Test Definition: F8NGS

F8 Gene, Full Gene NGS

Overview

Useful For

Molecular confirmation of a clinical diagnosis of hemophilia A in affected male patients

Identification of the causative alteration in the *F8* gene for prognostic and genetic counseling purposes

Helping determine hemophilia A carrier status for female patients with a family history of hemophilia A

Molecular prenatal confirmation of hemophilia A

Genetics Test Information

This test detects pathogenic alterations within the *F8* gene to delineate the underlying molecular defect in a patient with a laboratory diagnosis of hemophilia A and for determining carrier status for female patients with a family history of hemophilia A. This test may also be used to prenatally identify and confirm hemophilia A in a male fetus at risk for inheriting the disease. Diagnostic prenatal testing for female fetuses is typically medically unnecessary and not performed except in very rare cases where the fetus is known to be at risk of inheriting a pathogenic *F8* alteration from both parents.

The gene target for this test is:

Gene name (transcript): *F8* (GRCh37 [hg19] NM_000132)

Chromosomal location: Xq28

Testing Algorithm

Prenatal genetic testing is not routinely performed without the prior identification of a familial hemophilia alteration in an affected male relative or a female relative who is a confirmed carrier of the alteration. Requests for this prenatal testing without a known familial alteration are performed at the discretion of a Molecular Hematopathology Laboratory Director.

For any cord blood or prenatal specimen that is received, maternal cell contamination studies will be added. A maternal whole blood sample is required to perform this test.

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.

The following algorithms are available in Special Instructions:

-Hemophilia Testing Algorithm (for testing affected male patients)

-Hemophilia Carrier Testing Algorithm (for female patients with a family history of hemophilia)

Special Instructions

- Informed Consent for Genetic Testing
- Hemophilia Carrier Testing Algorithm
- Hemophilia Testing Algorithm
- Hemophilia A Patient Information
- Informed Consent for Genetic Testing (Spanish)

Highlights

-This test identifies pathogenic alterations in the *F8* gene associated with hemophilia A.

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

-This test allows for the prenatal identification of pathogenic alterations in the *F8* gene associated with hemophilia A. Prenatal genetic testing is not routinely performed without the prior identification of a familial hemophilia alteration.

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

Method Name

Custom Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing when appropriate.

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Genetic testing for hemophilia A should only be considered in males if clinical and family history, initial coagulation screens, and FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma) indicate a diagnosis of hemophilia A. Causes of acquired (non-genetic) hemophilia A should be excluded prior to genetic testing.

Most patients with less than 1% factor VIII activity and severe hemophilia A have large rearrangements in the *F8* gene called inversions that are not detectable by this testing method. Full gene sequencing for a patient with severe hemophilia A should be performed only if genetic testing for these inversions is negative. See F8INV / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, Whole Blood.

Genetic testing for hemophilia A in females should only be considered if a first-degree male relative has been diagnosed with hemophilia A, if there is a maternal family history of hemophilia A and her mother has not been excluded as a carrier, or if the patient has abnormally low FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma).

For females with bleeding symptoms and no known personal or family history of hemophilia A, consider BDIAL / Bleeding Diathesis Profile, Limited or the specific factor assays.

Prenatal genetic testing should NOT be performed without the prior identification of a familial hemophilia alteration because diagnostic prenatal testing requires an invasive procedure that carries a small but real risk of inducing spontaneous abortion.

Additional Testing Requirements

Due to the complexity of testing non-peripheral blood, consultation with the laboratory is required for all cord blood samples. All cord blood specimens **must be accompanied** by a maternal blood specimen. Order this test on the cord blood specimen (only 1 sample tube required) and order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Shipping Instructions

Prenatal Specimens: Advise Express Mail or equivalent if not on courier service. Prenatal specimens can be sent Monday through Thursday and **must be received by 3 p.m. CST on Friday** in order to be processed appropriately.

Blood: Ambient and refrigerate specimens **must** arrive within 7 days and frozen specimens must arrive within 14 days.

Collect and package specimen as close to shipping time as possible.

Necessary Information

Hemophilia A Patient Information **(T712) 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

Submit only 1 of the following specimens:

Specimen Type: Peripheral blood or cord blood

Container/Tube:

Preferred: Lavender top (EDTA)

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

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

Specimen Stability Information: Ambient (preferred) 7 days/Refrigerated 7 days/Frozen 14 days

Due to the complexity of prenatal testing, consultation with the laboratory is required for all prenatal testing.

Specimen Type: Amniotic fluid

Supplies: Refrigerate/Ambient Shipping Box, 5 lb (T329)

Container/Tube: Amniotic fluid container

Specimen Volume: 10-20 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.

3. Place the tubes in a Styrofoam container.

4. Fill remaining space with packing material.

5. Unavoidably, about 1% to 2% of mailed-in specimens are not viable.

6. Bloody specimens are undesirable.

7. If the specimen does not grow in culture, you will be notified within 7 days of receipt.

Additional Information:

A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid

Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient <24 hours

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.

Additional Information:

A separate culture charge will be assessed under CULFB / Fibroblast Culture for Genetic Testing

Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient <24 hours

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

Additional Information: There will be no culture charge.

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Forms

1. **Hemophilia A Patient Information (T712) is required,** see Special Instructions.

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 in Special Instructions:

-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.

Reject Due To

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

Specimen Minimum Volume

Blood: 1 mL

Amniotic fluid: 10 mL

Chorionic villi: 20 mg

Confluent cultured cells: 2 full flasks

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)		

Clinical & Interpretive

Clinical Information

Hemophilia A (HA) is a bleeding diathesis that most commonly affects male individuals. Symptoms include soft tissue bleeding and articular hemorrhage, such as joint bleeds, deep muscle hematomas, intracranial bleeding, prolonged oozing after surgery, and unexplained gastrointestinal (GI) bleeding. In male patients with severe deficiency, spontaneous bleeding may occur. In individuals with mild HA, bleeding may occur only after surgery or trauma. In severe hemophilia, affected male patients typically present with these symptoms within the first 2 years of life. In moderate hemophilia, affected male patients will usually present in their toddler years. Mild hemophiliacs are typically diagnosed later in life and sometimes well into adulthood.

HA is an X-linked recessive disorder that affects approximately 1 in 10,000 live male births, among all ethnic populations. Female carriers of HA alterations have a 50% chance of passing on the alteration to each child they have. If the child is male, there is a 50% chance of him inheriting the alteration and being affected by HA. If the child is female, there is a 50% chance of her inheriting the alteration and being a carrier of HA. While female carriers generally do not have bleeding symptoms, they are at risk of having affected sons. However, not all women with an affected son are germline carriers of a *F8* alteration as de novo alterations in *F8* occur. Overall, there is a 2% to 20% chance of her not being a carrier of an alteration associated with HA, depending on the type of alteration in the son. Importantly, there is a small risk for recurrence even when the familial *F8* alteration is not identified in the mother of the affected patient due to the possibility of germline mosaicism. All of the daughters of a man with HA will inherit the disease-causing alteration. None of his sons will inherit the alteration or be affected by HA. Daughters of a man with hemophilia are considered obligate carriers because it is a virtual certainty that they carry the alteration by virtue of their biological relationship with their father. If a woman has a brother or maternal nephew who is affected with hemophilia and then has a son with hemophilia, she too is considered an obligate carrier.

HA is caused by a deficiency of clotting factor VIII (FVIII), an essential blood coagulation protein. Factor VIII increases the catalytic activity of factor IXa to convert factor X to Xa by more than 100,000-fold, propelling further steps in the coagulation cascade. FVIII is encoded by the factor VIII (*F8*) gene. Approximately 98% of patients with a diagnosis of HA are found to have an alteration in *F8*.

Hemophilia is classified according to bleeding severity, which correlates with FVIII activity levels. Severe HA is associated with FVIII activity levels of less than 1% in a male. Moderate HA is associated with 1% to 5% activity. Mild hemophilia is associated with 5% to 40% of factor VIII activity.

Affected male patients are diagnosed with hemophilia A on the basis of their FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma) and clinical evaluation, while obligate carrier female patients are identified by family history assessment. For affected male patients, genetic testing to identify the causative alteration is indicated if factor studies confirm an abnormally low FVIII clotting activity (less than 40%) and von Willebrand factor antigen testing is normal. In affected male patients, there is good correlation between genotype, FVIII plasma levels, and hemorrhagic risk.

Genetic testing for HA in women should only be considered if she has a first-degree male relative diagnosed with HA, if there is a maternal family history of hemophilia A and her mother has not been excluded as a carrier, or if she has abnormally low FVIII activity (F8A / Coagulation Factor VIII Activity Assay, Plasma). Carrier status in females is not excluded if the female patient has normal FVIII activity. In females, the wide range of normal factor FVIII activity in women precludes an accurate assessment of carrier status, thus making molecular testing essential in assessment of carrier status in women maternally related to males affected by HA. Carrier testing is made much easier and conclusive when the specific familial alteration has been identified in an affected male relative of obligate carrier.

For prenatal testing, a specific familial alteration should be known in order to perform prenatal testing on any male fetus at risk of inheriting a genetic alteration causing hemophilia from his mother. This is because diagnostic prenatal testing requires an invasive procedure (ie, amniocentesis or chorionic villi sampling) that carries a small but real risk of inducing spontaneous abortion. Thus, prior to any prenatal genetic testing, every effort should be made to 1) identify the familial alteration in an affected male relative or in an obligate carrier and 2) confirm the mother carries the alteration. This ensures an invasive procedure is not performed unnecessarily on a pregnancy that is not at risk for hemophilia and that the test results are informative and conclusive.

Causes of acquired (non-genetic) HA that should be excluded prior to genetic testing include heparin use, disorders associated with antibodies to clotting factors such as systemic lupus erythematosus or antiphospholipid syndrome, pregnancy or the postpartum period, rheumatic disease, tumors or hematologic malignancies, use of certain drugs (eg, penicillin, sulfamides, phenytoin, interferon, fludarabine).

Obtaining a medical genetics or hematology (coagulation) consultation prior to ordering is advised.

Reference Values

An interpretive report will be provided

Interpretation

An interpretive report will be provided.

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

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.

Cautions

Clinical:

Some individuals may have a mutation that is not identified by the methods performed. The absence of a variant, therefore, does not eliminate the possibility of hemophilia A (HA). This assay does not distinguish between germline and somatic alterations, particularly with variant allele frequencies (VAF) significantly lower than 50%. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Technical Limitations:

Next-generation sequencing (NGS) may not detect all types of genetic variants. Additionally, rare alterations may be present that could lead to false negative or positive results. Therefore, test results should be interpreted in the context of activity and antigen measurements, clinical findings, family history, and other laboratory data. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If multiple alterations are identified, NGS is not able to distinguish between alterations that are found in the same ("in cis") and alterations found on different alleles ("in trans"). This limitation may complicate diagnosis or classification and has implications for inheritance and genetic counseling. To resolve these cases, molecular results must be correlated with clinical history, activity and antigen measurements, and family studies.

Unless reported or predicted to cause disease, alterations found deep in the intron or alterations that do not result in an amino acid substitution are not reported. These and common polymorphisms identified for this patient are available upon request.

Reclassification of Variants Policy:

At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

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(11):1485-1499

6. Konkle BA, Huston H, Fletcher SN: Hemophilia A. In GeneReviews. Edited by MP Adam, HH Ardinger, RA Pagon et al. University of Washington, Seattle. Updated 2017 June 17. Accessed March 2020. Available at ncbi.nlm.nih.gov/books/NBK1404/

Performance

Method Description

Next-generation sequencing and/or PCR and Sanger sequencing are performed.

Regions of homology, high guanine-cytosine (GC)-rich content, and repetitive sequences may not provide accurate sequence. Therefore, all reported alterations detected by next-generation sequencing in these regions are confirmed by an independent reference method. However, this does not rule out the possibility of a false-negative result in these regions.

Sanger sequencing is used to confirm alterations detected by next-generation sequencing when appropriate.(Unpublished Mayo method)

PDF Report

No

Specimen Retention Time

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

81407

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
F8NGS	F8 Gene, Full Gene NGS	94234-2

Result ID	Reporting Name	LOINC®
113044	F8NGS Result	50397-9
113038	Alterations Detected	82939-0
113036	Interpretation	69047-9
113039	Additional Information	48767-8
113040	Method	85069-3
113041	Disclaimer	62364-5
113042	Panel Gene List	21673-9
113043	Reviewed By	18771-6