Overview

Useful For
Detecting deficiencies in the alternative pathway that can cause atypical-hemolytic uremic syndrome, dense deposit disease, and C3 glomerulonephritis

A second-order test that aids in the differential diagnosis of thrombotic microangiopathies

Profile Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTGA</td>
<td>AHUS Interpretation</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>COM3</td>
<td>Complement, Total, S</td>
<td>Yes, (order COM)</td>
<td>Yes</td>
</tr>
<tr>
<td>AH503</td>
<td>Complement, Alternate Path, Func, S</td>
<td>Yes, (order AH50)</td>
<td>Yes</td>
</tr>
<tr>
<td>C3HUS</td>
<td>Complement C3, S</td>
<td>Yes, (order C3)</td>
<td>Yes</td>
</tr>
<tr>
<td>C4HUS</td>
<td>Complement C4, S</td>
<td>Yes, (order C4)</td>
<td>Yes</td>
</tr>
<tr>
<td>FBCA</td>
<td>Factor B Complement Antigen, S</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>FHCA</td>
<td>Factor H Complement Antigen, S</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C4D</td>
<td>C4d Complement, P</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>CBB</td>
<td>CBb Complement, P</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SC5B9</td>
<td>SC5b-9 Complement, P</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1QFX</td>
<td>C1Q Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C2FXN</td>
<td>C2 Complement, Functional, S, NR</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C3FX</td>
<td>C3 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C4FX</td>
<td>C4 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C5FX</td>
<td>C5 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C6FX</td>
<td>C6 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C7FX</td>
<td>C7 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
**Test Definition: AHUSD**
aHUS Complement Panel, S and P

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8FX</td>
<td>C8 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C9FX</td>
<td>C9 Complement, Functional, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C5AG2</td>
<td>C5 Complement, Antigen, S</td>
<td>Yes, (order C5AG)</td>
<td>No</td>
</tr>
</tbody>
</table>

**Method Name**
C3HUS, C4HUS, FBCA, FHCA, C5AG2: Nephelometry
COM3: Automated Liposome Lysis Assay
AH503, C4D, CBB, SC5B9: Enzyme-Linked Immunosorbent Assay (ELISA)

**NY State Available**
Yes

**Specimen**

**Specimen Type**
Plasma Na Cit
Serum Red

**Advisory Information**
For evaluating patients with possible thrombotic microangiopathies (TMA), the recommended first-order test is ADM13 / ADAMTS13 Activity and Inhibitor Profile. This test, AHUSD, should be a second-order test for TMA.

For patients who have received eculizumab or need to monitor response to eculizumab therapy, the recommended test is ECUMP / Eculizumab Monitoring Panel, Serum. Soluble membrane attack complex (sMAC) should not be used as a standalone assay to monitor eculizumab efficiency.

**Specimen Required**
Both plasma and serum are required for this test.

**Patient Preparation:**
1. Fasting preferred.
2. Samples should not be drawn earlier than 48 hours following plasma exchange.

**Specimen Type:** Plasma

**Collection Container/Tube:** Light-blue top (3.2% sodium citrate)

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 1.5 mL
Collection Instructions:

1. Immediately after specimen collection, place the tube on wet ice.
2. Centrifuge; 1,500 x g for 10 minutes at 4 degrees C, and separate plasma from cells.
3. Freeze specimen within 30 minutes.

Specimen Type: Serum

Collection Container/Tube: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 1.5 mL

Collection Instructions:

1. Immediately after specimen collection, place the tube on wet ice.
2. Centrifuge at 4 degrees C and separate serum from clot.
3. Freeze specimen within 30 minutes.

Forms
If not ordering electronically, complete, print, and send a Renal Diagnostics Test Request (T830) with the specimen.

Specimen Minimum Volume
Serum, Plasma: 1 mL each

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>OK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
</tr>
<tr>
<td>Gross icterus</td>
<td>OK</td>
</tr>
</tbody>
</table>

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na Cit</td>
<td>Frozen</td>
<td>14 days</td>
<td></td>
</tr>
<tr>
<td>Serum Red</td>
<td>Frozen</td>
<td>14 days</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and Interpretive

Clinical Information

Individuals presenting with thrombotic microangiopathies (TMAs) require clinical testing to identify the underlying cause. Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are both acute
syndromes with many overlapping clinical features. Reduced levels of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motives) activity is associated with TTP and is one laboratory feature that distinguishes TTP from HUS. HUS can also have a number of causes; one of the rarer forms of disease is caused by defects in the alternative pathway of the complement system, so called atypical-HUS (aHUS). Patients with defective alternative pathway regulation can benefit from biologics that suppress the complement system.

The purpose of this panel is to aid in the differential diagnosis of TMAs. The suggested approach is to rule-out other causes of TMAs first, since aHUS is one of the rarer causes of TMAs. Additionally, the assays can be used in the setting of membranoproliferative glomerulonephritis (MPGN), and help distinguish between immune-complex mediated or complement-mediated kidney disease. MPGN mediated by immune-complexes are the ones resulting from infectious processes, autoimmune diseases, or monoclonal gammopathies, whereas complement-mediated MPGN can then be subdivided in C3 glomerulonephritis (C3GN) and dense deposit disease (DDD), based on electron microscopy of the kidney biopsy histological findings. Despite phenotypic differences, these glomerular diseases share dysfunction of the alternative pathway as the defining pathophysiology.

**Reference Values**

**FACTOR B COMPLEMENT ANTIGEN**

15.2-42.3 mg/dL

**SC5b-9 COMPLEMENT**

< or =250 ng/mL

**FACTOR H COMPLEMENT ANTIGEN**

23.6-43.1 mg/dL

**C4d COMPLEMENT ACTIVATION FRAGMENT**

< or =9.8 mcg/mL

**CBb COMPLEMENT ACTIVATION FRAGMENT**

< or =1.6 mcg/mL

**COMPLEMENT C4**

14-40 mg/dL

**COMPLEMENT C3**

75-175 mg/dL

**COMPLEMENT, ALTERNATE PATHWAY (AH50), FUNCTIONAL**

> or =46% normal

**COMPLEMENT, TOTAL**

> or =16 years: 30-75 U/mL
Reference values have not been established for patients who are <16 years of age.

**Interpretation**
An interpretive report will be included.

**Cautions**
As with all complement assays, proper sample handling is of utmost importance to ensure that the complement system is not activated before clinical testing.

This (AHUSD) panel should be performed prior to treatment initiation, and in the absence of therapy with complement inhibitors, such as eculizumab. Complement inhibitors will affect performance of these assays.

**Clinical Reference**

**Performance**

**Method Description**

Complement, Total:
An automated method is performed using liposomes as the target for the serum complement system. The dinitrophenyl (DNP)-labeled liposomes are sensitized with antibody to DNP. Serum complement causes lysis and release of entrapped glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase reacts with glucose-6-phosphate and nicotinamide adenine dinucleotide (NAD). NAD is reduced to reduced nicotinamide adenine dinucleotide (NADH) and the conversion is measured at 340 nm. The assay correlates with the CH50 assay based on sheep RBC lysis, has lower variability, and is simpler to perform. (Package insert: Wako Autokit CH50. Wako Pure Chemical Industries, Ltd, 08/2007; Yamamoto S, Kubotsu K, Kida M, et al: Automated homogeneous liposome-based assay system for total complement activity. Clin Chem 1995;41:586-590)

Complement, Alternate Pathway (AH50), Functional:
For quantitation of alternative pathway function, immunoassay strips are coated with specific activators of the alternative pathway. Patient serum is diluted in diluent containing a specific blocker to ensure that only the alternative pathway is activated. During the incubation of the diluted patient serum in the wells, complement is activated by the specific coating generating a terminal complement complex (C5b-9, membrane attack complex: MAC). Strips are washed and the MAC is detected with a specific alkaline phosphatase-labeled antibody to the neoantigen expressed during MAC formation. After additional washing, color generation is accomplished by incubation with alkaline phosphatase substrate solution. The amount of complement activation correlates with the color intensity and is measured in terms of absorbance (optical density). (Nordin JG, Truebsson L, Sjoholm A: New procedure for detection of complement deficiency by ELISA, J Imm Methods 1993;166:655-668)

Complement C3:
C3 reagent is added to patient serum and quantitated on a Dade Behring BN II analyzer by fixed-time kinetic nephelometry. (Unpublished Mayo method; instruction manual: Siemens Nephelometer II, Version 3, 2008)
Complement C4:

C4 reagent is added to patient serum and quantitated on a Dade Behring BN II analyzer by fixed-time kinetic nephelometry.(Unpublished Mayo method; instruction manual: Siemens Nephelometer II, Version 3, 2008)

Factor B Complement Antigen:

Antifactor B (FB) reagent is added to patient serum and quantitated on a Dade Behring BN II analyzer by fixed-time kinetic nephelometry.(Unpublished Mayo method; instruction manual: Siemens Nephelometer II, Version 3, 2008)

Factor H Complement Antigen:

Antiproperdin factor H reagent is added to patient serum and quantitated on a Dade Behring BN II analyzer by fixed-time kinetic nephelometry.(Instruction manual: Siemens Nephelometer II, Version 3, 2008)

C4d Complement Activation Fragment:

Microtiter plates are coated with monoclonal antibody specific to the C4d fragment of the fourth component of the complement cascade. Controls, standards, and patient samples are exposed to the plate. After washing the plate, a horseradish peroxidase-conjugated polyclonal C4d antibody is added followed by a substrate to initiate color change.(Package insert: MicroVue C4d Fragment EIA Kit Quidel Corporation, San Diego, CA A5339H 03/2009)

CBb Complement Activation Fragment:

Microtiter plates are coated with monoclonal antibody specific to the complement factor Bb (CBb) fragment of the fourth component of the complement cascade. Controls, standards, and patient samples are exposed to the plate. After washing the plate, a horseradish peroxidase-conjugated polyclonal CBb antibody is added followed by a substrate to initiate color change.(Package insert: MicroVue CBb Plus EIA Kit Quidel Corporation, San Diego, CA 1307EN04 11/2011)

SC5b-9 Complement Activation Complex:

Microtiter plates are coated with monoclonal antibody specific to the C9 ring of the SC5b-9 complex. Controls, standards, and patient samples are exposed to the plate. After washing the plate, a horseradish peroxidase-conjugated anti-SC5b-9 complex antibody is added followed by a substrate to initiate color change.(Package insert: MicroVue SC5b-9 Plus EIA Kit Quidel Corporation, San Diego, CA 0980EN02 01/2009)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Varies

**Analytic Time**

12 days

**Maximum Laboratory Time**

21 days

**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
86160 x 7
86161
86162

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHUSD</td>
<td>aHUS Complement Panel, S and P</td>
<td>In Process</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>62584</td>
<td>C4d Complement, P</td>
<td>39565-7</td>
</tr>
<tr>
<td>62585</td>
<td>CBb Complement, P</td>
<td>4517-9</td>
</tr>
<tr>
<td>FBCA</td>
<td>Factor B Complement Antigen, S</td>
<td>2269-9</td>
</tr>
<tr>
<td>FHCA</td>
<td>Factor H Complement Antigen, S</td>
<td>4519-5</td>
</tr>
<tr>
<td>62586</td>
<td>SC5b-9 Complement, P</td>
<td>93244-2</td>
</tr>
<tr>
<td>38316</td>
<td>Complement, Alternate Path, Func, S</td>
<td>74520-8</td>
</tr>
<tr>
<td>COM3</td>
<td>Complement, Total, S</td>
<td>4532-8</td>
</tr>
<tr>
<td>C3HUS</td>
<td>Complement C3, S</td>
<td>4485-9</td>
</tr>
<tr>
<td>C4HUS</td>
<td>Complement C4, S</td>
<td>4498-2</td>
</tr>
<tr>
<td>39844</td>
<td>AHUS Interpretation</td>
<td>69048-7</td>
</tr>
<tr>
<td>ECPRO</td>
<td>Is patient receiving Eculizumab?</td>
<td>86955-2</td>
</tr>
</tbody>
</table>