

## **Hemophilia A**

**Background:** Hemophilia A is the most common severe bleeding disorder that is inherited as a classic X-linked recessive disease. In 45% of all cases of severe Hemophilia A (Factor VIII <1%), a characteristic inversion in intron 22 is detectable. In those cases where the inversion is not present, or if the Factor VIII level is not <1% (mild disease), direct mutation analysis by sequencing, deletion/duplication analysis, or linkage analysis is available.

**Assay:** Southern blot for inversion. Direct mutation analysis by full sequencing. Real-time PCR for deletion/duplication analysis

**Utility:** Diagnostic confirmation, carrier detection in at-risk females, and prenatal diagnosis

**Sensitivity:** If the patient has severe hemophilia A (<1% Factor VIII), the combination of Southern blot and full sequencing will identify the disease causing mutation ~93% of the time. If the mutation is in the coding sequence of the Factor VIII gene, sequencing will detect the mutation 99% of the time. A deletion or duplication is estimated to account for ~2% of mutations in severe hemophilia. Mutations in non-coding sequences, insertions, or novel rearrangements will not be detected in our current assays.

**Turn around:** 2-3 weeks for inversion, 4-6 weeks for direct mutation screen by full sequencing, 2-3 weeks for the deletion/duplication analysis, 2-3 weeks for a known familial mutation, 3-4 weeks for linkage analysis, 1-3 weeks for prenatal diagnosis

**Fees:** \$260-inversion testing  
\$1240-direct mutation screening by full sequencing  
\$500-deletion/duplication analysis  
\$340-known familial point mutation  
\$340-prenatal diagnosis for point mutation  
\$380-prenatal diagnosis for inversion  
\$640-linkage analysis (please call the laboratory before ordering this test)

**CPT Codes:** Inversion detection: 83891, 83892x2, 83894, 83896, 83897, 83912  
Full sequence analysis: 83891, 83894x14 83898x17, 83904x24, 83909x2, 83912  
Deletion/Duplication Analysis: 83891, 83900, 83901x5, 83909x2, 83912  
Known familial mutation: 83891, 83898x6 83904x6, 83912  
Prenatal diagnosis for point mutation: 83891x2, 83898x4, 80904x5, 83912  
Prenatal diagnosis for inversion: 83891x3, 83892x2, 83909, 83896, 83897, 83912  
Linkage analysis: 83891x3, 83894x10, 83898x11, 83912

**Additional Disease Information:** National Hemophilia Foundation

## Hemophilia A Testing

### INSTRUCTIONS FOR SAMPLE SUBMISSION

**Documentation:** Each sample must be accompanied by:

1. A requisition for DNA analysis completed by the physician, nurse or genetic counselor requesting screening. **Please note: ICD-9 code is required for billing purposes. If ICD-9 code is unknown, please provide patient's clinical symptom(s) or family history that prompted testing.**
2. The patient's pedigree including three generations, if possible.
3. An informed consent signed by the patient (if under 18 years of age, the parent or guardian should sign) and the professional obtaining the consent. Please have the patient initial at the top of each page and send **all** pages of the consent.
4. A verification of blood tubes form signed by the patient, parent or guardian. The form should be signed at the time of the blood draw.
5. A completed registration form with check, money order, credit card authorization or information for billing the referring institution.

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**IN THE EVENT THAT ALL PROPERLY COMPLETED FORMS DO NOT ACCOMPANY THE SPECIMEN, YOU WILL BE NOTIFIED, AND TESTING WILL BE HELD UNTIL PAPERWORK IS COMPLETE.**

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#### Preparing Sample:

- Obtain 2 EDTA tubes (lavender top) of blood - approx. 4 mL per tube
- Label each tube with the patient's name and date sample was obtained
- We accept banked or recently extracted DNA; please include the concentration
  
- For prenatal testing: cultured amniotic fluid or CVS cells, 2 confluent T-25 flasks. Please call the lab prior to sending a prenatal sample. We are often able to offer testing on a direct villi or amnio sample, and we can discuss the requirements with you. 5mL of whole blood from each parent should accompany the prenatal sample.

**Shipping Sample:** Ship at room temperature via Federal Express or other overnight courier that guarantees AM delivery to arrive Monday-Friday. There is no one in the laboratory evenings and weekends to receive samples. If sample is drawn on a Friday, please refrigerate it until shipment on the following business day.

**Shipping Address:** Genetic Diagnostic Laboratory  
University of Pennsylvania  
415 Anatomy-Chemistry Building  
3620 Hamilton Walk  
Philadelphia, PA 19104

**GENETIC DIAGNOSTIC LABORATORY  
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE  
DEPARTMENT OF GENETICS  
Tel: (215) 573-9161 • Fax: (215) 573-5940  
CLIA ID: 39D0893887**

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**REQUEST FOR HEMOPHILIA A (FACTOR VIII) TESTING**

PATIENT FIRST NAME \_\_\_\_\_ PATIENT LAST NAME \_\_\_\_\_

BIRTH DATE \_\_\_\_\_ SEX \_\_\_\_\_ RACE \_\_\_\_\_ ETHNICITY \_\_\_\_\_

STREET ADDRESS \_\_\_\_\_

CITY \_\_\_\_\_ STATE \_\_\_\_\_ ZIP \_\_\_\_\_ HOME PHONE \_\_\_\_\_

Patient is affected \_\_\_\_\_ or Patient is a possible carrier \_\_\_\_\_ Patient's Factor VIII level? \_\_\_\_\_

**Please include a family pedigree indicating affected individuals and relationship of at-risk female carriers to affected individuals.**

Has anyone in the patient's family ever had DNA testing for Hem A? \_\_\_\_\_ If yes, what was the result?

**Please include a copy of the affected relative's genetic test result.**

REFERRING PHYSICIAN \_\_\_\_\_ PHONE \_\_\_\_\_ FAX \_\_\_\_\_

GENETIC COUNSELOR \_\_\_\_\_ PHONE \_\_\_\_\_ FAX \_\_\_\_\_

EMAIL ADDRESS FOR COUNSELOR OR PHYSICIAN \_\_\_\_\_

INSTITUTION and  
DEPARTMENT \_\_\_\_\_

STREET ADDRESS \_\_\_\_\_

CITY \_\_\_\_\_ STATE \_\_\_\_\_ ZIP \_\_\_\_\_ COUNTRY \_\_\_\_\_

**ICD-9 CODE (or patient's clinical symptoms)** \_\_\_\_\_

**TEST REQUESTED**

- \_\_\_\_\_ Inversion Testing and, if negative, Direct Mutation Analysis by Full Sequencing
- \_\_\_\_\_ Inversion Testing Only
- \_\_\_\_\_ Direct Mutation Analysis by Full Sequencing Only
- \_\_\_\_\_ Deletion/Duplication analysis (reflex test if Inversion and Sequencing are negative for a mutation)
- \_\_\_\_\_ Screening for known familial mutation \_\_\_\_\_ Sequencing Mutation or \_\_\_\_\_ Inversion
- \_\_\_\_\_ Prenatal Diagnosis (please call the lab before sending sample to discuss fetal sample requirements; 5mL of whole blood from both parents is requested for MCC studies)
- \_\_\_\_\_ Linkage Analysis (please call the laboratory before ordering)



Initials \_\_\_\_\_

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***Informed Consent for DNA Testing for Hemophilia A Testing***

I, \_\_\_\_\_, hereby request testing for **Hemophilia A** using a DNA-based test. I understand that a sample of my/my child's blood will be obtained from a vein, a procedure that carries very little risk, or if this is a prenatal diagnostic test, fetal cells obtained by amniocentesis or chorionic villus sampling will be tested. I understand that the diagnostic samples will be used for the purpose of attempting to determine if I, my child (or my fetus) have an altered Factor VIII gene.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. The DNA test may not detect certain mutations even though they are present. If the mutation is in the coding sequencing of the Factor VIII gene, the testing will detect the mutation ~95% of the time. Mutations in non-coding sequences, insertions, deletions or rearrangements (with the exception of the characteristic inversion in severe hemophilia cases) will not be detected by sequencing.
2. The DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for Hemophilia A and in no way guarantees my health, the health of my child or of my unborn child.
3. These tests are relatively new and are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor that requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing cannot be attributed to identifiable patients and the results are not reportable. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the

*Hemophilia A Testing*

Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

**CONSENT OF PATIENT**

I have read and received a copy of this consent form. I agree to have genetic testing performed for my fetus or myself and accept the risks. I understand the information provided in this document and I have had the opportunity to ask questions I might have about the testing, the procedure, the associated risks and the alternatives.

\_\_\_\_\_  
Signature of Patient

\_\_\_\_\_  
Date

Printed Name of Patient \_\_\_\_\_

**CONSENT OF PARENT OR GUARDIAN**

I have read and received a copy of this consent form. I agree to have genetic testing performed for my child and accept the risks. I understand the information provided in this document and I have had the opportunity to ask questions I might have about the testing, the procedure, the associated risks and the alternatives.

\_\_\_\_\_  
Signature of Parent/Guardian

\_\_\_\_\_  
Date

Relationship to Child \_\_\_\_\_

Printed Name of Child \_\_\_\_\_

DOB \_\_\_\_\_

**Physician's/Counselor's Statement:** I have explained DNA testing to this individual. I have addressed the limitations outlined above, and I have answered this individual's questions.

\_\_\_\_\_  
Signature of Professional Obtaining Consent

\_\_\_\_\_  
Date

Printed Name \_\_\_\_\_

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***Verification of Correctly Identified Blood Tubes***

I am a participant in genetic DNA testing.

I have been shown the tubes containing my blood for this genetic testing and my name has been correctly placed on each one of these tubes.

I have signed a copy of the consent form regarding this genetic testing to be sent along with my blood samples. I have been given a copy of the consent form to keep.

Participant Name: \_\_\_\_\_

Participant/Parent Signature: \_\_\_\_\_

Date: \_\_\_\_\_

