MAYO CLINIC LABORATORIES

Test Definition: F81P

Hemophilia A F8 Gene, Intron 1 Inversion
Known Mutation Analysis, Prenatal

Overview

Useful For

Prenatal testing for hemophilia A when a *F8* intron 1 inversion has been identified in a family member

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

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

Intron 1 inversion known variant analysis on a prenatal specimen can only be performed when there is a known intron 1 inversion in the family.

Testing Algorithm

If an amniotic fluid specimen is received, an amniotic fluid culture will be performed at an additional charge.

If a chorionic villi specimen is received, a fibroblast culture will be performed at an additional charge.

For any prenatal specimen that is received, maternal cell contamination testing 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
Varies

Ordering Guidance
This test should be ordered for prenatal specimens when an intron 1 inversion has previously been identified in the family. For whole blood or postnatal umbilical cord specimens, order F81B / Hemophilia A *F8* Gene, Intron 1 Inversion Known Mutation, Blood.

If a familial variant has not been identified in a severely affected hemophilia A patient, order F8INP / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, Prenatal.

Additional Testing Requirements
Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

All prenatal specimens must be accompanied by a maternal blood specimen. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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

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
Results will be reported and 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 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.
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.
6. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid.
7. All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi
Supplies: CVS Media (RPMI) and Small Dish (T095)
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.
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
Additional Information:
1. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing, Chorionic Villi/Products of Conception/Tissue. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.
2. All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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: All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

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

Amniotic fluid: See Specimen Required; 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		

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 on a prenatal specimen can only be performed when there is a known intron 1 inversion in the family.

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. 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 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 amniotic fluid or chorionic villi 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
Monday through Friday

Report Available
28 to 35 days

Specimen Retention Time
Extracted 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
F81P	HA F8 Intron 1 Inversion, AF or CVS	82342-7

Result ID	Test Result Name	Result LOINC® Value
35138	HA F8 Int1 KM Reason for Referral	42349-1
35004	HA F8 Intron 1 Inversion, AF or CVS	82342-7
35005	F81P Interpretation	69047-9
35006	HA F8 Int1 KM Reviewed By	18771-6