Overview

Useful For

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

Identification of the causative alteration in the F9 gene for prognostic and genetic counseling purposes

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

Molecular prenatal confirmation of hemophilia B

Genetics Test Information

This test detects pathogenic alterations within the F9 gene to delineate the underlying molecular defect in a patient with a laboratory diagnosis of Hemophilia B and for determining carrier status for females with a family history of hemophilia B. This test may also be used to prenatally identify and confirm hemophilia B in a male fetus at risk for inheriting the disease. Diagnostic prenatal genetic 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 F9 alteration from both parents.

The gene target for this test is:

Gene name (transcript): F9 (GRCh37 [g19] NM_000133)

Chromosomal location: Xq27.1

Highlights

This test identifies pathogenic alterations in the F9 gene associated with hemophilia B, hemophilia B Leyden, and other rare bleeding and clotting phenotypes.

This test allows for the prenatal identification of pathogenic alterations in the F9 gene associated with hemophilia B.

Prenatal genetic testing is not routinely performed without the prior identification of a familial hemophilia alteration.

Reflex Tests

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<th>Always Performed</th>
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<td>Amniotic Fluid Culture/Genetic Test</td>
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<td>CULFB</td>
<td>Fibroblast Culture for Genetic Test</td>
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<tr>
<td>MATCC</td>
<td>Maternal Cell Contamination, B</td>
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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 the 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**

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

**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

**Advisory Information**

Genetic testing for hemophilia B should only be considered in males if clinical and family history, initial coagulation screens, and factor IX (FIX) activity (F_9 / Coagulation Factor IX Activity Assay, Plasma) indicate a diagnosis of hemophilia B. Causes of acquired (non-genetic) hemophilia B should be excluded prior to genetic testing.

Genetic testing for hemophilia B in females should only be considered if a first-degree male relative has been diagnosed with hemophilia B, if there is a maternal family history of hemophilia B and her mother has not been excluded as a carrier, or if the patient has abnormally low FIX activity (F_9 / Coagulation Factor IX Activity Assay, Plasma).

For females with bleeding symptoms and no known personal or family history of hemophilia B, 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 nonperipheral 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, Blood on the maternal specimen.

Shipping Instructions
1. Prenatal Specimens: Advise Express Mail or equivalent if not on courier service.

2. 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.

3. Blood: Ambient and refrigerated specimens must arrive within 7 days, and frozen specimens must arrive within 14 days of collection.

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

Necessary Information
Hemophilia B 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: 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 Mailer, 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 (T329).

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 / Amniotic Fluid Culture for Genetic Testing

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
Test Definition: NGSF9
F9 Gene, Full Gene NGS

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. 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
Blood: 1 mL
Amniotic fluid: 10 mL
Chorionic villi: 20 mg
Confluent cultured cells: 2 full flasks

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability

Specimen Stability Information

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Clinical and Interpretive

Clinical Information
Hemophilia B is a bleeding diathesis that most commonly affects males. 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 males with severe deficiency, spontaneous bleeding may occur. Males affected by severe hemophilia B typically present with these symptoms within the first 2 years of life. In individuals with mild hemophilia B, bleeding may occur only after surgery or trauma.

Hemophilia B is an X-linked recessive disorder that affects 1 in 30,000 live male births across all ethnic groups. Female carriers of hemophilia B 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 hemophilia B. If the child is female, there is a 50% chance of her inheriting the alteration and being a carrier of hemophilia B. While most female carriers of hemophilia B do not have bleeding symptoms, they are at risk of having affected sons. However, not all females with an affected son and no other family history of the disease are germline carriers of a F9 alteration as de
novel alterations in $F9$ occur.

Importantly, there is a small risk for recurrence even when the familial $F9$ 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 hemophilia B will inherit the disease-causing alteration. None of his sons will inherit the alteration or be affected by hemophilia B. 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.

Hemophilia B is caused by a deficiency of clotting factor IX (FIX), an essential blood coagulation protein that is synthesized in the liver and circulates in plasma as an inactive zymogen. The main function of factor IX is to activate factor $X$ to $Xa$, initiating thrombin formation necessary for the creation of an insoluble hemostatic thrombus at the site of vascular injury to help stop bleeding. FIX is encoded by the factor IX ($F9$) gene. Alterations in the $F9$ gene that reduce the amount of circulating FIX or result in the impairment of its function lead to the inability to form a clot to stop bleeding at the site of injury. Additionally, there are several unusual FIX variants that cause atypical phenotypes, such as hemophilia B Leyden (associated with age-dependent bleeding severity), the c.109G>A; $p$.Ala37Thr alteration (which induces warfarin sensitivity), and FIX Padua ($p$.Arg384Leu; associated with thrombophilia).

Hemophilia B is classified according to bleeding severity, which correlates with FIX activity levels. Severe hemophilia B is associated with FIX activity levels of less than 1%. Moderate hemophilia B is associated with 1% to 5% activity. Mild hemophilia is associated with 5% to 40% of factor IX activity.

Affected males are diagnosed with hemophilia B on the bases of their FIX activity ($F_9 / \text{Coagulation Factor IX Activity Assay, Plasma}$) and clinical evaluation, while obligate carrier females are identified by family history assessment. For affected males, genetic testing to identify the causative mutation is indicated if factor studies confirm an abnormally low FIX clotting activity (less than 40%). In affected males, there is good correlation between genotype, FIX plasma levels, and hemorrhagic risk.

Genetic testing for hemophilia B in females should only be considered if she has a first-degree male relative diagnosed with hemophilia B, if there is a maternal family history of hemophilia B and her mother has not been excluded as a carrier, or if she has abnormally low FIX ($F_9 / \text{Coagulation Factor IX Activity Assay, Plasma}$). Carrier status in females is not excluded if the female patient has normal FIX activity. In females, the wide range of normal factor IX 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 hemophilia B. Carrier testing is made much easier 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 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) factor IX deficiency that should be excluded prior to genetic testing include vitamin K deficiency. Given that FIX is a vitamin K-dependent protein, all patients with mild to moderate reductions in FIX activity should have vitamin K deficiency excluded. In addition, healthy normal children have a lower FIX activity that reaches adult reference ranges at puberty. A handful of cases have been described that involve an acquired deficiency of factor IX (acquired hemophilia B) related to an autoimmune disorder.

Obtaining a medical genetics of hematology (coagulation) consultation prior to ordering is advisable.
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 mutation, therefore, does not eliminate the possibility of hemophilia B. 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 polymorphisms 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 allele (“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


Performance

Method Description

Next-generation sequencing and/or 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

Day(s) and Time(s) Test Performed

Performed weekly, Varies

Analytic Time

21 days

Maximum Laboratory Time

28 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.
Test Definition: NGSF9
F9 Gene, Full Gene NGS

- 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
81238

LOINC® Information

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