Atypical hemolytic uremic syndrome (aHUS) is a disease characterized by non-immune hemolytic anemia, thrombocytopenia, and organ dysfunction, especially renal impairment. Many cases of aHUS are caused by uncontrolled activation of the complement system; both familial and sporadic forms of aHUS can be caused by complement-related genetic variants.

Loss of function variants in genes encoding inhibitors of the complement system, including complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP/CD46), complement factor H-related proteins (CFHR1, CFHR3, CFHR4, CFHR5), thrombomodulin (THBD), and C4b binding protein (C4BP), as well as gain of function variants in genes encoding complement factor B (CFB) and complement component 3 (C3) are associated with aHUS. Variants in the gene encoding the epsilon isoform of diacyl glycerol kinase, DGKe, (DGKE) have also been associated with aHUS. Clinical guidelines suggest that patients presenting with symptomatic thrombotic microangiopathy (as occurs with aHUS) should also be tested for ADAMTS13 deficiency or ADAMTS13 sequence variants. An ADAMTS13 deficiency would be indicative of thrombotic thrombocytopenic purpura (TTP), and while TTP and aHUS overlap clinically, patients with an ADAMTS13 deficiency are managed differently than those with aHUS. The unrelated renal disorder, membranoproliferative glomerulonephritis type II [also known as dense deposit disease (DDD)], can be caused by variants in C3, CFH, CFHR5 and LMNA. This panel will detect pathogenic variants for DDD in these genes.

Short-term response to plasma therapy, outcome after kidney transplant, and overall prognosis have been shown to correlate with the gene affected, and individuals with aHUS have been reported to have single or multiple sequence variants associated with the disease. Identification of novel sequence variants in patients with aHUS signifies the importance of comprehensive testing for genetic causes of aHUS.

**REASONS FOR REFERRAL:**
- Identification of pathogenic genetic variants in aHUS patients.
- Identification of pathogenic genetic variants in DDD patients.
- Targeted testing of at-risk relatives for known familial variants.

**REFERENCE INTERVAL:**
No variants detected.
Sequence variants are reported using standard nomenclature.
Sequence variants are classified using current recommendations from ACMG.
METHOD:
This test sequences all coding regions and splice sites and many untranslated regions (intrinsic regions and promoter sites) known to be associated with aHUS or DDD within the following genes: CFH, CFI, MCP (CD46), THBD, C4BPA, C4BPB, CFB, C3, LMNA, DGKE, ADAMTS13, CFHR1, CFHR3, CFHR4, and CFHR5. Multiplex PCR-based amplification is used to enrich genomic DNA followed by next-generation sequencing (NGS) (MiSeq) with >50 fold coverage at every target base. Supplemental Sanger sequencing is used to provide data for areas with insufficient coverage. All pathogenic and likely pathogenic sequence variants identified by NGS are confirmed by Sanger sequencing, as are all reported variants of unknown clinical significance. Deletions and duplications in exons of CFH, CFHR1, CFHR3, and CFHR5, including the most common CFHR3-1 deletion, are detected by Multiplex Ligation Probe Amplification (MLPA).

LIMITATIONS:
This test detects all pathogenic aHUS variants reported in the literature to date in the genes listed above with the exception of CFHR4 copy number variants (<1% of cases). Copy number variants in the genes/exons not covered by MLPA will not be detected. A negative result does not exclude a genetic basis of the patient's disease as pathogenic genetic variants are identified in only 50-60% of aHUS cases and ~10% of DDD cases.

REPORTING OF RESULTS:
While this assay is designed to detect genetic variants related to aHUS and DDD, some of the genes tested are also associated with other clinical conditions, such as certain forms of cardiomyopathy, muscular dystrophy and macular degeneration. A comprehensive database of gene-phenotype relationships listed by gene name can be found at http://www.omim.org. Results are reported in accordance with ACMG next-generation sequencing standards.8

• All aHUS or DDD related variants predicted to be pathogenic, likely pathogenic and of unknown significance will be reported. Variants classified as likely benign or benign are not be reported but are available upon request.

• Incidental findings (variants related to conditions other than aHUS or DDD) will only be reported for highly penetrant variants known to cause Mendelian disorders.

RECOMMENDED FORMS:
Consent form: Federal regulations governing clinical laboratories do not require a completed consent document to accompany the sample; however, laws in individual states may require written informed consent for certain tests. For the benefit of providers and patients, a consent form is available on our website. Submission of an order for testing constitutes certification by the ordering provider that informed consent has been obtained from the patient as required by any applicable state or federal laws with respect to each test ordered.

Clinical History Form: It is recommended that ordering providers submit the requested phenotypic information to allow for optimal analysis and interpretation of testing results. A completed aHUS Clinical History Form can be submitted with the sample. For samples submitted without a clinical history form, our genetic counselor will attempt to contact the ordering provider to obtain relevant clinical history; testing will be accepted and processed without delay.
SPECIMEN REQUIREMENTS:
Fetal: 7-15ml Amniotic Fluid, Cultured Amniocytes (2x10^6 minimum), 5-10gm CVS or Two T25 flasks Cultured CVS.
Parental/Patient: 3-5 ml EDTA (lavender top) whole blood.

SHIPPING REQUIREMENTS:
Place the room temperature specimen and requisition in plastic bags, seal and insert in a Styrofoam container. Seal the Styrofoam container, place in a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. Address package to:

Client Services/Molecular Diagnostics Laboratory
BloodCenter of Wisconsin
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

TURNAROUND TIME: 28 days

CPT CODES: 81479

REFERENCES: