MAYO CLINIC LABORATORIES

Test Definition: F8INV

Hemophilia A F8 Gene, Intron 1 and 22 Inversion Mutation Analysis, Blood

Overview

Useful For

First-tier molecular testing for male patients affected with severe hemophilia A when a variant has not been identified in the family

Determining hemophilia A carrier status for at-risk female patients, ie, individuals with a family history of severe hemophilia A

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No

Genetics Test Information

This test detects the common inversion variants within the *F8* gene. Approximately 50% of affected male patients with severe hemophilia A have been shown to have an inversion.

It is recommended that the *F8* inversion variant be confirmed in an affected male patient or obligate female carrier prior to testing at-risk individuals.

Testing Algorithm

For any postnatal umbilical cord blood specimen that is received, maternal cell contamination studies will be performed at an additional charge. **A maternal whole blood specimen is required to perform this test**. See Additional Testing Requirements.

Fore more information the following algorithms are available:
-Hemophilia Carrier Testing Algorithm
-Hemophilia Testing Algorithm

Special Instructions

- Informed Consent for Genetic Testing
- Hemophilia Carrier Testing Algorithm

- Hemophilia Testing Algorithm
- Hemophilia A Patient Information
- Informed Consent for Genetic Testing (Spanish)

Method Name
Polymerase Chain Reaction (PCR) or Inverse Shifting-Polymerase Chain Reaction (IS-PCR)

NY State Available
Yes

Specimen

Specimen Type
Whole blood

Ordering Guidance
This test should be ordered when a familial variant has not been identified in a severely affected hemophilia A patient.

If an intron 1 inversion has previously been identified in the family, order F81B / Hemophilia A *F8* Gene, Intron 1 Inversion Known Mutation, Blood.

If an intron 22 inversion has previously been identified in the family, order F822B / Hemophilia A *F8* Gene, Intron 22 Inversion Known Mutation, Blood.

For evaluation of a patient with bleeding symptoms and no known personal history of a bleeding disorder, consider ALBLD / Bleeding Diathesis Profile, Limited, Plasma or the specific factor assays.

Additional Testing Requirements
Due to the complexity of testing, consultation with the laboratory is required for all postnatal umbilical cord blood specimens; call 800-533-1710 to speak to a genetic counselor.

All postnatal umbilical cord specimens must be accompanied by a maternal blood specimen. Order this test on the cord blood specimen (only 1 specimen tube required) and order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Necessary Information
Hemophilia A Patient Information**(T712) is required.** Testing may proceed without the patient information, however, the information aids in providing a more thorough interpretation. Ordering healthcare professionals are strongly encouraged to fill out the form and send with the specimen.

Specimen Required
Patient Preparation: A previous bone marrow transplant from an allogeneic donor will interfere with testing. For

information about testing patients who have received a bone marrow transplant, call 800-533-1710.
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD) or blue top (3.2% sodium citrate)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
3. Whole blood collected postnatal from an umbilical cord is also acceptable if approved by the laboratory. See Additional Information.
Additional Information:
1. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
2. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are performed to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal blood specimen under a separate order number.

Forms

1. **Hemophilia A Patient Information** (T712) **is required.**
2. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
3. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	7 days	
	Refrigerated	7 days	
	Frozen	7 days	

Clinical & Interpretive

Clinical Information

Hemophilia A (HA) is caused by a deficiency of clotting factor VIII (FVIII). HA is an X-linked recessive bleeding disorder that affects approximately 1 in 5000 male individuals. Male patients are typically affected with bleeding symptoms, whereas female carriers generally do not have bleeding symptoms but are at risk of having affected sons. Approximately 10% of female carriers have FVIII activity levels below 35% and are at risk for bleeding.

Bleeding, the most common clinical symptom in individuals with HA, correlates with FVIII activity levels. FVIII activity levels below 1% are associated with severe disease, 1% to 5% activity with moderate disease, and 5% to 40% with mild disease. In male patients with severe deficiency, spontaneous bleeding may occur. In individuals with mild HA, bleeding may occur only after surgery or trauma.

Clotting factor VIII is encoded by the factor VIII (*F8*) gene. Approximately 98% of patients with a diagnosis of HA are found to have a variant in *F8* (ie, intron 1 and 22 inversions, point mutations, insertions, and deletions). The intron 1 and 22 inversion variants account for approximately 50% of variants associated with severe HA. These inversions are typically not identified in patients with mild or moderate HA.

It is recommended that the *F8* variant be confirmed in the affected male patient or obligate female carrier prior to testing at-risk individuals. Affected male patients are identified by FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma) and clinical evaluation, while obligate female carriers are identified by family history assessment. If the intron inversion assays do not detect an inversion in these individuals, additional analysis (ie, *F8* sequencing) may be able to identify the familial variant. Of note, not all women with an affected son are germline carriers of a *F8* variant, as *de novo* variants in *F8* do occur. Approximately 20% of mothers of isolated cases do not have an identifiable germline *F8* variant. Importantly, there is a small risk for recurrence even when the familial *F8* variant is not identified in the mother of the affected patient due to the possibility of germline mosaicism.

Reference Values

An interpretive report will be provided.

Interpretation

The interpretive report will include assay information, background information, and conclusions based on the test results.

Cautions

Obtaining a medical genetics or hematology (coagulation) consultation prior to ordering is advisable. Consultations with the Mayo Clinic Special Coagulation Clinic, Molecular Hematopathology Laboratory, or Thrombophilia Center are available for DNA diagnosis cases. This may be especially helpful in complex cases or in situations where the diagnosis is atypical or uncertain.

This assay detects only *F8* intron 1 and 22 inversion variants. Thus, a negative result does not exclude the presence of other variants in *F8*.

The intron 1 and 22 inversion variants targeted by this assay are found in approximately 50% of individuals with severe hemophilia A; the assay may be uninformative for a number of families.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in

the interpretation of results may occur if the information provided is inaccurate or incomplete.

Clinical Reference

1. Antonarakis SE, Rossiter JP, Young M, et al. Factor VIII gene inversions in severe hemophilia A: results of an international consortium study. Blood 1995;86(6):2206-2212
2. Rossiter JP, Young M, Kimberland ML, et al. Factor VIII gene inversions causing severe hemophilia A originate almost exclusively in male germ cells. Hum Mol Genet 1994;3(7):1035-1039
3. Castaldo G, D'Argenio V, Nardiello P, et al. Haemophilia A: molecular insights. Clin Chem Lab Med 2007;45(4):450-461
4. 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. doi:10.1182/bloodadvances.2016002923
5. Pruthi RK: Hemophilia. A practical approach to genetic testing. Mayo Clin Proc 2005;80(11):1485-1499

Performance

Method Description

Genomic DNA from whole blood is amplified by PCR with primers specific for the *F8* intron 1 inversion mutation.(Bagnall RD, Waseem N, Green PM, Giannelli F: Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood 2002;99[1]:168-174; Meijer P, Verbruggen, Spannagi M: Clotting factors and inhibitors: Assays and Interpretation. In: Kottke-Marchant K, ed. Laboratory Hematology Practice. Wiley Blackwell Publishing. 2012:435-446)

Genomic DNA from whole blood is digested with restriction enzyme, ligated with T4 DNA ligase, and amplified by polymerase chain reaction (PCR) with primers specific for the *F8* intron 22 inversion variants.(Rosetti LC, Radic CP, Larripa IB, De Brasi CD: Developing a new generation of tests for genotyping hemophilia-causative rearrangements involving int22h and int1h hotspots in the factor VIII gene. J Thromb Haemost. 2008 May;6(5):830-836)

PDF Report

No

Day(s) Performed

Weekly

Report Available

14 to 21 days

Specimen Retention Time

Whole blood: 2 weeks; Extracted DNA: Indefinitely; from New York State: 90 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81403

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
F8INV	HA F8 Intron 1/22 Inversion, B	81761-9

Result ID	Test Result Name	Result LOINC® Value
35760	HA F8 Int 1/22 Reason for Referral	42349-1
35761	HA F8 Intron 1/22 Inversion, B	81761-9
35762	F8INV Interpretation	69047-9
35763	HA F8 Intron 1/22 Reviewed By	18771-6