

# Hemophilia A (F8) Genetic Testing

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Hemophilia A is a heritable bleeding disorder typically affecting males that is characterized by a deficiency of factor VIII (FVIII) clotting activity. FVIII activity level is related to the age of diagnosis as well as the frequency and severity of bleeding episodes. Severe deficiency (less than 1% of normal FVIII activity) is usually diagnosed in the first two years of life and is associated with spontaneous joint or deep muscle bleeding. Moderate deficiency (1-5% of normal activity) is typically diagnosed by age 6 due to prolonged or delayed oozing after minor trauma, with episodic frequency varying from once a month to once a year. Spontaneous bleeding is rare. Mild deficiency (6-40% of normal activity) is often diagnosed in adulthood and is characterized by abnormal bleeding after tooth extractions, surgery, or injuries, and recurrent or delayed wound healing. Female carriers of hemophilia A may have increased bleeding tendencies. Affected individuals should be followed at a hemophilia treatment center. The World Federation of Hemophilia has published treatment guidelines for the management of individuals with hemophilia.<sup>1</sup>

## Genetics

### Etiology

Pathogenic *F8* germline variants

### Penetrance

100% in males; disease severity is typically similar to other affected males in the family

Approximately 30% of female carriers have FVIII activity levels of <40% and are at risk for bleeding symptoms typically consistent with mild hemophilia A.<sup>2</sup> Rarely, females can have severe disease due to skewed X-chromosome inactivation or biallelic pathogenic variants.

### Epidemiology

1 in 5,000 live male births worldwide

The frequency of severe, moderate and mild disease is 60%, 15%, and 25%, respectively.<sup>3</sup>

### Inheritance

X-linked recessive

In approximately 30% of cases that appear to be de novo, the mother is found to be a carrier >80% of the time.

### Variant Spectrum by Disease Severity

Severe hemophilia A:

- Intron 22A inversion in approximately 45% of cases and intron 1 inversion in 2-5% of cases
- Sequence variants in 43-51% of cases
- Large deletions/duplications in <2% of cases

Mild to moderate hemophilia A:

- Sequence variants in 76-99% of cases
- Intron 22A and intron 1 inversions 0-4% of cases
- Large deletions/duplications <1% of cases

## Featured ARUP Testing

### [Hemophilia A \(F8\) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication 3004232](#)

**Method:** Inverse Polymerase Chain Reaction/Massively Parallel Sequencing/Multiplex Ligation-dependent Probe Amplification

- Use to identify causal F8 variant in individuals with established severe hemophilia A.
- Use to determine carrier status in at-risk females with severely affected male relatives.
- Clinical sensitivity: 98%

If a familial sequence variant has been previously identified, targeted sequencing for that variant may be appropriate; refer to the [Laboratory Test Directory](#) for additional information.

For detailed information on the testing strategy for hemophilia, refer to [Hemophilia - Factor VIII or IX Deficiency](#).

# Test Interpretation by Component/Methodology

## Inversion Analysis (Inverse Polymerase Chain Reaction/Electrophoresis)

### Clinical Sensitivity

Approximately 50% for severe hemophilia A

### Analytic Sensitivity

99%

### Limitations

- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- Variants other than the *F8* type 1 or type 2 intron 22-A and intron 1 inversions will not be evaluated.
- Rare *F8* intron 22-A and intron 1 inversions with different breakpoints may not be detected by this assay.

## Sequence Analysis (Massively Parallel Sequencing)

### Clinical Sensitivity

76-99% for mild or moderate hemophilia A

43-51% for severe hemophilia A

### Analytic Sensitivity

Variant Class	Analytic Sensitivity (PPA) Estimate <sup>a</sup> (%)	Analytic Sensitivity (PPA) 95% Credibility Region <sup>a</sup> (%)
SNVs	99.2	96.9-99.4
Deletions 1-10 bp	93.8	84.3-98.2
Deletions 11-44 bp	99.9	87.8-100
Insertions 1-10 bp	94.8	86.8-98.5
Insertions 11-23 bp	99.9	62.1-100

<sup>a</sup>Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.

bp, base pairs; PPA, positive percent agreement; SNVs, single nucleotide variants

### Limitations

- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Variants outside the *F8* coding regions and intron-exon boundaries
  - Variants in regions that are not included in the preferred transcript (NM\_000132.4)
  - Regulatory region and deep intronic variants
  - Noncoding transcripts
- The following may not be detected:
  - Deletions/duplications/insertions of any size by massively parallel sequencing
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

## Deletion/Duplication Analysis (Multiplex Ligation-dependent Probe Amplification [MLPA])

### Clinical Sensitivity

Up to 2%

### Analytic Sensitivity

99%

### Limitations

- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - *F8* base pair substitutions, small deletions/duplications, deep intronic, and regulatory region variants
  - Breakpoints of large deletions/duplications
  - Single exon deletions/duplications based on the breakpoints of the rearrangement

### References

1. Srivastava A, Santagostino E, Dougall A, et al. [WFH Guidelines for the management of hemophilia, 3rd edition](#) [published correction appears in *Haemophilia*. 2021;27(4):699]. *Haemophilia*. 2020;26 Suppl 6:1-158.
2. Konkle BA, Huston H, Nakaya Fletcher S. [Hemophilia A](#). In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*. University of Washington, Seattle.
3. Johnsen JM, Fletcher SN, Huston H, et al. [Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative](#). *Blood Adv*. 2017;1(13):824-834.

### Related Information

[Hemophilia - Factor VIII or IX Deficiency](#)

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