MAYO CLINIC LABORATORIES

Test Definition: GNBLF

Bleeding Disorders, Focused Gene Panel, Next-Generation Sequencing, Varies

Overview

Useful For

Evaluating hereditary bleeding in patients with a personal or family history suggestive of a hereditary bleeding disorder and initial laboratory testing results are suggestive for factors VII, VIII, IX, or XI deficiency, or a von Willebrand disease

Confirming a hereditary bleeding disorder diagnosis with the identification of a known or suspected disease-causing alteration in one or more of 6 genes associated with a variety of hereditary bleeding disorders

Determining the disease-causing alterations within one or more of these 6 genes to delineate the underlying molecular defect in a patient with a laboratory diagnosis of a bleeding disorder

Identifying the causative alteration for genetic counseling purposes

Prognosis and risk assessment based on the genotype-phenotype correlations

Carrier testing for close family members of an individual with a hereditary bleeding disorder diagnosis

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 6 genes associated with a variety of hereditary bleeding disorders: *F7*, *F8*, *F9*, *F11*, *GP1BA*, and *VWF*. See Targeted Genes and Methodology Details for Bleeding Disorders, Focused Gene Panel and 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 a variety of hereditary bleeding disorders.

Testing Algorithm

A systematic diagnosis through conventional coagulation testing is recommended prior to considering genetic testing for

any suspected bleeding disorder.

Genetic testing for a hereditary bleeding disorder is indicated if:
-Coagulation tests indicate a deficiency or functional abnormality (note these tests are best performed in medically stable patients who are not receiving particular anticoagulants)
-There is a clinical suspicion of a hereditary bleeding disorder due to family history or atypical clinical presentation
-Acquired causes of deficiencies associated with bleeding have been excluded (eg, multiple myeloma, liver disease, warfarin therapy, vitamin K deficiency, systemic amyloidosis, or inhibitors)

If the bleeding tendency is a concern, sets of clinical guidelines on testing for heritable bleeding disorders, both common and rare, are freely available.(1-5)

For prenatal specimens only:
Prenatal genetic testing is not routinely performed without the prior identification of familial alterations. 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
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Rare Coagulation Disorder Patient Information
- Targeted Genes and Methodology Details for Bleeding Disorders, Focused Gene Panel

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

Special coagulation testing for evaluating patients with bleeding or hypocoagulability states should be performed prior to genetic testing. For more information see ALBLD / Bleeding Diathesis Profile, Limited, Plasma.
This test is designed to evaluate factor (F) VII, FVIII, FIX, FXI, *GP1BA,* and von Willebrand disease bleeding disorders.

If testing for hereditary bleeding disorders using a larger panel is desired, a 25-gene bleeding panel is available; order GNBLC / Bleeding Disorders, Focused Gene Panel, Next-Generation Sequencing, Varies

This test is not designed to evaluate for a single common hereditary bleeding disorder, such as when an individual has a known family history of hemophilia A or B or von Willebrand disease, specifically. If testing for a particular common hereditary bleeding disorder is desired, single gene tests are available for the *F8*, *F9*, and *VWF* genes. See GNHMA / Hemophilia A, *F8* Gene, Next-Generation Sequencing, Varies; GNHMB / Hemophilia B, *F9* Gene, Next-Generation Sequencing, Varies; or GNVWD von Willebrand Disease, *VWF* and *GP1BA* Genes, Next-Generation Sequencing, Varies

This test does not evaluate for the presence of inversions in the *F8* gene that can cause hemophilia A. If testing for possible inversions in the *F8* gene is desired, order F8INV / Hemophilia A *F8* Gene, Intron 1 and 22 Inversion Mutation Analysis, Whole Blood

This test is not designed to evaluate for hereditary thrombosis disorders. If thrombosis is the indication for testing and testing for hereditary thrombosis disorders is desired, order GNTHR / Thrombosis Disorders, Comprehensive Gene Panel, Next-Generation Sequencing, Varies

This test is not designed to evaluate for inherited platelet disorders. If a platelet disorder is suspected and comprehensive testing for platelet disorders is desired, order GNPLT / Platelet Disorders, Comprehensive Gene Panel, Next-Generation Sequencing, Varies

Customization of this panel and single gene analysis for any gene present on this panel are 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 mutations testing) is available for the genes on this panel. 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

Rare Coagulation Disorder 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

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. 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.

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. **Rare Coagulation Disorder Patient Information (T824) 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

Congenital or acquired bleeding diatheses are caused by a wide variety of coagulation abnormalities. The clinical presentation of an underlying bleeding disorder may include epistaxis, easy bruising, ecchymoses, muscle hematomas, menorrhagia, post-operative bleeding, bleeding into joint spaces, mucosal tract bleeds, intracranial bleeding, or gastrointestinal bleeding.

Determination of a hereditary bleeding disorder contributing to bleeding events in an individual or family can be useful for prognosis and risk assessment. Identification of an alteration that is known or suspected to cause disease can also be useful for determining the risk of bleeding for family members.

This panel evaluates 6 genes associated with a variety of hereditary bleeding disorders, including factor VII deficiency, hemophilia A, hemophilia B, factor XI deficiency, platelet-type von Willebrand disease, and von Willebrand disease.

The risk for developing bleeding associated with these syndromes varies. For example, in symptomatic individuals with factor VII deficiency, the most common symptoms have been reported as mucocutaneous, soft tissue, and joint and gastrointestinal bleeding; while those in individuals with factor X deficiency were bleeding after surgery and trauma; additionally, heavy menstrual bleeding was reported as common in both disorders.(1) Several of the genes on this panel have established bleeding risk or expert group guidelines.(1-7)

Indications for testing include, but are not limited to:
-Individuals with a suspected bleeding disorder for which there is no specific coagulation assay readily available
-Individuals who are at risk for being a carrier of a bleeding disorder, especially those bleeding disorders where carrier status cannot be easily determined by available coagulation assays
-Individuals whose personal or family histories indicate coinheritance of multiple hereditary bleeding disorders

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(8) 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.(8) 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. Mumford AD, Ackroyd S, Alikhan R, et al: Guideline for the diagnosis and management of the rare coagulation disorders: a United Kingdom Haemophilia Centre Doctors' Organization guideline on behalf of the British Committee for Standards in Haematology. Brit J Haematol. 2014 Nov;167(3):304-326
2. Pruthi RK: Hemophilia: a practical approach to genetic testing. Mayo Clin Proc. 2005 Nov;80(11):1485-1499
3. 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. doi: 10.1111/hae.14046
4. Laffan MA, Lester W, O'Donnell JS, et al: The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology.

Brit J Haematol. 2014 Nov;167(4):453-465
5. James PD, Connell NT, Ameer B, et al, ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021 Jan 12;5(1):280-300
6. International Society on Thrombosis and Haemostasis: Bleeding Thrombotic and Platelet Disorder TIER1 genes. ISTH; 2018. Updated July 2022. Accessed October 6, 2022. Available at:www.isth.org/page/GinTh_GeneLists
7. Megy K, Downes K, Simeoni I, et al: Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. J Thromb Haemost. 2019 Aug;17(8):1253-1260
8. 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 genes analyzed, 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 deletion-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 genes analyzed.

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. See Targeted Genes and Methodology Details for Bleeding Disorders, Focused Gene Panel and Methodology Details for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.(Unpublished Mayo method)

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.

Genes analyzed: *F7*, *F8*, *F9*, *F11*, *GP1BA*, and *VWF*

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
81407
81408
81479
81479 (if appropriate for government payers)
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
GNBLF	Bleeding Focused Gene Panel, NGS	105331-3

Result ID	Test Result Name	Result LOINC® Value
619244	Test Description	62364-5
619245	Specimen	31208-2
619246	Source	31208-2
619247	Result Summary	50397-9
619248	Result	82939-0
619249	Interpretation	59465-5
619250	Additional Results	82939-0
619251	Resources	99622-3

619252	Additional Information	48767-8
619253	Method	85069-3
619254	Genes Analyzed	82939-0
619255	Disclaimer	62364-5
619256	Released By	18771-6