

Overview

Useful For

Monitoring of complement blockage by ravulizumab

Assessing the response to ravulizumab therapy

Assessing the need for dose escalation

Evaluating the potential for dose deescalation or discontinuation of therapy in remission states

Monitoring patients who need to be above a certain ravulizumab concentration in order to improve the odds of a clinical response for therapy optimization

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
RAVU	Ravulizumab, S	Yes	Yes
RAVUM	Ravulizumab Complement Blockage, S	No	Yes
RAVIN	Ravulizumab Interpretation, S	No	Yes

Highlights

Ravulizumab is a therapeutic monoclonal antibody targeting complement C5 with a longer half-life than eculizumab. Monitoring the 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.

Therapeutic drug monitoring of ravulizumab may be useful when patients need to be above a certain target or therapeutic threshold of the monoclonal antibody concentration to improve odds of a clinical response for therapy optimization or potential dose deescalation or discontinuation of therapy in remission states.

Method Name

RAVUM: Enzyme-Linked Immunosorbent Assay (ELISA)

RAVU: Liquid Chromatography Tandem Mass Spectrometry, High Resolution Accurate Mass (LC-MS/MS HRAM)

NY State Available

Yes

Specimen

Specimen Type

Serum
Serum Red

Ordering Guidance

To measure only serum concentration of ravulizumab, order RAVU / Ravulizumab, Serum.

Specimen Required**Patient Preparation:**

1. Fasting preferred.
2. Suggest discontinuing natalizumab at least 4 weeks prior to testing for ravulizumab quantitation in serum. Patient should consult the healthcare provider who prescribed this drug to determine if discontinuation is an option. If not, ok to proceed with testing while taking natalizumab.

Supplies: Aliquot Tube, 5 mL (T914)

Collection Container/Tube: Red top (serum gel/SST are **not acceptable**)

Submission Container/Tube: 2 Plastic vials

Specimen Volume: 2 mL in 2 plastic vials, each vial containing 1 mL

Collection Instructions:

1. Draw blood immediately before next scheduled dose.
2. Immediately after specimen collection, place the tube on wet ice.
3. After sample has clotted on wet ice, centrifuge at 4 degrees C and aliquot serum into two 5 mL plastic vials.
4. Freeze specimen within 30 minutes of centrifugation. Sample must be placed on dry ice if not frozen immediately.

Specimen Minimum Volume

1 mL in 2 plastic vials, each vial containing 0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Frozen	14 days	
Serum Red	Frozen	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.

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 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 undetectable activity was measured at all subsequent tested concentrations up to 1000 mcg/mL.(1)

Some patients whose serum concentrations persist above therapeutic targets with complete complement blockade could benefit from dose deescalation or prolonged infusion intervals. Therapeutic drug monitoring of ravulizumab could result in cost-savings and improved quality of life if target therapeutic concentrations can be achieved with complete complement system blockage at less frequent dosing intervals.

Reference Values

RAVULIZUMAB COMPLEMENT BLOCKAGE:

> or =46% normal

RAVULIZUMAB:

Lower limit of quantitation =5.0 mcg/mL

>175 mcg/mL: Therapeutic concentration for paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome

Interpretation

Target trough therapeutic concentrations (immediately before next infusion) of ravulizumab are expected to be above 175 mcg/mL for paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Pharmacodynamic studies of complement blockage may also be recommended for patients undergoing therapy.

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 below 10% 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

The complement blockage 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 falsely low function results. These are difficult to differentiate from real complement dysregulation or blockage, and in the event of poor preanalytical handling, ravulizumab concentrations are a more reliable indicator, as they are not subject to stringent temperature stability.

While preanalytic handling can lead to falsely low results, it is far less likely that it would lead to false normal results.

Complement testing may be ordered in several circumstances where standard treatment includes plasmapheresis or

plasma exchange. The procedure itself, if traumatic, may activate complement and, therefore, may not be a true reflection of 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.

Results must be interpreted within the clinical context of the patient.

Patients in transition between eculizumab (ECULI / Eculizumab, Serum) and ravulizumab administration will have a result that is the sum of eculizumab plus ravulizumab in circulation. This assay will not clearly differentiate between these specific analytes and must be interpreted with caution.

Patients actively undergoing therapy with both natalizumab and ravulizumab (extremely rare scenario) could present with an assay interference. It is suggested patients discuss with their doctors the possibility of discontinuing natalizumab 4 weeks prior to testing. If discontinuation is not possible, it is ok to proceed with testing.

This test should not form the sole basis for a diagnosis or treatment decisions.

Clinical Reference

1. Willrich MAV, Ladwig PM, Martinez MA, et al. Monitoring ravulizumab effect on complement assays. *J Immunol Methods*. 2021;490:112944. doi:10.1016/j.jim.2020.112944
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. 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
4. 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;160(2):237-243
5. 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
6. Ladwig PM, Barnidge DR, Willrich MA. Quantification of the IgG2/4 kappa monoclonal therapeutic eculizumab from serum using isotype specific affinity purification and microflow LC-ESI-Q-TOF Mass Spectrometry. *J Am Soc Mass Spectrom*. 2017;28(5):811-817. doi: 10.1007/s13361-016-1566-y
7. Kulasekararaj AG, Hill A, Rottinghaus ST, et al. Ravulizumab (ALXN1210) vs eculizumab in C5-inhibitor-experienced adult patients with PNH: the 302 study. *Blood*. 2019;133(6):540-549. doi:10.1182/blood-2018-09-876805
8. Stern RM, Connell NT: Ravulizumab. a novel C5 inhibitor for the treatment of paroxysmal nocturnal hemoglobinuria. *Ther Adv Hematol*. 2019;10:2040620719874728. doi:10.1177/2040620719874728
9. Alexion Pharmaceuticals. BLA 761108-S1 Multi-disciplinary review and evaluation: Ultomiris (ravulizumab-cwvz). FDA; April 2, 2019. Accessed August 7, 2023. Available at www.fda.gov/media/135113/download
10. Vu, T, Meisel, A, Mantegazza, R, et al. Terminal complement inhibitor ravulizumab in generalized myasthenia gravis. *N Engl J Med Evid*. 2022;1(5):1-12. doi:10.1056/EVIDoa2100066

11. Sridharan M, Go RS, Willrich MAV. Clinical utility and potential cost savings of pharmacologic monitoring of eculizumab for complement-mediated thrombotic microangiopathy. *Mayo Clin Proc Innov Qual Outcomes*. 2022;6(5):458-464. doi:10.1016/j.mayocpiqo.2022.03.005

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;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)

Ravulizumab concentration is extracted from serum and measured by liquid chromatography high-resolution accurate-mass mass spectrometry. (Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Varies

Report Available

3 to 10 days

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

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

80299

86161

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RAVMP	Ravulizumab Monitoring Panel, S	101923-1

Result ID	Test Result Name	Result LOINC® Value
609500	Ravulizumab Complement Blockage, S	74520-8
609420	Ravulizumab, S	97184-6
619952	Ravulizumab Interpretation	59462-2