# Test Definition: GNHMB

Hemophilia B, F9 Gene, Next-Generation Sequencing, Varies

## Overview

### Useful For

Confirming a clinical diagnosis of hemophilia B in affected male patients with the identification of a disease-causing variant in the *F9* gene

Determining the disease-causing alteration within the *F9* gene to delineate the underlying molecular defect in a male patient with a laboratory diagnosis of hemophilia B

Identifying the causative alteration for prognostic and genetic counseling purposes

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

Prenatal testing for hemophilia B when a familial *F9* variant has been previously identified in a family member

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| _STR1 | Comp Analysis using STR (Bill only) | No, (Bill only) | No |
| _STR2 | Add'l comp analysis w/STR (Bill Only) | No, (Bill only) | No |
| CULFB | Fibroblast Culture for Genetic Test | Yes | No |
| CULAF | Amniotic Fluid Culture/Genetic Test | Yes | No |
| MATCC | Maternal Cell Contamination, B | Yes | No |

### Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *F9* gene associated with hemophilia B (also known as factor IX deficiency). See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for hemophilia B.

### Testing Algorithm

The clinical workup for hemophilia B in symptomatic male patients should begin with special coagulation testing for factor IX (FIX) activity. Genetic testing is indicated if FIX activity is less than 40% of normal (Note: reference range may vary depending on the locally established reference range) and von Willebrand factor antigen testing is normal. For more information see Hemophilia Testing Algorithm.

FIX clotting activity does not correlate as well with bleeding severity in female patients, and therefore is unreliable in the detection of female carriers of hemophilia B. Carrier status is determined by identification of a heterozygous disease-causing variant in *F9* by molecular genetic testing. For female patients with a suspected or confirmed diagnosis of hemophilia B in a family member, carrier testing is recommended as specified by the Hemophilia Carrier Testing Algorithm.(1-3)

Acquired (nongenetic) causes of hemophilia B that should be excluded prior to genetic testing include vitamin K deficiency autoimmune disorders, malignancy, and infections such as HIV and hepatitis B.

**For prenatal specimens only:**
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.

-If amniotic fluid (nonconfluent cultured cells) is received, amniotic fluid culture/genetic test will be added at an additional charge.
-If chorionic villus specimen (nonconfluent cultured cells) is received, fibroblast culture for genetic test will be added at an additional charge.

For any prenatal specimen that is received, maternal cell contamination testing will be performed at an additional charge.

### 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

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

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

## Ordering Guidance

For male patients, this test should only be considered if clinical and family history, initial coagulation screens, and/or initial activity tests indicate a diagnosis of hemophilia B. For female patients, this test should only be considered if there is a confirmed diagnosis of hemophilia B in a family member or the patient has abnormally low factor IX (FIX) activity.

This test does not measure FIX activity levels. For assessment of FIX activity, F_9 / Coagulation Factor IX Activity Assay, Plasma.

For individuals with bleeding symptoms and no known personal or family history of hemophilia B, consider ALBLD / Bleeding Diathesis Profile, Limited, Plasma or the specific factor assays.

If genetic testing for hereditary bleeding disorders using a larger panel is desired, both a 6-gene focused bleeding panel and a 25-gene comprehensive bleeding panel are available. For more information see GNBLF / Bleeding Disorders, Focused Gene Panel, Next-Generation Sequencing, Varies or GNBLC / Bleeding Disorders, Comprehensive Gene Panel, Next-Generation Sequencing, Varies.

Testing for the *F9* gene as part of a customized panel is available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for the *F9* gene. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

## Additional Testing Requirements

**All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen as this must be a different order number than the prenatal specimen.

## Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

## Necessary Information

**Hemophilia B Patient Information 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

**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.

**Submit only 1 of the following specimens:**

**Specimen Type:** Whole blood
**Container/Tube:**
**Preferred**: Lavender top (EDTA)

**Acceptable:** Yellow top (ACD)
**Specimen Volume:** 3 mL
**Collection Instructions:**
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
**Specimen Stability Information:** Ambient (preferred) 4 days/Refrigerated

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

**Specimen Type:** Amniotic fluid
**Container/Tube:** Amniotic fluid container
**Specimen Volume:** 20 mL
**Specimen Stability Information:** Refrigerated (preferred)/Ambient
**Additional information:**
1. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid.
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:** Chorionic villi
**Container/Tube:** 15-mL tube containing 15 mL of transport media
**Specimen Volume:** 20 mg
**Specimen Stability Information:** Refrigerated
**Additional Information:** 1. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing.
2. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

**Acceptable:**
**Specimen Type:** Confluent cultured cells
**Container/Tube:** T-25 flask
**Specimen Volume:** 2 Flasks
**Collection Instructions:** Submit confluent cultured cells from another laboratory.
**Specimen Stability Information:** Ambient (preferred)/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 B Patient Information (T518) 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 an Coagulation Test Request (T753) with the specimen.

### Specimen Minimum Volume
Blood: 1 mL; Amniotic fluid: 10 mL; Other specimen types: see Specimen Required

### 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 B (HB) is a hereditary bleeding disorder associated with germline variants in the *F9* gene. It is inherited in an X-linked recessive manner with variable expressivity and is estimated to affect 1 in 30,000 live male births.(2,3)

HB is characterized by a deficiency in clotting factor IX (FIX), a vitamin K–dependent enzyme essential for clot formation. Symptomatic male patients may experience mild to severe bleeding problems, including excessive bruising, prolonged epistaxis, post-operative bleeding, hemarthrosis, deep-muscle hematomas, and intracranial or gastrointestinal tract bleeding. Female carriers are not typically affected but some may experience increased bleeding tendencies, especially after medical procedures and surgery. Note that FIX activity may not correlate with the severity of symptoms in female patients.(2-7)

Acquired (nongenetic) hemophilia B is quite rare; most cases result from the development of autoantibodies toward FIX. Causes to exclude prior to genetic testing include vitamin K deficiency, autoimmune disorders, malignancies, and infections such as HIV and hepatitis B.(8)

The World Federation of Hemophilia provides guidelines regarding diagnosis, management, and laboratory testing for individuals with HB.(9)

### Reference Values
An interpretive report will be provided.

### Interpretation
All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(10) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

### Cautions
Clinical Correlations:

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the

classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(9) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

### Clinical Reference

1. Pruthi RK: Hemophilia: a practical approach to genetic testing. Mayo Clin Proc. 2005 Nov;80(11):1485-1499
2. Berntorp E, Fischer K, Hart DP: Haemophilia. Nat Rev Dis Primers. 2021 Jun 24;7(1):45
3. Konkle BA, Huston H, Fletcher SN: Hemophilia B. In: Adam MP, Everman DB, Mirzaa GM, et al. GeneReviews [Internet]. University of Washington, Seattle; 2000. Updated June 15, 2017. Accessed December 1, 2022. Available at www.ncbi.nlm.nih.gov/books/NBK1495/
4. Sidonio RF Jr, Malec L: Hemophilia B (factor IX deficiency). Hematol Oncol Clin North Am. 2021 Dec;35(6):1143-1155
5. Dolan G, Benson G, Duffy A, et al: Haemophilia B: Where are we now and what does the future hold? Blood Rev. 2018 Jan;32(1):52-60
6. Plug I, Mauser-Bunschoten EP, Brocker-Vriends AHJT, et al: Bleeding in carriers of hemophilia. Blood. 2006 Jul 1;108(1):52-56
7. Goodeve AC: Hemophilia B: molecular pathogenesis and mutation analysis. J Thromb Haemost. 2015 Jul;13(7):1184-1195
8. Alshaikhli A. Hemophilia B: In: Rokkam VR, ed. StatPearls [Internet]. StatPearls Publishing; 2021. Updated February 8, 2022. Accessed December 1, 2022. Available at https://www.statpearls.com/ArticleLibrary/viewarticle/22744/
9. Srivastava A, Santagostino E, Dougall A, et al: WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia. 2020 Aug;26 Suppl 6:1-158
10. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-424

## Performance

### Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *F9* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp, and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the *F9* gene.

There may be regions of the *F9* gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

The reference transcript for the *F9* gene is NM_000133.4. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

### PDF Report

Supplemental

### Day(s) Performed

Varies

### Report Available

28 to 42 days

### Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Amniotic fluid, cultured amniocytes, chorionic villi, cultured chorionic villi: 1 month

### 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

81238
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)
88235-Amniotic fluid culture (if appropriate)
81265-Maternal cell contamination (if appropriate)

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| GNHMB | F9 Gene, Full Gene NGS | 93811-8 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 619118 | Test Description | 62364-5 |
| 619119 | Specimen | 31208-2 |
| 619120 | Source | 31208-2 |
| 619121 | Result Summary | 50397-9 |
| 619122 | Result | 82939-0 |
| 619123 | Interpretation | 59465-5 |
| 619124 | Additional Results | 82939-0 |
| 619125 | Resources | 99622-3 |
| 619126 | Additional Information | 48767-8 |
| 619127 | Method | 85069-3 |
| 619128 | Genes Analyzed | 82939-0 |
| 619129 | Disclaimer | 62364-5 |
| 619130 | Released By | 18771-6 |