Overview

Useful For
First-tier molecular testing for males affected with severe hemophilia A when a mutation has not been identified in the family

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

Genetics Test Information
Detects the common inversion mutations within the F8 gene. Approximately 50% of affected males with severe hemophilia A have been shown to have an inversion.

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

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATCC</td>
<td>Maternal Cell Contamination, B</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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

NY State Available
Yes

Specimen
Test Definition: F8INV
HA F8 Intron 1/22 Inversion, B

Specimen Type
Whole blood

Advisory Information
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 F8INV / Hemophilia A F8 Gene, Intron 1 and 22 Inversion Mutation Analysis, 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
Specimen Stability Information

<table>
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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>Whole blood</td>
<td>Ambient (preferred)</td>
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<td></td>
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<tr>
<td></td>
<td>Frozen</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
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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 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 and 22 inversion mutations account for approximately 50% of mutations associated with severe HA. These inversions are typically not identified in patients with mild or moderate HA.

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.

This assay detects only \( F^8 \) intron 1 and 22 inversion mutations. Thus, a negative result does not exclude the presence of other mutations in \( F^8 \).

The intron 1 and 22 inversion mutations 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 our interpretation of results may occur if information given is inaccurate or incomplete.

**Clinical Reference**


**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 \( F^8 \) 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)


**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Performed weekly; Varies

**Analytic Time**

14 days
Test Definition: F8INV
HA F8 Intron 1/22 Inversion, B

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

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<td>F8INV</td>
<td>HA F8 Intron 1/22 Inversion, B</td>
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