Test Definition: F81B

Hemophilia A F8 Gene, Intron 1 Inversion
Known Mutation, Blood

Overview

Useful For

First-tier molecular testing for male patients affected with severe hemophilia A, when a familial intron 1 inversion has been previously identified

Determining hemophilia A carrier status for at-risk female patients, ie, individuals with a family history of severe hemophilia A due to *F8* intron 1 inversion

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 intron 1 inversion in the *F8* gene. The intron 1 inversion variant accounts for approximately 5% of the variants associated with severe hemophilia A.

Intron 1 inversion known variant analysis can only be performed for individuals when an intron 1 inversion has already been identified in the family. For testing options, see Ordering Guidance.

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.

For 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)

NY State Available
Yes

Specimen

Specimen Type
Whole blood

Ordering Guidance
This test should be ordered for whole blood or postnatal umbilical cord specimens when an intron 1 inversion has previously been identified in the family. For prenatal specimens order F81P / Hemophilia A *F8* Gene, Intron 1 Inversion Known Mutation Analysis, Prenatal.

If a familial variant has not been identified in a severely affected hemophilia A patient, order F8INV / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, 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.

Additional Testing Requirements
Due to the complexity of testing nonperipheral blood, consultation with the laboratory is required for all cord blood samples. Order this test on the cord blood specimen (only 1 specimen tube required) and order MATCC / Maternal Cell Contamination, Molecular Analysis, Blood 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 providers 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. Rarely, 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 inversion variant accounts for approximately 5% of variants associated with severe HA. These inversions are typically not identified in patients with mild or moderate HA.

Intron 1 inversion known variant analysis is only recommended for individuals when an intron 1 inversion has already been identified in the family.

If a familial mutation has not been identified in a severely affected HA patient, the *F8* gene intron 1 and 22 inversion analysis (F8INV / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, Blood) should be ordered.

If the intron 1 inversion analysis is negative, the tested individual has not inherited the familial variant.

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.

Intron 1 inversion known variant analysis is only recommended for individuals when an intron 1 inversion has already been identified in the family.

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

The intron 1 inversion variant targeted by this assay is found in approximately 5% of individuals with severe hemophilia A; if an intron 1 inversion has not been already identified in the family, the assay may be uninformative.

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 polymerase chain reaction with primers specific for the *F8* intron 1 inversion variant.(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)

PDF Report

No

Day(s) Performed

Weekly

Report Available

14 to 21 days

Specimen Retention Time

Whole blood: 2 weeks; 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
F81B	HA F8 Intron 1 Inversion KM, B	81762-7

Result ID	Test Result Name	Result LOINC® Value
35137	HA F8 Int1 KM Reason for Referral	42349-1
35001	HA F8 Intron 1 Inversion KM, B	81762-7
35002	F81B Interpretation	69047-9
35003	HA F8 Int1 KM Reviewed By	18771-6