Genes Tested

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**Description:** This panel detects the most common genetic causes of atypical hemolytic uremic syndrome (aHUS).

Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia, thrombocytopenia and renal failure secondary to thrombotic microangiopathy (TMA). The most common form of HUS is associated with infection by Shiga toxin-producing E. coli (STEC-HUS, ~90% of cases). Atypical HUS (aHUS) is a rarer form (~10% of cases) of HUS, and is associated with genetic or acquired defects in the regulation of the alternative pathway of the complement system. Specific genetic susceptibility mutations may be identified in up to 60% of affected individuals. Age at onset of aHUS is variable and recurrence of the disease following initial recovery is common. Atypical HUS is a systemic disease, and while at least one-half of patients with aHUS develop permanent renal damage, 20% of patients have extrarenal symptoms that may involve the cardiovascular, gastrointestinal and central nervous systems.

Mutations in proteins that regulate the alternate complement pathway, as well as autoantibodies that neutralize the function of these proteins, are associated with aHUS. Loss of function mutations in complement regulatory proteins Factor H (CFH), Factor H-related 5 (CFHR5), Factor I (CFI), Membrane Cofactor Protein/CD46 (CD46), and Thrombomodulin (THBD), as well as gain of function mutations in alternative pathway components Factor B (CFB) and C3 (C3) have all been identified in patients with atypical HUS. Mutational analysis by sequencing of the ten known genes associated with aHUS (CFH, CFI, MCP/CD46, C3, CFB, THBD, CFHR1, CFHR3, CFHR5, and most recently identified DGKE), as well as deletion/duplication analysis to identify the common CFHR3-CFHR1 deletion provides a comprehensive assessment of defects in complement regulation. This informs not only the disease process as likely complement-mediated, but also provides therapeutic and prognostic information in patients with aHUS with regards to risk of progression to end stage renal disease, risk of relapse, and risk of recurrence in kidney transplant.

One, two and even three mutations have been reported in individuals with aHUS. Individuals with mutations in two or more genes (digenic inheritance), and uniparental disomy have also been reported. In most families (excepting those with DGKE mutations), susceptibility to aHUS is inherited as an autosomal dominant trait with reduced penetrance and variable expressivity. In general, the presence of a single mutation confers about a 50% risk of developing renal disease; other factors...
including additional complement gene variants, pregnancy, exposure to certain medications and severe infections may trigger aHUS in at-risk individuals. aHUS secondary to DGKE mutations is inherited as an autosomal recessive disorder with a 25% recurrence risk in siblings, and heterozygous carriers are at low risk of developing aHUS.

Indications:
- Identification of genetic risk factors in a patient with suspected atypical hemolytic uremic syndrome to aid in medical management
- Identification of at-risk individuals for future medical management
- Identification of risk status in family members who are potential kidney donors.

Specimen: At least 3 mLs whole blood in a lavender top (EDTA) tube. Label tube with patient’s name, birth date, and date of collection.

Testing Methodology:
This test is performed by enrichment of the exons, flanking intronic and untranslated regions (5’ and 3’) of the genes specified above (except for DGKE) using microdroplet PCR technology followed by next-generation sequencing with > 40 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing. For DGKE, testing is performed by PCR-based sequencing of the entire coding regions and intron/exon boundaries of the DGKE gene. The common CFHR3-CFHR1 deletion is detected by multiple ligation-dependent probe amplification (MLPA) analysis. Each gene on this panel may also be ordered individually as a Sanger sequencing-based test.

Sensitivity:
Clinical Sensitivity: This test detects 60-99% of the reported mutations in these genes. A single mutation in one of these genes confers approximately a 50% risk

* aHUS can still be made as a diagnosis with completely normal genetic testing because all contributing genetic factors are not yet known. Both patients with and without mutations seem to have the same response to eculizumab.
of developing aHUS, except for the CFHR3-CFHR1 deletion that is equally common in affected and non-affected individuals.

**Analytical Sensitivity:** The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes and small deletions and insertions (<10 bases) in the regions analyzed. MLPA accurately detects the common CFHR3-CFHR1 deletion, but neither the CFHR1-CFHR4 deletion nor the CFH-CFHR1 hybrid allele are detected by this test. Similarly, larger deletions, exonic deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in CFH and CF1 and will not be identified by this test. Mutations in regulatory regions or other untranslated regions are not detected by this test. Rare primer site variants may lead to erroneous results. Parental studies are sometimes necessary to determine the phase of identified sequence variants in order to determine clinical significance.

**Note:** Single gene sequencing is available for all genes in the panel.

**Turn-Around Time:**
- 42 days for NGS of the panel
- 28-84 days for analysis of any gene by sanger sequencing.

**Cost:** Please call 1-866-450-4198 for current pricing, or with any billing questions.

**CPT Codes:**
Atypical Hemolytic Uremic Syndrome (aHUS) Genetic Susceptibility Panel 81479x10
Single gene sequencing of any gene in this panel 81479

**Results:** Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for clinical management and additional testing, if warranted. Biochemical and functional test results will be incorporated, when available. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

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**Ancillary Testing:**
1. Thrombotic Microangiopathy (aHUS & TTP) Profile (for preliminary screening of suspected aHUS patients)
2. aHUS Complement Activation Panel (useful for confirming complement activation and for monitoring patients on eculizumab therapy)
3. ADAMTS13 biochemical, functional and genetic testing (to rule out TTP as an alternate cause of TMA).

Ancillary tests are available by calling 513-636-4530 or by visiting www.cincinnatichildrens.org/tma.

**Shipping Instructions**
Please enclose test requisition with sample. All information must be completed before sample can be processed. Place samples in Styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

**Ship to:**
Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

**References:***
Loirat, C., et al. (2011). Orphanet J Rare Dis, 6, 60.
Maga, T. K., et al. (2010). Hum Mutat, 31(6), E1445-1460