequence analysis: 81405
  - Analysis of F9 promoter region for Factor IX Leyden: 81404
  - Known familial mutation: 81403
  - Prenatal diagnosis for point mutation: 81403, 81265
  - Prenatal F9 promoter analysis: 81404, 81265

Resources:
- National Hemophilia Foundation: www.hemophilia.org