

Hemophilia B (*F9*) Sequencing and Deletion/Duplication

Indications for Ordering

- Determine
 - Causal *F9* gene variant in established cases of hemophilia B
 - Carrier status for women with a family history of hemophilia B
- Contraindicated for
 - Diagnostic or carrier testing of individuals with a previously identified familial *F9* gene variant

Test Description

- Bidirectional sequencing of entire coding region, intron/exon boundaries, and proximal promoter variants of *F9* gene
- Multiplex ligation-dependent probe amplification (MLPA) to detect large deletions/duplications in *F9* gene

Tests to Consider

Typical testing strategy

- Initial testing for hemophilia B – factor IX activity, partial thromboplastin time, and prothrombin time
- Molecular genetic testing of *F9* gene – sequencing and deletion/duplication analysis to detect variants
 - *F9* gene encodes coagulation factor IX
 - Factor IX deficiency leads to reduced blood clotting and abnormal bleeding

Primary tests

[Hemophilia B \(*F9*\) Sequencing and Deletion/Duplication 2010494](#)

- Most comprehensive test to confirm diagnosis or determine carrier status for *F9* gene variants

[Hemophilia B \(*F9*\) Sequencing 2001578](#)

- Acceptable test to confirm diagnosis or determine carrier status for *F9* gene variants

Related tests

Initial testing for hemophilia B

- [Factor IX, Activity 0030100](#)
- [Partial Thromboplastin Time 0030235](#)
- [Prothrombin Time 0030215](#)

Molecular testing when pathogenic familial variant identifiable by sequencing is known

- [Familial Mutation, Targeted Sequencing 2001961](#)
- [Familial Mutation, Targeted Sequencing, Fetal 2001980](#)

Disease Overview

Prevalence – 1/25,000 males worldwide

Symptoms

Mild hemophilia B

- 6-30% factor IX activity
- ~15% of cases
- Usually not diagnosed until adulthood
- Abnormal bleeding observed after surgery, tooth extraction, or major injuries
- Spontaneous bleeding does not occur
- Bleeding frequency may vary from once a year to once in 10 years
- 10% of carrier females are symptomatic (typically have factor IX activity level <30%)
 - Usually mildly affected
 - Should be monitored postpartum for delayed bleeding unless baseline factor IX activity is normal

Moderate hemophilia B

- 1-5% factor IX activity
- ~25% of cases
- Typically diagnosed by age 6 due to
 - Prolonged bleeding after surgery, tooth extractions, or injuries
 - Delayed wound healing
- Spontaneous bleeding is rare
- Episode frequency varies from once a month to once a year

Severe hemophilia B

- <1% factor IX activity
- ~60% of cases
- Usually diagnosed in first year of life due to spontaneous joint or deep-muscle bleeding occurring 2-5 times/month
- Life expectancy for untreated individuals with severe disease is 11 years
 - With proper treatment, life expectancy increases to 63 years
- Leading cause of death is intracranial hemorrhage
- Major cause of disability from bleeding is joint disease

Diagnostic issues

- Hemophilia B is not clinically distinguishable from hemophilia A
- Diagnosis of hemophilia B is established by deficiency of factor IX coagulation activity
 - Lower activity levels correspond with earlier age of diagnosis and higher frequency of bleeding episodes
- First-line testing in most individuals is not molecular
 - Molecular genetic testing may be helpful in predicting clinical phenotype and risk of developing a factor IX inhibitor
- Carrier testing cannot be accurately performed by measuring factor IX activity
 - Molecular genetic studies must be performed

Genetics

Gene – *F9*

Inheritance – X-linked recessive

Penetrance

- 100% in males
- 10% in females

De novo variants

~1/3 to 1/2 of individuals with hemophilia B have no family history of the disease

- Disease in such individuals is caused by de novo variants

Variants

- *F9* gene variants are the only cause of hemophilia B
 - ~3% of causative variants are whole gene or large gene deletions/duplications
- Rare *F9* promoter variants may result in hemophilia B Leyden
 - Typically results in a decreased bleeding tendency in males after puberty
- Somatic mosaicism is more common in hemophilia B than in hemophilia A
 - May reduce the variant detection rate in males

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – 97% for sequencing, 3% for deletion/duplication
- Analytical sensitivity/specificity – 99%

Results

- Positive – pathogenic variant detected
 - Predictive of mild, moderate, or severe hemophilia B disease in males and carrier status in females
 - 10% of carrier females are affected (typically with mild disease)
- Negative – no pathogenic gene variant detected
 - Hemophilia diagnosis is less likely
 - Does not rule out hemophilia B due to the possibility of an undetectable variant in the *F9* gene
- Uncertain – sequencing may reveal novel variant(s)
 - Determination of clinical significance (benign or pathogenic) may not be possible

Limitations

- Deep intronic variants will not be detected
- Rare diagnostic errors may occur due to primer-site variants