

# Hemophilia A (*F8*) Inversion, Sequencing, and Deletion/Duplication

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

### Disease Overview

- Clinical characteristics of severe hemophilia A include spontaneous joint or deep-tissue bleeding. Findings of moderate to mild hemophilia A may include prolonged or recurrent bleeding after surgery, tooth extraction, or injuries.
- Severe hemophilia A, defined by less than 1 percent factor VIII 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 VIII concentrate every other day to maintain factor VIII clotting activity higher than 1 percent to prevent spontaneous bleeding. If bleeding does occur, intravenous infusion of plasma-derived or recombinant factor VIII concentrate is needed within the first hour of onset.
- Moderate hemophilia A, characterized by 1–5 percent of factor VIII 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.
- Mild hemophilia A, with 6–35 percent factor VIII 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. Immediate treatment of bleeding is recommended, or patient may receive prophylaxis with intravenous or nasal desmopressin or factor VIII concentrate.
- 10 percent of carrier females are symptomatic. They are usually mildly affected. Carriers should be monitored postpartum for delayed bleeding, unless their baseline factor VIII activity is normal.
- Diagnosis of hemophilia A is established by documenting low factor VIII activity with a normal von Willebrand factor level.
- Carrier testing cannot be accurately performed by measuring factor VIII activity. Molecular studies must be performed.
- 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.
- The major cause of disability from bleeding is joint disease.

### Prevalence

One in 4,000–5,000 live male births worldwide.

### Genetics

- *F8* gene mutations are the only cause of hemophilia A.
- Inheritance is X-linked recessive.
- In the approximately 30 percent of cases that appear to be de novo, the mother is found to be a carrier more than 80 percent of the time.
- The etiology for severe hemophilia A is an inversion occurring at intron 22A or intron 1 in 48 percent and 3 percent of affected individuals, respectively. Large gene deletions represent 6 percent of severe mutations, while smaller point mutations cause another 43 percent of severe mutations.
- Mild to moderate hemophilia is caused by mutations detected by sequence analysis in 98 percent of cases. Deletion analysis detects less than 1 percent of such cases.

### Indications for Ordering

- To determine the specific *F8* gene mutation in affected individuals.
- To determine carrier status for women with a family history of hemophilia.

### Contraindications

- Testing for individuals with a previously identified familial *F8* mutation.
- Prenatal testing.

### Additional Ordering Notes

If there is a family history of hemophilia A, please provide the relationship of the proband to the individual being tested, as well as the specific mutation identified in the proband. Knowledge of the specific mutation in the proband would be a contraindication for ordering this test.

## Interpretation

- For optimal test interpretation, a Patient History for Hemophilia form documenting the patient's symptoms and family history of hemophilia A is required with sample submission.
- Detection of one copy of the *F8* intron 22A or intron 1 inversion is predictive of severe hemophilia A disease in males and carrier status in females.
- Identification of a large *F8* deletion or duplication is predictive of severe hemophilia A disease in males and carrier status in females.
- Detection of a deleterious mutation by sequence analysis predicts hemophilia A disease in males and carrier status in females. Mutations detected by sequencing may result in mild, moderate, or severe disease.
- 10 percent of carrier females are affected, typically with mild disease.
- Gene sequencing may reveal novel mutation(s); thus, the determination of clinical significance (benign or deleterious) may be unclear.
- A negative result does not rule out hemophilia A, due to the possibility of an undetectable mutation in the *F8* gene.
- For individuals with unclear or negative results, medical management should rely on clinical findings and family history.

## Methodology

- Inversion analysis of F8 introns 1 and 22A by BclI digest, followed by ligation, then PCR; products are analyzed by size using eGene.
- Bidirectional sequencing of the entire *F8* coding region and intron-exon borders.
- Multiplex ligation-dependent probe amplification (MLPA) for large deletion/duplication analysis of the *F8* gene.
- Clinical sensitivity is as high as 98 percent for all ethnicities.
- Analytical sensitivity and specificity are 99 percent for sequencing, MLPA, and inversion analysis.

## Limitations

- Breakpoints of large *F8* deletions/duplications will not be determined.
- *F8* deep intronic or promoter mutations, with the exception of the common intron 1 and 22A inversions, will not be detected.
- Rare diagnostic errors may occur due to primer- or probe-site mutations.

## Related Tests

- Hemophilia A (F8) 2 Inversions (2001759)
- Hemophilia A (F8) 2 Inversions, Fetal (2001755)
- Hemophilia A (F8) Sequencing (2001747)
- Hemophilia A (F8) Deletion/Duplication (2001751)
- Familial Mutation, Targeted Sequencing (2001961)
- Factor VIII, Activity (0030095)
- vonWillebrand Factor Activity (0030250)
- Partial Thromboplastin Time (0030235)

## References

1. Bogdanova, et al. Spectrum of molecular defects and mutation detection rate in patients with mild and moderate hemophilia A. *Human Mutation* 2007;29:54–60.
2. Online GeneTests: Hemophilia A. [www.genetests.org](http://www.genetests.org) (accessed on November 26, 2008).

## Test Information

2001614

### Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication

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).