Test Definition: AHUSD

Atypical Hemolytic Uremic Syndrome
Complement Panel, Serum and Plasma

Overview

Useful For

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

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

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
INTGA	AHUS Interpretation	No	Yes
COM3	Complement, Total, S	Yes, (order COM)	Yes
AH503	Alternative Complement Path Func, S	Yes, (order AH50)	Yes
C3HUS	Complement C3, S	Yes, (order C3)	Yes
C4HUS	Complement C4, S	Yes, (order C4)	Yes
FBCA	Factor B Complement Antigen, S	No	Yes
FHCA	Factor H Complement Antigen, S	No	Yes
CBB	CBb Complement, P	No	Yes
SC5B9	SC5b-9 Complement, P	Yes, (C5B9)	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
C1Q	Complement C1q, S	Yes	No
C1QFX	C1Q Complement, Functional, S	Yes	No
C2FXN	C2 Complement, Functional, S, NR	Yes	No
C3FX	C3 Complement, Functional, S	Yes	No
C4FX	C4 Complement, Functional, S	Yes	No
C5FX	C5 Complement, Functional, S	Yes	No
C6FX	C6 Complement, Functional, S	Yes	No
C7FX	C7 Complement,	Yes	No

	Functional, S		
C8FX	C8 Complement, Functional, S	Yes	No
C9FX	C9 Complement, Functional, S	Yes	No
C5AG2	C5 Complement, Antigen, S	Yes, (order C5AG)	No

Method Name

C3HUS, C4HUS, FBCA, FHCA; C5AG2: Nephelometry
COM3: Turbidimetric Measurement of Liposome Lysis
AH503, CBB, SC5B9: Enzyme-Linked Immunosorbent Assay (ELISA)
INTGA: Medical Interpretation

NY State Available

Yes

Specimen

Specimen Type

Plasma EDTA
Serum

Ordering Guidance

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

For evaluating patients with possible thrombotic microangiopathies (TMA), the recommended first-tier test is ADAMP / ADAMTS13 Activity with Reflex Inhibitor Profile, Plasma. This test should be a second-tier test for TMA.

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

For patients who have received ravulizumab or need to be monitored for response to ravulizumab therapy, the recommended test is RAVMP / Ravulizumab Monitoring Panel, Serum. sMAC testing should not be used as a standalone assay to monitor ravulizumab efficiency.

Specimen Required

Both serum and plasma are required for this test.

Patient Preparation:

1. Fasting: 12 hours, preferred but not required

2. Do **not** collect specimens for at least 48 hours following plasma exchange.
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)

Specimen Type: Serum
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1.5 mL total serum in **3 separate plastic vials**, each containing 0.5 mL serum
Collection Instructions:
1. Immediately after specimen collection, place the tube on wet ice and allow specimen to clot.
2. Centrifuge at 4 degrees C.
3. Aliquot serum into **3 separate plastic vials**, each containing 0.5 mL.
4. Within 30 minutes of centrifugation, freeze specimen. Specimen must be placed on dry ice if not frozen immediately.
Note: If a refrigerated centrifuge is not available, it is acceptable to use a room temperature centrifuge, provided the sample is kept on ice before centrifugation, and immediately afterward, the serum is aliquoted and frozen.

Specimen Type: Plasma
Collection Container/Tube:
Preferred: Lavender top (K2 EDTA)
Acceptable: Lavender top (K3 EDTA), light-blue top (sodium citrate)
Submission Container/Tube: Plastic vial
Specimen Volume: 1.5 mL total plasma in **2 separate plastic vials**, each containing 0.75 mL plasma
Collection Instructions:
1. Immediately after specimen collection, place the tube on wet ice.
2. Centrifuge between 1000 and 2000 x *g* for 10 minutes at 4 degrees C.
3. Aliquot plasma into **2 separate plastic vials**, each containing 0.75 mL.
4. Within 30 minutes of centrifugation, freeze specimen. Specimen must be placed on dry ice if not frozen immediately.
Note: If a refrigerated centrifuge is not available, it is acceptable to use a room temperature centrifuge, provided the sample is kept on ice before centrifugation, and immediately afterward, the plasma is aliquoted and frozen.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Renal Diagnostics Test Request (T830)
-Coagulation Test Request (T753)

Specimen Minimum Volume

Serum: 1.5 mL; Plasma: 1.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Plasma EDTA	Frozen	14 days	
Serum	Frozen	14 days	

Clinical & Interpretive

Clinical Information

Individuals presenting with thrombotic microangiopathies (TMA) 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, member 13) 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 TMA. The suggested approach is to rule-out other causes of TMA first, since aHUS is one of the rarer causes of TMA. Additionally, the assays can be used in the setting of membranoproliferative glomerulonephritis (MPGN) and can help distinguish between immune-complex mediated or complement-mediated kidney disease. MPGN mediated by immune-complexes are ones resulting from infectious processes, autoimmune diseases, or monoclonal gammopathies; whereas complement-mediated MPGN can be subdivided in C3 glomerulonephritis and dense deposit disease, 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
18.5 to 40.8 mg/dL

CBb COMPLEMENT ACTIVATION FRAGMENT
< or =1.6 mcg/mL

COMPLEMENT C4
14-40 mg/dL

COMPLEMENT C3
75-175 mg/dL

ALTERNATIVE COMPLEMENT, PATHWAY (AH50) FUNCTIONAL
> or =46% Normal

COMPLEMENT, TOTAL
> or =41 U/mL

Interpretation
Results from all associated testing performed as part of the atypical hemolytic uremic syndrome complement panel will be reviewed by a laboratory director and an interpretive report provided.

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

Clinical Reference
1. Daha MR. Role of complement in innate immunity and infections. Crit Rev Immunol. 2010;30(1):47-52. doi:10.1615/critrevimmunol.v30.i1.30
2. Prohaszka Z, Varga L, Fust G. The use of 'real-time' complement analysis to differentiate atypical haemolytic uraemic syndrome from other forms of thrombotic microangiopathies. Br J Haematol. 2012;158(3):424-425. doi:10.1111/j.1365-2141.2012.09168.x
3. Cataland SR, Holers VM, Geyer S, Yang S, Wu HM. Biomarkers of terminal complement activation confirm the diagnosis of aHUS and differentiate aHUS from TTP. Blood. 2014;123(24):3733-3738. doi:10.1182/blood-2013-12-547067
4. Go RS, Winters JL, Leung N, et al. Thrombotic microangiopathy care pathway: A consensus statement for the Mayo Clinic Complement Alternative Pathway-Thrombotic Microangiopathy (CAP-TMA) Disease-Oriented Group. Mayo Clin Proc. 2016;91(9):1189-1211. doi:10.1016/j.mayocp.2016.05.015
5. Willrich MAV, Andreguetto BD, Sridharan M, et al. The impact of eculizumab on routine complement assays. J Immunol Methods. 2018;460:63-71. doi:10.1016/j.jim.2018.06.010

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 NADH, which causes a measurable change in absorbance proportional to the complement activity in the sample. The assay correlates with the CH50 assay based on sheep red blood cell lysis, has lower variability, and is simpler to perform.(Package insert: Optilite CH50 Reagent, The Binding Site Group, Ltd.; INS095.OPTA, 08/2024; Yamamoto S, Kubotsu K, Kida M, et al. Automated homogeneous

liposome-based assay system for total complement activity. Clin Chem. 1995;41[4]:586-590)

Alternative Complement Pathway, Functional:
The Wieslab enzyme-linked immunosorbent assay complement assay for the alternative pathway combines principles of the hemolytic assay for complement activation with the use of labeled antibodies specific for neoantigens produced as a result of complement activation. The micro titer plate strips are coated with lipopolysaccharide. Patient serum is diluted in diluent containing specific blocker to ensure that only the alternative pathway is activated. During the first incubation, the diluted patient serum in the wells is activated by the coating. The wells are then washed and C5b-9 (membrane attack complex: MAC) is detected with a specific alkaline phosphatase labeled antibody to the neoantigen expressed during MAC formation. After a final wash, an alkaline phosphatase substrate is added. The amount of alternative pathway complement activity correlates with the color intensity of the solution and is measured in terms of absorbance (optical density).(Frazer-Abel A, Sepiashvili L, Mbughuni MM, Willrich MA. Overview of laboratory testing and clinical presentations of complement deficiencies and dysregulation. Adv Clin Chem. 2016;77:1-75. doi:10.1016/bs.acc.2016.06.001)

C3 and C4; C5, Factor B and Factor H Antigens:
In these Siemens Nephelometer II methods, the light scattered onto the antigen-antibody complexes is measured. The intensity of the measured scattered light is proportional to the amount of antigen-antibody complexes in the sample under certain conditions. If the antibody volume is kept constant, the signal behaves proportionally to the antigen volume.

A reference curve is generated by a standard with a known antigen content on which the scattered light signals of the samples can be evaluated and calculated as an antigen concentration. Antigen-antibody complexes are formed when a sample containing antigen and the corresponding antiserum are put into a cuvette. A light beam is generated with a light emitting diode, which is transmitted through the cuvette. The light is scattered onto the immuno-complexes that are present. Antigen and antibody are mixed in the initial measurement, but no complex is formed yet. An antigen-antibody complex is formed in the final measurement.
The result is calculated by subtracting value of the final measurement from the initial measurement. The distribution of intensity of the scattered light depends on the ratio of the particle size of the antigen-antibody complexes to the radiated wavelength.(Instruction manual: Nephelometer II Operations. Siemens, Inc; Version 2.3, 2008; Addendum to the Instruction Manual 2.3, 08/2017)

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; PIA027044EN00, 07/2022)

SC5b-9 Complement Activation Complex:
Microtiter plates are coated with monoclonal antibody specific to the C9 ring of the soluble C5b-9 (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; PIA020004EN00, 06/2022)

PDF Report
No

Day(s) Performed
Varies

Report Available
12 to 21 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
86160 x 6
86161
86162

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AHUSD	aHUS Complement Panel, S and P	34547-0

Result ID	Test Result Name	Result LOINC® Value
62585	CBb Complement, P	4517-9
FBCA	Factor B Complement Antigen, S	2269-9
FHCA	Factor H Complement Antigen, S	4519-5
62586	SC5b-9 Complement, P	93244-2
38316	Alternative Complement Path Func, S	74520-8
COM3	Complement, Total, S	4532-8

C3HUS	Complement C3, S	4485-9
C4HUS	Complement C4, S	4498-2
39844	AHUS Interpretation	69048-7
ECPRO	Is Eculizumab or Ravulizumab taken?	86955-2