

# Atypical Hemolytic Uremic Syndrome (aHUS) Genetic Susceptibility Panel

<i>ADAMTS13</i>	<i>C3</i>	<i>C4BPA</i>	<i>CD46 (MCP)</i>
<i>CD59</i>	<i>CFB</i>	<i>CFH</i>	<i>CFHR1</i>
<i>CFHR2</i>	<i>CFHR3</i>	<i>CFHR4</i>	<i>CFHR5</i>
<i>CFI</i>	<i>DGKE</i>	<i>MMACHC</i>	<i>PLG</i>
<i>THBD</i>	<i>CFHR3-CFHR1</i> structural variants		
C5 (c.2653C>T(p.Arg885Cys) and c.2654G>A(p.Arg885His))			

## 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 is a rarer form (~10% of cases) of HUS, and is associated with genetic or acquired defects in the proteins that regulate the alternate complement pathway, as well as autoantibodies that neutralize the function of these proteins. Specific genetic susceptibility variants may be identified in up to 60% of symptomatic 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.

Loss of function variants in complement regulatory proteins CD46/ Membrane Cofactor Protein (*CD46*), Factor H (*CFH*), Factor H-related proteins (*CFHR1*, *CFHR2*, *CFHR3*, *CFHR4*, *CFHR5*), Factor I (*CFI*), Diacylglycerol kinase epsilon (*DGKE*) and Thrombomodulin (*THBD*), as well as gain of function variants in alternate pathway components Factor B (*CFB*) and C3 (*C3*) have all been implicated in patients

with aHUS. Genes associated with conditions clinically indistinguishable from aHUS *ADAMTS13* (Thrombotic Thrombocytopenic Purpura or TTP), *CD59* (CD59-mediated hemolytic anemia with or without immune-mediated polyneuropathy), *MMACHC* (Methylmalonic aciduria and homocystinuria, cb1C type) and *PLG* (Plasminogen deficiency) are also included in this panel. Additionally, *C4BPA* (associated with aHUS as a secondary modifier gene) and two reported variants in *C5* which are associated with poor response to Eculizumab (a medication used in the treatment of aHUS) are part of this panel. Sequence analysis of these eighteen genes as well as deletion/ duplication analysis of *CFHR3-CFHR1* for structural variants provide a comprehensive genetic assessment for patients suspected to have aHUS. This informs not only the disease process, 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 pathogenic variants have been reported in individuals with aHUS. Individuals with pathogenic variants in two or more genes (digenic inheritance), and uniparental disomy have also been reported. In most families (except in those with pathogenic variants in *DGKE*), susceptibility to aHUS is inherited as an autosomal dominant trait with reduced penetrance and variable expressivity. In general, the presence of a single pathogenic variant 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. Atypical HUS secondary to *DGKE* pathogenic variants is inherited as an autosomal recessive condition and heterozygous carriers are at low risk of developing aHUS. The genes *ADAMTS13*, *CD59*, *MMACHC* and *PLG* are also associated with autosomal recessive traits.

## Indications:

- Confirmation 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
- Evaluate response to eculizumab treatment based on reported C5 variants, c.2653C>T(p.Arg885Cys) and c.2654G>A(p.Arg885His).

## 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.

**Note:** For post-transplant patients, we prefer pre-transplant samples or post-transplant skin fibroblasts. Culturing of skin fibroblasts is done at an additional charge. For alternate sample types, please contact the laboratory.

## Testing Methodology:

**aHUS Panel by NGS:** This test is performed by enrichment of the coding exons, flanking intronic

and untranslated regions (5' and 3'), as well as known pathogenic variants (HGMD 2017.3) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >50X coverage at every target base. For C5, only two variants that are reported in association with poor Eculizumab response are reported. The common *CFHR3-CFHR1* deletion is detected by multiple ligation-dependent probe amplification (MLPA) analysis. All pathogenic and novel variants, as well as variants of unknown significance, as determined bioinformatically, are confirmed by Sanger sequencing.

## Gene Specific Sequencing/ Variant Specific Analysis:

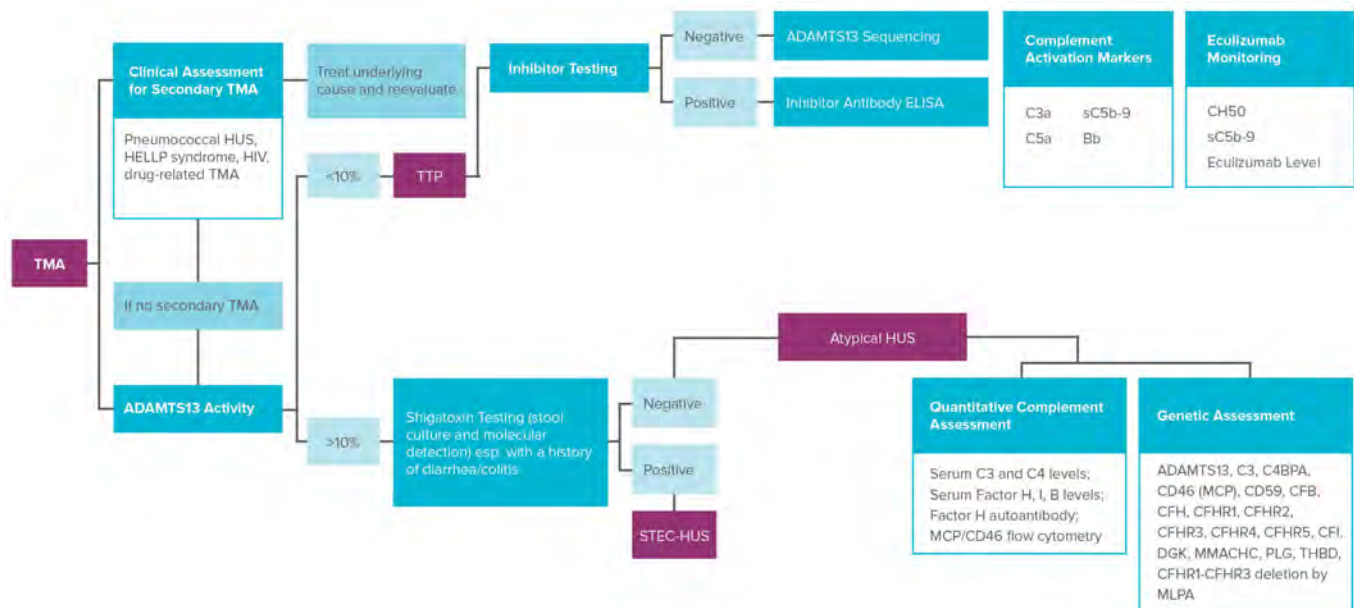
Sanger sequencing following PCR amplification of the specified coding and exon/ intron boundaries of the specified gene is performed.

## Sensitivity:

**Clinical Sensitivity:** This test detects pathogenic variants in 66-83% of genetic aHUS. Pathogenic variants in these genes typically confer an approximately 50% risk of developing aHUS.

**Analytical Sensitivity:** The sensitivity of DNA sequencing is over 99% for the detection of nucleotide

## Diagnostic Algorithm



\*Patients with a diagnosis of aHUS can have completely normal genetic testing because not all contributing genetic factors are known yet. Both patients with and without pathogenic variants in aHUS genes (except C5) seem to have the same response to Eculizumab.

base changes and small deletions and insertions (<10 bases) in the regions analyzed. MLPA accurately detects the common *CFHR3-CFHRI* deletion, but is not validated for other structural variants in the *CFHR* region. Also, parental studies may be sometimes necessary to determine the phase of identified variants in order to determine their clinical significance.

**Note:** Single gene sequencing is available for all genes in the panel. Deletion/ duplication analysis by targeted array comparative genomic hybridization (aCGH) is available for *CFHR2*, *CFHR4* and *CFHR5*.

### Turn-Around Time:

- 28–42 days for NGS of the panel
- Up to 28 days for analysis of any gene by Sanger sequencing.

### CPT Codes:

- **Atypical Hemolytic Uremic Syndrome (aHUS) Genetic Susceptibility Panel:** 81443
- **Single gene sequencing and targeted variant analysis:** Call for information
- **Deletion/Duplication analysis of *CFHR1/CFHR3* by MLPA:** 81403
- **Deletion/Duplication analysis for all other genes available:** call for information

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

### 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 Saturday.

### Ship to:

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

### Results:

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

### References:

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