MAYO CLINIC LABORATORIES

Test Definition: RAVMP

Ravulizumab Monitoring Panel, Serum

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

RAVU: Liquid Chromatography Tandem Mass Spectrometry, High Resolution Accurate Mass (LC-MS/MS HRAM)
RAVUM: Enzyme-Linked Immunosorbent Assay (ELISA)
RAVIN: Technical Interpretation

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: 8 hours, preferred but not required
2. Suggest discontinuing natalizumab at least 4 weeks before specimen collection for ravulizumab testing. Patient should consult the healthcare professional who prescribed this drug to determine if discontinuation is an option. If not, it is okay 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 Serum 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 and allow specimen to clot.
3. Centrifuge at 4 degrees C and aliquot serum into two 5 mL plastic vials.
4. Freeze serum within 30 minutes of centrifugation. Serum 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.

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

Specimen Minimum Volume
Serum: 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:
Lower limit of quantitation =5.0 mcg/mL
>175 mcg/mL: Therapeutic concentration for paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome

RAVULIZUMAB COMPLEMENT BLOCKAGE:
> or =46% Normal

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 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 okay to proceed with testing.

Clinical Reference

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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

Monoclonal immunoglobulin rapid accurate mass measurement (miRAMM) is used to quantify intact light chains from the therapeutic monoclonal antibodies (mAb) in human serum. Briefly, IgG4 along with IgG4 monoclonal or therapeutics are extracted from patient sample using a human IgG4 affinity matrix that contains a 12-kDa llama antibody fragment recognizing human IgG4. Wash steps significantly reduce background and remove all non-IgG4s as well as other proteins from the sample. After elution, the mixture undergoes a reduction step to release the light chains from the heavy chains by reducing the disulfide bonds that keep them together. While full scan data is collected, targeted selected ion monitoring occurs for the +10, 11, and 12 charge states for the eculizumab/ravulizumab light chain along with the +11-charge state for natalizumab, the surrogate internal standard. Multiple isotopes of each charge state are combined to be used for quantitation. A standard curve of the pharmaceutical mAb spiked into normal human serum is used for quantitation.(Unpublished Mayo method)

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, Sjoholm 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)

PDF Report

No

Day(s) Performed

Varies

Report Available
3 to 10 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
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