

# Hemophilia B (*F9*) Sequencing

## *TO DETERMINE THE CAUSATIVE FACTOR IX MUTATION IN AFFECTED INDIVIDUALS AND CARRIER STATUS IN AT-RISK INDIVIDUALS*

### Disease Overview

- Clinical characteristics of severe hemophilia B include spontaneous joint or deep-muscle bleeding. Findings of moderate to mild hemophilia B may include the following: prolonged bleeding after tooth extractions, surgery, or injuries, and recurrent or delayed wound healing. The presentation of hemophilia B is not clinically distinguishable from hemophilia A.
- Diagnosis of hemophilia B is established by deficiency of factor IX clotting activity; lower activity levels correspond with earlier age of diagnosis and higher frequency of bleeding episodes.
- Severe hemophilia B, defined by less than 1 percent factor IX activity, is usually diagnosed in the first year of life due to spontaneous joint or deep muscle bleeding occurring two to five times per month. Treatment involves prophylactic infusions of factor IX concentrate twice weekly to maintain factor IX clotting activity higher than 1 percent to prevent spontaneous bleeding. If bleeding does occur, intravenous infusion of plasma-derived or recombinant factor IX concentrate is needed within the first hour of onset.
- Moderate hemophilia B, characterized by 1–5 percent of factor IX activity, is typically diagnosed by age 6 due to prolonged or delayed oozing after minor trauma, with episode frequency varying from once a month to once a year. These individuals seldom have spontaneous bleeding.
- Mild hemophilia B, with 6–30 percent factor IX activity, is not usually diagnosed until adulthood. Although spontaneous bleeding does not occur, abnormal bleeding is observed after surgery, tooth extraction, or major injuries. Bleeding frequency may vary from once a year to once in 10 years.
- Severe hemophilia B accounts for approximately 60 percent of cases, while moderate and mild disease is present in 25 percent and 15 percent of patients, respectively.
- The major cause of disability from bleeding is joint disease.
- The leading cause of death due to bleeding is intracranial hemorrhage. Life expectancy for untreated individuals with severe disease is 11 years; when adequately treated, life expectancy increases to 63 years.
- Patients with hemophilia B should be followed at a hemophilia treatment center.
- Ten percent of carrier females are symptomatic, typically having a factor IX activity level below 30 percent. Symptomatic carriers are usually mildly affected, but should be monitored postpartum for delayed bleeding unless their baseline factor IX activity is normal.
- Carrier testing cannot be accurately performed by measuring factor IX activity. Molecular studies must be performed.

### Prevalence

One in 25,000 males worldwide.

### Genetics

- *F9* gene mutations are the only cause of hemophilia B.
- Inheritance is X-linked recessive. Penetrance is 100 percent in males and 10 percent in females.
- Approximately one-third to one-half of hemophilia B cases have no family history of the disease and result from de novo mutations.
- *F9* sequence analysis detects 97 percent of causative mutations, which may result in mild, moderate, or severe disease.
- Approximately 3 percent of causative mutations are whole gene or large gene deletions/ duplications.
- Rare *F9* promoter mutations may result in hemophilia B Leyden, which typically results in a decreased bleeding tendency in males after puberty.
- Somatic mosaicism is more common in hemophilia B than hemophilia A and may reduce the mutation-detection rate in males.

### Indications for Ordering

- To determine the specific *F9* gene mutation in affected individuals.
- To determine carrier status for women with a family history of hemophilia B.
- To determine if a male fetus is affected with hemophilia B when there is a known familial *F9* gene mutation.

### Contraindication

- For diagnostic or carrier testing of individuals with a previously identified familial *F9* mutation, order Custom PCR and Sequencing (ARUP test #0050358).
- Provide a copy of the affected relative's laboratory report documenting the familial mutation.

### Additional Ordering Note

If there is a family history of hemophilia B, please provide the relationship of the proband to the individual being tested, as well as the specific mutation identified in the proband.

### Interpretation

- For optimal test interpretation, a Patient History for Hemophilia A and B form documenting the patient's symptoms and family history of hemophilia B is required with sample submission.
- Detection of a deleterious mutation by sequence analysis predicts hemophilia B disease in males and carrier status in females. Mutations detected by sequencing may result in mild, moderate, or severe disease in males.
- Ten percent of carrier females are affected, typically with mild disease.
- Gene sequencing may reveal novel mutations; thus, the determination of clinical significance (benign or deleterious) may be unclear.
- A negative result does not rule out hemophilia B, due to the possibility of an undetectable mutation in the *F9* gene.
- For individuals with unclear or negative results, medical management should rely on clinical findings and family history.

### Methodology

- Bidirectional sequencing of the entire *F9* coding region, intron-exon boundaries, and proximal promoter.
- Analytical sensitivity and specificity are 99 percent.
- Clinical sensitivity is 97 percent.

### Limitations

- Deep intronic mutations and gene duplications will not be detected in patients of either sex; large deletions will not be detected in females.
- Rare diagnostic errors may occur due to primer-site mutations.

### Related Tests

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

### References

1. Online GeneTests: Hemophilia B. [www.genetests.org](http://www.genetests.org) (accessed on January 5, 2009).
2. Online Mendelian Inheritance in Man: #306900, Hemophilia B. <http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim> (accessed on January 15, 2009).
3. Mitchell M, et al. The molecular analysis of haemophilia B: a guideline from the UK haemophilia centre doctors' organization haemophilia genetics laboratory network. *Haemophilia* 2005;11:398–404.
4. Tagariello G, et al. The Italian haemophilia B mutation database: a tool for genetic counselling, carrier detection and prenatal diagnosis. *Blood Transfus* 2007;5:158–63.

## Test Information

2001578

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For specific collection, transport, and testing information, refer to the ARUP Web site at [www.aruplab.com](http://www.aruplab.com).

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at [www.arupconsult.com](http://www.arupconsult.com).