

Overview

Useful For

Monitoring of complement blockage by ravulizumab

Investigation of suspected alternative pathway complement deficiency, atypical hemolytic uremic syndrome, C3 glomerulonephritis, dense-deposit disease

Highlights

Ravulizumab is a new complement C5 inhibitor therapeutic monoclonal antibody with a longer half-life than eculizumab. Monitoring complete complement blockade by eculizumab has allowed personalized therapy in specific settings. Similar action is expected with ravulizumab. Ravulizumab has 4 different amino acids from eculizumab, which allow greater affinity for the FcRn immunoglobulin receptor and change the affinity of the molecule for C5.

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

No

Specimen

Specimen Type

Serum Red

Ordering Guidance

To measure therapeutic concentrations of ravulizumab, order RAVU / Ravulizumab, Serum.

Specimen Required

Patient Preparation: Fasting preferred.

Supplies: Aliquot Tube, 5 mL (T465)

Collection Container/Tube: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 1 mL

Collection Instructions:

1. Immediately after specimen collection, place the tube on wet ice.
2. Centrifuge at 4 degrees C and aliquot serum into 5 mL plastic vial.
3. Freeze specimen within 30 minutes.

Forms

[If not ordering electronically, complete, print, and send a Coagulation Test Request \(T753\)](#) with the specimen.

Reject Due To

Gross hemolysis OK
Gross lipemia OK
Gross icterus OK

Specimen Minimum Volume

0.2 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum Red	Frozen (preferred)	14 days	

Clinical & Interpretive

Clinical Information

Ravulizumab (Ultomiris, Alexion Pharmaceuticals) is a humanized hybrid monoclonal antibody (IgG2/IgG4) that blocks complement C5 cleavage, thereby preventing the activation of the proinflammatory effects of C5a and the cytolytic effects of the membrane attack complex (MAC) formed by C5b-C9. It is FDA-approved for atypical hemolytic uremic syndrome,(1) and paroxysmal nocturnal hemoglobinuria.(2)

Compared to its predecessor eculizumab, ravulizumab is a longer-acting therapeutic monoclonal antibody. There are 4 amino acid changes in ravulizumab's heavy chain in comparison to eculizumab. These changes resulted in more affinity for the FcRn receptor which recycles immunoglobulins instead of degrading them, and changes in the variable region of the heavy chain made it possible for C5 to be released from ravulizumab molecule, so that C5 is left alone inside the endosome to be degraded. The dosing regimen for ravulizumab is weight-based, and after a loading dose schedule, the maintenance therapy requires administration intravenously every 8 weeks. Therapy efficacy may be monitored by

measuring efficiency of complement blockade. Ravulizumab will affect complement function assays that rely on the formation of the MAC to generate cell lysis. Validation studies performed by the Mayo Clinic show that the alternative pathway (AH50) enzyme-linked immunosorbent assay is the most helpful of the complement tests to monitor efficacy of the complement blockage by ravulizumab. Ravulizumab serum concentrations greater than 200 mcg/mL inhibited the AH50 activity completely, and 0% activity was detected at all subsequent tested concentrations up to 1000 mcg/mL.

Reference Values

> or =46% normal

Interpretation

In clinical trials for paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, therapeutic concentrations of ravulizumab were established as greater than 175 mcg/mL.

For the complement blockage monitoring of ravulizumab:

-When ravulizumab is present in serum at concentrations around 50 mcg/mL, the results range from 20% to 29% of normal.

-When ravulizumab concentrations are around 100 mcg/mL, the results range from 8% to 13% of normal.

-When ravulizumab concentrations are greater than 200 mcg/mL, the results are below the limit of quantitation of the assay (<10% of normal).

Cautions

This assay is a functional test and is dependent on correct sampling, storage, and shipping conditions. Both degradation by temperature and consumption of complement components will lead to false low function results. These are difficult to differentiate from real complement dysregulation.

While pre-analytic handling can lead to false positive results, it is far less likely that it would lead to false normal results. If more than one component is measured as low, it is important to look for technical errors.

Complement testing may be ordered in several circumstances where standard treatment includes plasmapheresis or plasma exchange. The procedure itself, if traumatic, may activate complement so may not reflect what is going on with the patient's complement system. The recommendation is to collect blood prior to the plasma exchange whenever possible.

Functional results inconsistent with the clinical history should be verified with a new blood draw.

Specimens should be frozen immediately after collection.

Long term stability is optimal when the sample is kept at -70 degrees Celsius or lower prior to testing.

Clinical Reference

1. 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
2. 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
3. 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.
4. Ardissino G, Tel F, Sgarbanti M, et al: Complement functional tests for monitoring eculizumab treatment in patients with atypical hemolytic uremic syndrome: an update. *Pediatr Nephrol.* 2018;33(3):457-461
5. Volokhina EB, van de Kar NC, Bergseth G, et al: Sensitive, reliable and easy-performed laboratory monitoring of eculizumab therapy in atypical hemolytic uremic syndrome. *Clin Immunol.* 2015 Oct;160(2):237-243
6. Sridharan M, Willrich MA, Go RS: Personalized Dosing of Eculizumab Using C5 Functional Activity and Eculizumab Level in Complement-mediated Thrombotic Microangiopathy: A Safe and Cost-saving Approach. XXVIII Congress of the International Society on Thrombosis and Haemostasis. Virtual ISTH 2020; July 12-14, 2020.
7. Cataland S, Ariceta G, Chen P, et al: Discordance between free C5 and CH50 complement assays in measuring complement C5 inhibition in patients with aHUS treated with ravulizumab. *Blood.* 2019;134(Supplement_1):1099

Performance

Method Description

The Wieslab enzyme-linked immunosorbent assay (ELISA) 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 microtiter 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).(Nordin JG, Truedsson L, Sjöholm A: New procedure for detection of complement deficiency by ELISA. Analysis of activation pathways and circumvention of rheumatoid factor influence. J Immunol Methods. 1993 Dec 3;166(2):263-270; 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)

PDF Report

No

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

Fees & Codes**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 US Food and Drug Administration.

CPT Code Information

86161

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
RAVUM	Ravulizumab Complement Blockage, S	74520-8

Result ID	Reporting Name	LOINC®
609500	Ravulizumab Complement Blockage, S	74520-8