MAYO CLINIC LABORATORIES

Test Definition: AHUGP

Atypical Hemolytic Uremic Syndrome (aHUS)/Thrombotic Microangiopathy (TMA) /Complement 3 Glomerulopathy (C3G) Gene Panel, Varies

Overview

Useful For

Providing a genetic evaluation for patients with a personal or family history suggestive of atypical hemolytic uremic syndrome (aHUS), thrombotic microangiopathy (TMA), or complement 3 glomerulopathy (C3G)

Establishing a diagnosis of genetic aHUS, TMA, or C3G and, in some cases, allowing for appropriate management and surveillance for disease features based on the gene involved

Identifying variants in genes encoding complement alternate pathway components and specific coagulation pathway genes known to be associated with increased risk for aHUS, TMA, and C3G allowing for predictive testing of at-risk family members

Providing genetic information that may be considered when making treatment decisions, including duration of therapy and recurrence risk, as well as consideration of transplantation

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide, small deletion-insertion, and copy number variants in 15 genes associated with atypical hemolytic uremic syndrome (aHUS), thrombotic microangiopathy (TMA), and complement 3 glomerulopathy (C3G): *ADAMTS13, C3, C5*[Chr9(GRCh37):g.123759950-123759973 only], *CD46 (MCP), CFB, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, DGKE, MMACHC,* and *THBD*. See Targeted Genes and Methodology Details for Atypical Hemolytic Uremic Syndrome / Thrombotic Microangiopathy / Complement 3 Glomerulopathy Gene Panel in Method Description for additional details.

Identification of a pathogenic variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for aHUS, TMA, and C3G.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

- Hereditary Renal Genetic Testing Patient Information
- Targeted Genes and Methodology Details for Atypical Hemolytic Uremic Syndrome / Thrombotic Microangiopathy / Complement 3 GlomerulopathyGene Panel

Method Name
Sequence Capture and Amplicon-Based Next-Generation Sequencing (NGS)

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
Due to atypical hemolytic uremic syndrome genotype-phenotype complexity, targeted testing for familial variants will not be accepted without approval from the laboratory; call 800-533-1710 to discuss testing options with a genetic counselor.

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

Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

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

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant
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

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin. Tubes of culture media can be supplied upon request (Eagle's minimum essential medium with 1% penicillin and streptomycin).
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing, Tissue. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing, Tissue. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Hereditary Renal Genetic Testing Patient Information (T918)
3. If not ordering electronically, complete, print, and send a Renal Diagnostics Test Request (T830) with the specimen.

Specimen Minimum Volume

Blood: 1 mL; Skin biopsy or cultured fibroblasts: 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

Thrombotic microangiopathy (TMA) is a pathologic condition characterized by abnormalities in the walls of small blood vessels (arterioles and capillaries) that result in microvascular thrombosis. Typically, they feature microangiopathic hemolytic anemia (MAHA) and thrombocytopenia, but these features may not be apparent in kidney-limited disease. Laboratory findings may include anemia, thrombocytopenia, presence of schistocytes on peripheral smear, elevated lactate dehydrogenase, and elevated serum creatinine.(1,2) The main categories of TMA include complement-mediated thrombotic microangiopathy (CM-TMA; also known as atypical hemolytic syndrome [aHUS]), thrombotic thrombocytopenic purpura (TTP), Shiga toxin-mediated hemolytic uremic syndrome, and drug-induced TMA. Due to the overlapping clinical features, laboratory testing is useful in differentiated these disorders.(3)

CM-TMA (aHUS) is a well-recognized disease entity characterized by complement activation in the microvasculature. Abnormalities of the alternate pathway of complement, which may be inherited (genetic) or acquired, underlie both the sporadic and familial forms of the disease and are identified in approximately 60% of patients.(3,4) Unlike many other monogenic disorders of the immune system, multiple hits may be required for disease manifestation, which may include a trigger event (transplantation, pregnancy, malignant hypertension, autoimmune disorders, sepsis, malignancy, etc) and one or more contributing genetic variants or risk haplotypes in the alternate pathway complement genes.(3) Individuals with genetic CM-TMA (aHUS) may experience relapse even after complete recovery following the presenting episode.

TTP is a rare clinical entity but is important to diagnose properly since it is associated with very high mortality (90%) if untreated. Mortality can be reduced by early plasma exchange. Congenital TTP is due to genetic defects in the *ADAMTS13* gene, while acquired TTP is related to autoantibodies against ADAMTS13, which reduces function. While TTP was initially characterized by thrombocytopenia, MAHA, fluctuating neurological signs, kidney failure and fever, not all of these features may be present in the manifestation of the disease.(1,2)

The hereditary form of CM-TMA is characterized by the presence of disease-causing variants in one or more of the genes known to be associated with aHUS, irrespective of familial history, or when two or more members of the same family are affected by the disease at least 6 months apart and exposure to a common triggering infectious agent has been excluded.(3) A patient may have both genetic variants in the alternative complement pathway and autoantibodies. While genetic testing may be used during the diagnostic work-up, the presence of disease-causing variants may also alter recurrence risk and impact decisions related to continuation of anti-complement therapy after resolution of symptoms.

Complement 3 glomerulopathies (C3G) include dense deposit disease and C3 glomerulonephritis and are characterized by C3 deposition within the glomeruli. In these disorders, the activity of the C3 convertase is increased by C3 nephritic factors, which are antibodies that stabilize the convertase, or loss of complement regulator activity, which may be due to genetic variants, autoantibodies, or other immunoglobulins. C3G may be preceded by an upper respiratory tract infection in some cases. Patients typically have proteinuria or hematuria and may present with variable kidney impairment. In addition to medical therapy, patients may be treated with kidney transplantation; however, disease recurrence and graft loss may occur.

It is important to note that while TMA and C3G are associated with complement dysregulation, disease-causing variants

in these genes may also result in complement deficiency, which is associated with recurrent infections with encapsulated pathogens or connective tissue diseases with no evidence of aHUS/TMA.(5)

Two risk alleles associated with increased susceptibility to aHUS/TMA and variants in *C5* associated with poor response to anticomplement therapy are also included on this panel to aid in risk assessment:

-*CFH*-H3 Risk Haplotype: The variants that comprise this risk haplotype are common in the general population, but in the context of additional pathogenic genetic and environmental factors, the presence of this risk haplotype is associated with an increased risk for development or progression of atypical hemolytic uremic syndrome. (6)
-*MCP*/*CD46* Risk Haplotype: The variants that comprise this risk haplotype are common in the general population, but in the context of additional pathogenic genetic and environmental factors, the presence of this risk haplotype is associated with an increased risk for development or progression of atypical hemolytic uremic syndrome.6)
-*C5* Genotype: Two variants, p.Arg885His and p.Arg885Cys, have been associated with poor response to eculizumab.(7)

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), including hybrid alleles formed between *CFH* and *CFHR* genes, 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.

Genes may be added or removed based on updated clinical relevance. Refer to the Targeted Genes and Methodology Details for Atypical Hemolytic Uremic Syndrome / Thrombotic Microangiopathy / Complement 3 Glomerulopathy Gene Panel for the most up to date list of genes included in this test. 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:
At this time, 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.

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 are interpreted with caution and professional clinical judgement.

Rarely, incidental findings or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. George JN, Nester CM: Syndromes of thrombotic microangiopathy. N Engl J Med. 2014 Aug 14;371(7):1654-1666
2. Go RS, Winters JL, Leung N, et al: Thrombotic microangiopathy care pathway: A consensus statement for the Mayo Clinic Complement Alternative Pathway-Thrombotic Microangiopathy (CAP-TMA) Disease-Oriented Group. Mayo Clin Proc. 2016 Sep;91(9):1189-1211
3. Noris M, Bresin E, Mele C, Remuzzi G: Genetic atypical hemolytic-uremic syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 2007. Updated September 23, 2021. Accessed June 7, 2022. Available at www.ncbi.nlm.nih.gov/books/NBK1367/
4. Kavanagh D, Goodship TH. Atypical hemolytic uremic syndrome, genetic basis, and clinical manifestations. Hematology Am Soc Hematol Educ Program. 2011:15-20. doi: 10.1182/asheducation-2011.1.15
5. Picard C, Gaspar HB, Al-Herz W, et al: International Union of Immunological Societies: 2017 Primary Immunodeficiency Disease Committee report on inborn errors of immunity. J Clin Immunol. 2018 Jan;38(1):96-128
6. Bernabeu-Herrero ME, Jimenez-Alcazar M, Anter J, et al. Complement factor H, FHR-3 and FHR-1 variants associate in an extended haplotype conferring increased risk of atypical hemolytic uremic syndrome. Mol Immunol. 2015;67(2 Pt B):276-286. doi: 10.1016/j.molimm.2015.06.021
7. Nishimura J, Yamamoto M, Hayashi S, et al. Genetic variants in C5 and poor response to eculizumab. N Engl J Med. 2014 Feb 13;370(7):632-639 doi: 10.1056/NEJMoa1311084
8. Richards S, Aziz N, Bale S, et al: 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

Capture-based and amplicon-based next-generation sequencing (NGS) are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as 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 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 Atypical Hemolytic Uremic Syndrome / Thrombotic Microangiopathy / Complement 3 Glomerulopathy Gene Panel for details regarding the targeted genes

analyzed for each test and specific gene regions not routinely covered.(Unpublished Mayo method)

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

Genes analyzed: *ADAMTS13, C3, C5* [Chr9(GRCh37):g.123759950-123759973 only],*CD46 (MCP), CFB, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, DGKE, MMACHC, THBD*

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; Cultured fibroblasts: 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
81404
81479
81479 (if appropriate for government payers)

LOINC® Information

MAYO CLINIC LABORATORIES

Test Definition: AHUGP

Atypical Hemolytic Uremic Syndrome (aHUS)/Thrombotic Microangiopathy (TMA) /Complement 3 Glomerulopathy (C3G) Gene Panel, Varies

Test ID	Test Order Name	Order LOINC® Value
AHUGP	aHUS/TMA/C3G Gene Panel	99967-2

Result ID	Test Result Name	Result LOINC® Value
618017	Test Description	62364-5
618018	Specimen	31208-2
618019	Source	31208-2
618020	Result Summary	50397-9
618021	Result	82939-0
618022	Interpretation	69047-9
618023	Additional Results	82939-0
618024	Resources	99622-3
618025	Additional Information	48767-8
618026	Method	85069-3
618027	Genes Analyzed	48018-6
618028	Disclaimer	62364-5
618029	Released By	18771-6