


Specimen Number	Specimen Type <b>Peripheral Blood</b>		Control Number	Account Number	Account Phone Number	Route
Patient Last Name						
Patient First Name <b>Alexei</b>		Patient Middle Name				
Patient SS#	Patient Phone	Total Volume				
Age (Y/M/D) <b>8 y.o.</b>	Date of Birth	Sex <b>Male</b>				
Patient Address			<b>Indication: Hemophilia, possibly type B</b> <b>Family History: Family history of uncontrolled bleeding in males</b> <b>Ethnicity: Western European Caucasian</b>			
Date and Time Collected	Date Entered	Date and Time Reported	Physician Name <b>Jane Ferreiro, MD</b>	NPI	Physician ID	
<b>Hemophilia Mutation Evaluation</b>			Tests Ordered			
<p style="text-align: center;">General Comments</p> <b>Please send a copy of the final report to the Molecular Science/M1 Training office via Fax at (202) 555-1212</b>						

## Clinical test results for DNA Hemophilia Mutation Evaluation

GENE	TEST RESULTS	EXPLANATION
<b>F9 (Xq27.1)</b>	c.278-3A>G	<p><b>This result supports the diagnosis of Hemophilia B.</b> This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., APTT, Factor IX Activity, Factor IX Inhibitor, etc.).</p> <p>A PCR/sequencing study has confirmed one copy of a F9 gene with putative splice-site variation (F9: g.15338A&gt;G, c.278-3A&gt;G). This variation is an A to G change at nucleotide position 15338 of the F9 gene. It has been reported that this nucleotide variant impacts post-transcriptional processing of the mRNA transcript. The presence of this variant induces the use of this position as a novel splice acceptor for exon 2, causing a -2bp frameshift. The new in-frame coding sequence of exon 2 produces an abnormal 11 amino acid peptide (ending in a termination codon) - resulting in an abnormally short, truncated protein.</p> <p>As males have only one copy of the X chromosome, a variation in an X-linked gene renders the patient with only a mutated form of the gene. Thus, they are susceptible to the most severe form of the disease.</p>
<b>F8 (Xq28)</b>	Negative	

### INDICATIONS FOR TESTING

Individuals with a diagnosis of hemophilia B, appropriate at-risk female relatives of probands with identified mutations, and hemophilia B carriers with genetic counseling, are candidates for testing.

### METHODOLOGY

Factor IX sequencing: All coding exons (1-8) and associated intron junctions of the Factor IX gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions.

## **PERFORMANCE**

Factor IX sequencing: From previous experience, we have been able to detect factor IX gene mutations in about 99% of individuals with the diagnosis of hemophilia B with specificity of mutation detection in probands and carrier detection is also estimated to be greater than 99%.

## **LIMITATIONS**

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.

## **CLINICAL DESCRIPTION**

Hemophilia B is characterized by deficiency in factor IX clotting activity that results in prolonged oozing after injuries, tooth extractions, or surgery, and delayed or recurrent bleeding prior to complete wound healing. This is an X-linked recessive bleeding disorder with an incidence of about 1 per 30,000 live male births. Hemophilia B affects males, however, all male offspring will be normal, and although all female offspring will be obligatory carriers, they rarely have symptomatic bleeding. In contrast, female offspring of carriers of hemophilia B have a 50% chance of being carriers themselves, and each male offspring has a 50% chance of being affected.

The age of diagnosis and frequency of bleeding episodes are related to the level of factor IX clotting activity. In severe hemophilia B, spontaneous joint or deep-muscle bleeding is the most frequent symptom. Individuals with severe hemophilia B are usually diagnosed during the first two years of life; without prophylactic treatment, they may average up to two to five spontaneous bleeding episodes each month. Individuals with moderate hemophilia B seldom have spontaneous bleeding; however, they do have prolonged or delayed oozing after relatively minor trauma and are usually diagnosed before age five to six years; the frequency of bleeding episodes varies from once a month to once a year. Individuals with mild hemophilia B do not have spontaneous bleeding episodes; however, without pre- and post-operative treatment, abnormal bleeding occurs with surgery or tooth extractions; the frequency of bleeding may vary from once a year to once every ten years. Individuals with mild hemophilia B are often not diagnosed until later in life. In any individual with hemophilia B, bleeding episodes may be more frequent in childhood and adolescence than in adulthood. Approximately 10% of carrier females are at risk for bleeding (even if the affected family member has mild hemophilia B) and are thus symptomatic carriers, although symptoms are usually mild. After major trauma or invasive procedures, prolonged or excessive bleeding usually occurs, regardless of severity.

## **REFERENCES**

1. Yoshitake S, Schach BG, Foster DC, et al: Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). *Biochemistry* 1985 July 2;24(14):3736-3750
2. Giannelli F, Green PM, Sommer SS, et al: Haemophilia B: database of point mutations and short additions and deletions-eighth edition. *Nucleic Acids Res* 1998 Jan 1;26(1):265-268
3. Ketterling RP, Bottema CD, Phillips JA 3rd, et al: Evidence that descendants of three founders constitute about 25% of hemophilia B in the United States. *Genomics* 1991 Aug;10(4):1093-1096