Overview

Useful For

First-tier molecular testing for males affected with severe hemophilia A, when a familial intron 22 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 22 inversion

Genetics Test Information

Detects the intron 22 inversion within the *F8* gene. The intron 22 inversion mutation accounts for approximately 45% of mutations associated with severe hemophilia A.

Intron 22 inversion known mutation analysis can only be performed for individuals when an intron 22 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.

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
MATCC	Maternal Cell Contamination, B	Yes	No

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)

Method Name

Inverse Shifting-Polymerase Chain Reaction (IS-PCR)

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Advisory Information

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, Limited or the specific factor assays.

Additional Testing Requirements

Due to the complexity of testing non-peripheral 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.

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	
	Frozen	7 days	
	Refrigerated	7 days	

Clinical and 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 levelsÂ 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 22 inversion mutations account for approximately 45% of mutations associated with severe HA. These inversions are typically not identified in patients with mild or moderate HA.

Intron 22 inversion known mutation analysis is only recommended for individuals when an intron 22 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 22 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 22 inversion known mutation analysis is only recommended for individuals when an intron 22 inversion has already been identified in the family.

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

The intron 22 inversion mutation targeted by this assay is found in approximately 45% of individuals with severe hemophilia A; if an intron 22 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 digested with Ksp 22 I restriction enzyme, ligated with T4 DNA ligase, and amplified by PCR with primers specific for the *F8* intron 22 inversion mutations.(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;6:830-836; 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

Day(s) and Time(s) Test Performed

Performed weekly; Varies

Analytic Time

14 days

Maximum Laboratory Time

21 days

Specimen Retention Time

Whole Blood: 2 weeks; DNA: Indefinitely

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81403

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
F822B	HA F8 Intron 22 Inversion KM, B	91679-1

Result ID	Test Result Name	Result LOINC Value
35139	HA F8 Int22 KM Reason for Referral	42349-1
35007	HA F8 Intron 22 Inversion KM, B	50397-9
35008	F822B Interpretation	69047-9

Result ID	Test Result Name	Result LOINC Value
35009	HA F8 Int22 KM Reviewed By	18771-6