MAYO CLINIC LABORATORIES

Test Definition: F8INP

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

Overview

Useful For

Prenatal testing for hemophilia A when a mutation has not been identified in the family.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
CULFB	Fibroblast Culture for 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

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 the affected male or obligate carrier female prior to testing at-risk individuals.

Testing Algorithm

If amniotic fluid is received, amniotic fluid culture for genetic testing will be added and charged separately. If chorionic villus specimen is received, fibroblast culture for genetic testing will be added and charged separately. For any prenatal specimen that is received, maternal cell contamination studies will be added. A maternal whole blood sample is required to perform this test.

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

Specimen Type
Varies

Additional Testing Requirements
Due to the complexity of prenatal testing, consultation with the laboratory is required for all prenatal testing.
Prenatal specimens can be sent Monday through Thursday and **must be received by 5 p.m. CST on Friday** in order to be processed appropriately. All prenatal specimens must be accompanied by a maternal blood specimen. Order MATCC / Maternal Cell Contamination, Molecular Analysis on the maternal specimen.

Shipping Instructions
Advise Express Mail or equivalent if not on courier service

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
Results will be reported and also telephoned or faxed, if requested.

Submit only 1 of the following specimens:

Specimen Type: Amniotic fluid
Container/Tube: Amniotic fluid container
Specimen Volume: 5-10 mL
Collection Instructions:
1. Optimal timing for specimen collection is during 14 to 18 weeks of gestation, but specimens collected at other weeks of gestation are also accepted.
2. Discard the first 2 mL of amniotic fluid. If the culture will be performed in conjunction with chromosome analysis and alpha-fetoprotein, a total of approximately 25 mL to 30 mL will be needed for the combined studies.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated
Additional Information:
1. Place the tubes in a Styrofoam container (T329).

2. Fill remaining space with packing material.
3. Unavoidably, about 1% to 2% of mailed-in specimens are not viable.
4. Bloody specimens are undesirable.
5. If the specimen does not grow in culture, you will be notified within 7 days of receipt.

Specimen Type: Chorionic villi
Container/Tube: 15-mL tube containing 15 mL of transport media
Specimen Volume: 20-30 mg
Collection Instructions:
1. Collect specimen by the transabdominal or transcervical method.
2. Transfer the chorionic villi specimen to a Petri dish containing transport medium (T095).
3. Using a stereomicroscope and sterile forceps, assess the quality and quantity of the villi and remove any blood clots and maternal decidua.
Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient

Specimen Type: Confluent cultured cells
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks approximately 90% confluent
Collection Instructions: Submit confluent cultured cells from another laboratory
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated
Additional Information: There will be no culture charge.

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

Amniotic fluid: 10 mL
Chorionic Villi: 5 mg

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)	0 hours	

Clinical & Interpretive

Clinical Information

Hemophilia A (HA) is due to a deficiency of clotting factor VIII (FVIII). HA is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 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 of <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

Not applicable

Interpretation

An interpretive report will be provided.

Cautions

Obtaining a medical genetics or hematology (coagulation) consultation prior to ordering is advisable. Molecular genetic or hemophilia center consultation is available for all possible hemophilia A cases and is particularly indicated in complex cases or in situations in which the diagnosis is atypical or uncertain.

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

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

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. Oldenburg J, Rost S, El-Maarri O, et al: De novo factor VIII gene intron 22 inversion in a female carrier presents as a somatic mosaicism. Blood 2000;96(8):2905-2906
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)

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)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

28 to 35 days

Specimen Retention Time

Extracted DNA indefinitely, patient must opt-out.

Performing Laboratory Location

Rochester

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 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 US Food and Drug Administration.

CPT Code Information

81403

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
F8INP	HA F8 Int 1/22 Inversion, AF or CVS	82343-5

Result ID	Test Result Name	Result LOINC® Value
35161	HA F8 Int 1/22 Reason for Referral	42349-1
35162	HA F8 Int 1/22 Inversion, AF or CVS	82343-5
35163	F8INP Interpretation	69047-9
35164	HA F8 Intron 1/22 Reviewed By	18771-6