Test Definition: ECMP

Eculizumab Monitoring Panel, Serum

Overview

Useful For

Monitoring of complement blockage by eculizumab

Assessing the response to eculizumab therapy

Assessing the need for dose escalation

Evaluating the potential for dose de-escalation or discontinuation of therapy in remission states

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

This test is **not useful** as the sole basis for a diagnosis or treatment decisions.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
ECULI	Eculizumab, S	Yes	Yes
EAH50	Eculizumab Complement Blockage, S	No	Yes
ECUIN	Eculizumab Interpretation, S	No	Yes

Highlights

Monitoring complete complement blockade by eculizumab has allowed personalized therapy in specific settings.

Therapeutic drug monitoring of eculizumab is helpful when providers are considering personalized treatment decisions such as therapy discontinuation or extending dose intervals when patients are in remission states. In paroxysmal nocturnal hemoglobinuria, a minimum therapeutic concentration is expected to be above 35 mcg/mL, and in atypical hemolytic uremic syndrome, the therapeutic concentrations are expected to be above 50 to 100 mcg/mL of eculizumab. Complement blockage studies can aid in determining that a therapeutic concentration of the drug has blocked the complement function and subsequent production of sC5b-9. Here we offer a panel of eculizumab concentration plus alternative pathway function (AH50) to monitor eculizumab therapy efficacy.

Method Name

EAH50: Enzyme-Linked Immunosorbent Assay (ELISA)
ECULI: Liquid Chromatography Tandem Mass Spectrometry, High Resolution Accurate Mass (LC-MS/MS HRAM)
ECUIN: Technical Interpretation

NY State Available

Yes

Specimen

Specimen Type
Serum
Serum Red

Ordering Guidance
To measure only the serum concentration of eculizumab, order ECULI / Eculizumab, Serum.

Specimen Required
Patient Preparation:
1. Fasting: 8 hours, preferred but not required
2. Suggest discontinuing natalizumab at least 4 weeks prior to testing for eculizumab 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: Sarstedt 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.
3. After specimen has clotted on wet ice, 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 1 of the following forms with the specimen:
-Coagulation Test Request (T753)
-Renal Diagnostics Test Request (T830)

Specimen Minimum Volume
Serum: 1 mL total 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

Eculizumab (Soliris, Alexion Pharmaceuticals), a humanized monoclonal IgG2/4 kappa antibody therapeutic directed against complement component C5, has been heralded as a breakthrough treatment for paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). More recently, eculizumab has been approved to treat aquaporin-4 (AQP4) IgG positive neuromyelitis optica spectrum disorder and generalized myasthenia gravis. By association with C5, eculizumab inhibits the terminal complement pathway through simultaneous blockade of the generation of the potent prothrombotic and proinflammatory molecule, C5a, and the formation of membrane attack complex initiator, C5b.

Eculizumab is administered as an intravenous infusion and the dosing regimen prescribed for an average adult diagnosed with PNH is 600 mg weekly for the first 4 weeks, followed by 900 mg for the fifth dose 1 week later, then 900 mg every 2 weeks thereafter. Eculizumab has been evaluated in aHUS patients through 2 prospective, open-label, single-arm studies (C08-002 and C08-003) as well as a single-arm retrospective study. In aHUS, it is prescribed for an average adult at 900 mg weekly for the first 4 weeks, followed by 1200 mg for the fifth dose 1 week later, then 1200 mg every 2 weeks thereafter. Eculizumab was generally well tolerated, and no significant adverse effects were attributed to drug treatment; some adverse reactions included upper respiratory tract infections and diarrhea in prospective and retrospective studies, hypertension, headache, and leucopenia (C08-002/C08-003), and fever (C09-001R). Additional case reports suggest that eculizumab may prevent post-transplantation recurrence of aHUS, even in those patients harboring CFH/CFHR1 hybrid gene variants who are at very high risk of recurrence. Further research is needed to determine the duration of eculizumab therapy in the context of the genetic background of aHUS cases and risk of disease relapse.

Therapeutic drug monitoring of eculizumab is helpful when providers are considering personalized treatment decisions such as therapy discontinuation or extending dose intervals when patients are in remission states. In PNH, a minimum therapeutic concentration is expected to be above 35 mcg/mL and in aHUS, the therapeutic concentrations are expected to be above 50 to 100 mcg/mL of eculizumab. Complement blockage studies can aid in determining that a therapeutic concentration of the drug has blocked the complement function and subsequent production of sC5b-9. Here we offer a panel of eculizumab concentration plus alternative pathway function to monitor eculizumab therapy efficacy.

Reference Values

ECULIZUMAB QUANTITATION:
Lower limit of quantitation =5.0 mcg/mL
>35 mcg/mL: Therapeutic concentration for paroxysmal nocturnal hemoglobinuria (PNH)
>50 mg/mL: Therapeutic concentration for atypical hemolytic uremic syndrome (aHUS)

ECULIZUMAB COMPLEMENT BLOCKAGE:

> or =46% normal

Interpretation

Minimum trough therapeutic concentrations (immediately before next infusion) of eculizumab are expected to be above 35 mcg/mL for paroxysmal nocturnal hemoglobinuria and above 50 mcg/mL for atypical hemolytic uremic syndrome.

For the complement blockage monitoring of eculizumab:
-When eculizumab is present in serum at concentrations around 100 mcg/mL, the results are below the limit of quantitation of the assay (<10% of normal).

Cautions

Eculizumab complement blockage monitoring 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 or blockage, and in the event of poor pre-analytical handling, eculizumab concentrations are a more reliable indicator, as not subject to stringent temperature stability.

While pre-analytic handling can lead to falsely low results, it is far less likely that it would lead to falsely 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 eculizumab (extremely rare scenario) could present as 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.

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;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
8. Willrich MA, Murray DL, Barnidge DR, Ladwig PM, Snyder MR. Quantitation of infliximab using clonotypic peptides and selective reaction monitoring by LC-MS/MS. Int Immunopharmacol. 2015;28(1):513-520. doi:10.1016/j.intimp.2015.07.007
9. 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
10. Ladwig PM, Barnidge DR, Willrich MA. Mass spectrometry approaches for identification and quantitation of therapeutic monoclonal antibodies in the clinical laboratory. Clin Vaccine Immunol. 2017;24(5):e00545-16. doi:10.1128/CVI.00545-16
11. 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
12. 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
13. Alexion Pharmaceuticals. BLA 761108-S1 Multi-disciplinary review and evaluation: Ultomiris (ravulizumab-cwvz). FDA; April 2, 2019. Available at www.fda.gov/media/135113/download
14. 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
15. Wong EK, Goodship TH, Kavanagh D. Complement therapy in atypical haemolytic uraemic syndrome (aHUS). Mol Immunol. 2013;56(3):199-212
16. Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat Biotechnol. 2007;25(11):1256-1264
17. Zuber J, Le Quintrec M, Krid S, et al. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. Am J Transplant. 2012;12(12):3337-3354
18. Andreguetto B, Murray D, Snyder M, et al: The impact of eculizumab in complement assays. Mol Immunol. 2015;67:119-120
19. Willrich MAV, Braun KMP, Moyer AM, Jeffrey DH, Frazer-Abel A. Complement testing in the clinical laboratory. Crit Rev Clin Lab Sci. 2021;58(7):447-478. doi:10.1080/10408363.2021.1907297
20. Pittock SJ, Berthele A, Fujihara K, et al: Eculizumab in aquaporin-4-positive neuromyelitis optica spectrum disorder. N Engl J Med. 2019;381(7):614-625. doi:10.1056/NEJMoa1900866
21. Dhillon S. Eculizumab: A review in generalized myasthenia gravis. Drugs. 2018;78(3):367-376. doi:10.1007/s40265-018-0875-9
22. Howard JF Jr, Utsugisawa, K, Benatar M, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. Lancet Neurol. 2017;16(12):976-986. doi:10.1016/ S1474-4422(17)30369-1

Performance

Method Description

Eculizumab is extracted from serum and measured by liquid chromatography (high-resolution accurate-mass) mass spectrometry.(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 12 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 Definition: ECMP

Eculizumab Monitoring Panel, Serum

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
ECMP	Eculizumab Monitoring Panel, S	101922-3

Result ID	Test Result Name	Result LOINC® Value
65676	Eculizumab, S	90240-3
619951	Eculizumab Interpretation	59462-2
618697	Eculizumab Complement Blockage, S	74520-8