Overview

Useful For
Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of complement-mediated HUS/atypical HUS (aHUS) or thrombotic microangiopathies (TMA)

Establishing a diagnosis 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 allowing for predictive testing of at-risk family members

Genetics Test Information
This test includes next-generation sequencing and supplemental Sanger sequencing to evaluate for the genes listed on the panel.

Highlights
This test uses next-generation sequencing to test for variants in the ADAMTS13, C3, CD46 (MCP), CFB, CFD, CFH, CFHR1, CFHR3, CFHR5, CFI, DGKE, PLG, and THBD genes.

This test uses Sanger sequencing to test for variants in certain exons of the following genes:

- **PLG** Exons 1, 4, and 19
- **CFH** Exons 20-22
- **CFHR1** Exons 4
- **CFHR3** Exons 4 and 5

Identification of a pathogenic variant may assist with prognosis, clinical management, familial screening, and genetic counseling.

Reflex Tests

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<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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<td>Fibroblast Culture</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>CRYOB</td>
<td>Cryopreserve for Biochem Studies</td>
<td>No</td>
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</table>

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture and cryopreservation testing 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](#)
Method Name
Custom Sequence Capture and Targeted Next-Generation Sequencing followed by Polymerase Chain Reaction (PCR) and Supplemental Sanger Sequencing

NY State Available
Yes

Specimen

Specimen Type
Varies

Advisory Information
Targeted testing for familial variants (also called site-specific or known mutation testing) is available for the genes on this panel. See:

-KVAR1 / Known Variant Analysis-1 Variant, Varies
-KVAR2 / Known Variant Analysis-2 Variants, Varies
-KVAR3 / Known Variant Analysis-3+ Variants, Varies

Call 800-533-1710 to confirm the appropriate test for targeted testing.

Necessary Information
1. Primary Immunodeficiencies Patient Information (T791) is required. See Special Instructions. Testing may proceed without the Patient Information, however, the information aids in providing a more thorough interpretation. Ordering physicians are strongly encouraged to fill out the form.

2. Include physician name and phone number with specimen.

Specimen Required
Submit only 1 of the following specimens:

Preferred:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.
Test Definition: AHUSP
Complement aHUS/TMA Gene Panel

**Specimen Stability Information:** Ambient (preferred) 4 days/Refrigerated 14 days

**Specimen Type:** Blood spot

**Supplies:** Card-Blood Spot Collection Filter Paper (T493)

**Container/Tube:**

- **Preferred:** Collection card (Whatman Protein Saver 903 Paper)
- **Acceptable:** Whatman FTA Classic paper, Ahlstrom 226 filter paper, or Blood Spot Collection Card (T493)

**Specimen Volume:** 2 to 5 blood spots on collection card

**Collection Instructions:**

1. An alternative blood collection option for a patient <1 year of age is finger stick.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.

**Specimen Stability Information:** Ambient (preferred)/Refrigerated

**Specimen Type:** Peripheral blood mononuclear cells (PBMCs)

**Container/Tube:** Cell pellet

**Collection Instructions:** Send as a suspension in freezing medium or cell pellet frozen on dry ice.

**Specimen Stability Information:** Frozen

**Specimen Type:** Cultured fibroblasts

**Container/Tube:** T-75 or T-25 flask

**Specimen Volume:** 1 Full T-75 or 2 full T-25 flasks

**Additional Information:** Indicate the tests to be performed on the fibroblast culture cells. A separate culture charge will be assessed under FIBR / Fibroblast Culture. An additional 4 weeks is required to culture fibroblasts before genetic testing can occur.

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

**Specimen Type:** Skin biopsy

**Supplies:** Fibroblast Biopsy Transport Media (T115)
**Test Definition: AHUSP**

**Complement aHUS/TMA Gene Panel**

**Container/Tube:** Sterile container with any standard cell culture media (e.g., 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 [T115]).

**Specimen Volume:** 4-mm punch

**Additional Information:** A separate culture charge will be assessed under FIBR / Fibroblast Culture. An additional 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Stability Information:** Refrigerated (preferred)/Ambient

**Specimen Type:** DNA

**Container/Tube:** 2 mL screw top tube

**Specimen Volume:** 100 mcL (microliters)

**Collection Instructions:**

1. The preferred volume is 100 mcL at a concentration of 250 ng/mcL

2. Include concentration and volume on tube.

**Specimen Stability Information:** Frozen (preferred) 1 year/Ambient/Refrigerated

**Forms**

1. **Primary Immunodeficiencies Patient Information (T791) 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)

**Specimen Minimum Volume**

Whole blood: 1 mL

**Reject Due To**

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<td>Lipemia</td>
<td>NA</td>
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<td>Icterus</td>
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<td>Other</td>
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**Specimen Stability Information**

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Document generated August 28, 2019 at 2:32am CDT
Complement-mediated hemolytic uremic syndrome, also known as atypical hemolytic uremic syndrome (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 at least two-thirds (approximately 60%) of patients. 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 1 or more contributing genetic variants or haplotypes in the alternate pathway complement genes. The overall prognosis is poor with most patients developing end-stage renal disease (ESRD) or permanent kidney injury within 1 year of diagnosis despite plasma exchange (PLEX/PEX) or plasma infusion (PI) therapy. Renal transplantation in most patients is also associated with a poor prognosis with loss of the allograft. Drugs targeting the complement pathway, notably Eculizumab, have achieved success in modulating clinical remission and there are a few reports of combined liver-kidney transplants for these patients. Newer therapies are also likely to emerge over time. Individuals with genetic aHUS frequently experience relapse even after complete recovery following the presenting episode. Complement-mediated HUS presents with clinical features that are nearly identical to thrombotic thrombocytopenic purpura (TTP) and Shiga-toxin HUS (ST-HUS), making laboratory differentiation essential. TTP is a rare clinical entity but is an important diagnosis as it is associated with very high mortality (90%) if untreated. Mortality can be reduced by early PLEX. 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, microangiopathic hemolytic anemia (MAHA), fluctuating neurological signs, renal failure and fever, the disease can present with only some of these features. The thrombotic microangiopathies (TMA) cover both aHUS and TTP and the clinical distinctions are not always clear-cut. Besides the thrombocytopenia, which is one of the key features of TMA, there is presence of schistocytes and highly increased levels of lactate dehydrogenase (LDH).

Complement-mediated HUS is considered genetic when 2 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, or when pathogenic variants are identified in 1 or more of the genes known to be associated with aHUS, irrespective of familial history. A patient may have both autoantibodies to complement alternate pathway proteins and genetic defects in these genes.

It is important to note that certain genetic defects in these genes, eg, complement C3 (C3), may be associated with a more classic immunodeficiency phenotype with recurrent infections with encapsulated pathogens and connective tissue diseases with no evidence of aHUS/TMA.

Table 1. Genes included in the Complement aHUS/TMA PID Gene Panel

<table>
<thead>
<tr>
<th>GENE SYMBOL (ALIAS)</th>
<th>PROTEIN</th>
<th>OMIM</th>
<th>INCIDENCE</th>
<th>INHERITANCE</th>
<th>PHENOTYPE DISORDER</th>
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<tbody>
<tr>
<td>ADAMTS13</td>
<td>A disintegrin and metalloprotease with</td>
<td>604134</td>
<td>Not available</td>
<td>AR</td>
<td>Familial thrombotic thrombocytopenic purpura</td>
</tr>
<tr>
<td></td>
<td>thrombospondin motifs 13 isoform 1 preproprotein</td>
<td></td>
<td></td>
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<tr>
<td>Genes</td>
<td>Description</td>
<td>Protein Name</td>
<td>Deficiency</td>
<td>Susceptibility</td>
<td></td>
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<tr>
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<tr>
<td><strong>C3</strong></td>
<td>Complement C3 proprotein</td>
<td>120700</td>
<td>Approximately 5% of aHUS</td>
<td>AD, AR</td>
<td>C3 deficiency (AR), susceptibility to aHUS (AD)</td>
</tr>
<tr>
<td><strong>CD46 (MCP)</strong></td>
<td>Membrane cofactor protein isoform 1 precursor</td>
<td>120920</td>
<td>Approximately 12% of aHUS</td>
<td>AD, AR</td>
<td>Susceptibility to aHUS 2</td>
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<tr>
<td><strong>CFB</strong></td>
<td>Complement factor B proprotein</td>
<td>138470</td>
<td>Rare</td>
<td>AD</td>
<td>Complement factor B deficiency, susceptibility to aHUS 4</td>
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<tr>
<td><strong>CFD</strong></td>
<td>Complement factor D isoform 1 proprotein</td>
<td>134350</td>
<td>Rare</td>
<td>AR</td>
<td>Complement factor D deficiency</td>
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<tr>
<td><strong>CFH</strong></td>
<td>Complement factor H isoform a precursor</td>
<td>134370</td>
<td>Approximately 30% of aHUS patients</td>
<td>AD, AR</td>
<td>Complement factor H deficiency, susceptibility to aHUS 1</td>
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<td><strong>CFHR1</strong></td>
<td>Complement factor H-related protein 1 precursor</td>
<td>134371</td>
<td>Rare</td>
<td>AD, AR</td>
<td>Susceptibility to aHUS</td>
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<tr>
<td><strong>CFHR3</strong></td>
<td>Complement factor H-related protein 3 isoform 1 precursor</td>
<td>605336</td>
<td>Rare</td>
<td>AD, AR</td>
<td>Susceptibility to aHUS</td>
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<td><strong>CFHR5</strong></td>
<td>Complement factor H-related protein 5 precursor</td>
<td>608593</td>
<td>3% of aHUS</td>
<td>AD</td>
<td>Nephropathy due to CFHR5 deficiency</td>
</tr>
<tr>
<td><strong>CFI</strong></td>
<td>Complement factor I isoform 2 proprotein</td>
<td>217030</td>
<td>4-10% of aHUS</td>
<td>AD, AR</td>
<td>Complement factor I deficiency (AR), susceptibility to aHUS (AD)</td>
</tr>
<tr>
<td><strong>DGKE</strong></td>
<td>Diacylglycerol kinase epsilon</td>
<td>601440</td>
<td>Rare</td>
<td>AR</td>
<td>Nephrotic syndrome Type 7, susceptibility to aHUS</td>
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<tr>
<td><strong>PLG</strong></td>
<td>Plasminogen isoform 1 precursor</td>
<td>173350</td>
<td>Rare</td>
<td>AR</td>
<td>Dysplasminogenemia, plasminogen deficiency Type I</td>
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<tr>
<td><strong>THBD</strong></td>
<td>Thrombomodulin precursor</td>
<td>188040</td>
<td>Approximately 3-5% of aHUS</td>
<td>AD</td>
<td>Thrombophilia due to thrombomodulin defect, susceptibility to aHUS</td>
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</table>
Reference Values

An interpretive report will be provided.

Interpretation

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.

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 predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Cautions

Clinical Correlations:

Some individuals who have involvement of one or more of the genes on the panel may have a variant that is not identified by the methods performed (e.g., promoter variants, deep intronic variants). The absence of a variant, therefore, does not eliminate the possibility of disease. Test results should be interpreted in context of clinical findings, family history, and other laboratory data (especially complement serological analyses). Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Note, several genes included on this panel (C3, CFB, CFH, CFHR1, CFHR3, and CFI) have high frequency (>1%) sequence variants or haplotypes that have been identified as protective alleles or susceptibility alleles for age-related macular degeneration (ARMD). High frequency variants in these genes (>1%) are not included on this report.

For predictive testing of asymptomatic individuals, it is often useful to first test an affected family member. Identification of a pathogenic variant in an affected individual allows for more informative testing of at-risk individuals.

Technical Limitations:

Next-generation sequencing may not detect all types of genetic variants. The variant detection software has lower detection efficiency for insertion/deletion variants as compared to single nucleotide variants. Therefore, small deletions and insertions greater than 8 nucleotides in length may not be detected by this test. Copy number variations (CNV) are not currently reported for any of the genes on this panel. Additionally, rare polymorphisms may be present that could lead to false-negative or false-positive results. In some cases, DNA variants of undetermined significance may be identified. If results do not match clinical findings, consider alternative methods for analyzing
these genes, such as Sanger sequencing or large deletion/duplication analysis. If the patient has had an allogeneic blood or bone marrow transplant or a recent (ie, <6 weeks from time of sample collection) heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants Policy:

At this time, it is not standard practice for the laboratory to systematically review likely deleterious alterations 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. Consultation with a healthcare provider, or team of healthcare providers, with expertise in genetics and primary immunodeficiencies, is recommended for interpretation of this result.

A list of benign and likely benign variants detected for this patient is available from the lab upon request.

Contact the laboratory if additional information is required regarding the transcript or human genome assembly used for the analysis of this patient's results.

Clinical Reference


Performance

Method Description

Next-generation sequencing (NGS) is performed using an Illumina instrument with paired-end reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with a bioinformatics software pipeline. Supplemental Sanger sequencing is performed in select regions including CFH (exons 21-22), CFHR1 (exon 4), CFHR3 (exons 4-5) and PLG (exons 1, 4, and 19). Additional Supplemental Sanger sequencing may be performed occasionally in regions where NGS is insufficient for data capture or not specific enough to correctly identify a variant.(Unpublished Mayo Method)

The following genes are evaluated in this multigene panel: ADAMTS13, C3, CD46 (MCP), CFB, CFD, CFH, CFHR1, CFHR3, CFHR5, CFI, DGKE, PLG, THBD.

PDF Report

No
Day(s) and Time(s) Test Performed
Varies

Analytic Time
4 weeks

Maximum Laboratory Time
8 weeks

Specimen Retention Time
Extracted DNA: 2 months

Performing Laboratory Location
Rochester

Fees and 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 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
81479

LOINC® Information

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