MAYO CLINIC LABORATORIES

Test Definition: F81B

HA F8 Intron 1 Inversion KM, B

Overview

Useful For

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

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

Genetics Test Information

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

Intron 1 inversion known mutation analysis can only be performed for individuals when an intron 1 inversion has already been identified in the family. If a mutation has not already been identified in the family, order F8INV / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, Whole Blood.

Testing Algorithm

Maternal cell contamination testing will be performed for all cord blood specimens. A maternal whole blood sample with an order for MATCC / Maternal Cell Contamination, Molecular Analysis, Blood is also required to perform this test. (See Specimen Required for more details.)

The following algorithms are available in Special Instructions:

-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)

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed

MATCC	Maternal Cell Contamination, B	Yes	No

Method Name
Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Whole blood

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

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

Additional Testing Requirements
Due to the complexity of testing nonperipheral blood, consultation with the laboratory is required for all cord blood samples. Order F822B / Hemophilia A *F8* Gene, Intron 22 Inversion Known Mutation, Whole Blood on the cord blood specimen (only 1 sample tube required) and order MATCC / Maternal Cell Contamination, Molecular Analysis, Blood on the maternal specimen.

Necessary Information
Hemophilia A Patient Information **is required,** see Special Instructions. 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 allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD) or blue top (sodium citrate)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

Forms

1. **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 in Special Instructions:

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

2. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK

Specimen Minimum Volume

1 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	7 days	
	Frozen	7 days	
	Refrigerated	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 males. Males are typically affected with bleeding symptoms, whereas carrier females generally do not have bleeding symptoms but are at risk of having affected sons. Rarely, approximately 10% of carrier females 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 males with severe deficiency, spontaneous bleeding may occur. In individuals with mild HA, bleeding may occur only after surgery or trauma.

FVIII is encoded by the factor VIII (*F8*) gene. Approximately 98% of patients with a diagnosis of HA are found to have a mutation in *F8* (ie, intron 1 and 22 inversions, point mutations, insertions, and deletions). The intron 1 inversion mutation accounts for approximately 5% of mutations associated with severe HA. These inversions are typically not identified in patients with mild or moderate HA.

Intron 1 inversion known mutation 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, Whole Blood) should be ordered.

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

It is recommended that the *F8* mutation be confirmed in the affected male or obligate carrier female prior to testing at-risk individuals. Affected males are identified by FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma) and clinical evaluation, while obligate carrier females 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 mutation. Of note, not all females with an affected son are germline carriers of a *F8* mutation, as de novo mutations in *F8* do occur. Approximately 20% of mothers of isolated cases do not have an identifiable germline *F8* mutation. Importantly, there is a small risk for recurrence even when the familial *F8* mutation 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 mutation 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 mutation. Thus, a negative result does not exclude the presence of other mutations in *F8*.

The intron 1 inversion mutation 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 our interpretation of results may occur if information given 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 May;1(13):824-834. doi:10.1182/bloodadvances.2016002923

5. Pruthi RK: Hemophilia: A Practical Approach to Genetic Testing. Mayo Clin Proc 2005;80:1485-1499

Performance

Method Description

Genomic DNA from whole blood or cord 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. Chapter 33. In Laboratory Hematology Practice. Edited by K Kottke-Marchant. Wiley Blackwell Publishing. 2012, pp 435-446)

PDF Report

No

Specimen Retention Time

Whole Blood: 2 weeks; DNA: Indefinitely

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test 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	Reporting Name	LOINC®
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