Test Definition: AH50

Alternative Complement Pathway, Functional, Serum

Overview

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

Method Name
Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available
Yes

Specimen

Specimen Type
Serum

Ordering Guidance
COM / Complement, Total, Serum and this test are the most appropriate primary assays to use as screening methods for complement deficiencies. Abnormal results in one or the other, neither or both assays will help direct further testing.

This test is rarely useful when ordered in isolation.

Specimen Required
Patient Preparation:
Fasting: 8 hours, preferred but not required
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Instructions:
1. Immediately after specimen collection, place the tube on wet ice and allow specimen to clot.
2. Centrifuge at 4 degrees C and aliquot serum into a plastic vial.
3. Freeze specimen within 30 minutes of centrifugation. 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 specimen is kept on ice before centrifugation, and immediately afterward, the serum aliquoted and frozen.

Forms

If not ordering electronically, complete, print, and send a Renal Diagnostics Test Request (T830) with the specimen.

Specimen Minimum Volume

0.75 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Frozen	14 days	

Clinical & Interpretive

Clinical Information

Complement proteins are components of the innate immune system. There are 3 pathways to complement activation:

1. The classical pathway
2. The alternative (or properdin) pathway
3. The lectin (or mannose-binding lectin, MBL) pathway

The total complement (CH50) assay (COM / Complement, Total, Serum) assesses the classical complement pathway including early components that activate the pathway in response to immune complexes (C1q, C2 and C4), as well as the terminal complement components (C3, C5, C6, C7, C8, C9) involved in the formation of the membrane attack complex (MAC). The CH50 assay will be abnormal if there are specific hereditary or acquired C1-C9 complement component deficiencies or if there is consumption of complement due to immune (or autoimmune) complexes.

This assay is a screening test for complement abnormalities in the alternative pathway. The alternative complement (AH50) pathway shares C3 and C5-C9 components but has unique early complement components designated factors D, B, and properdin, as well as control proteins factor H and factor I. This pathway can be activated by spontaneous hydrolysis of C3 or by microbial polysaccharides and does not require immune complex formation. Patients with disseminated infections with pyogenic bacteria in the presence of a normal CH50 may have a decreased AH50 due to hereditary or acquired deficiencies of the alternative pathway. Patients with deficiencies in the alternative pathway factors (D, B, properdin, H, and I) or late complement components (C3, C5-C9) are highly susceptible to recurrent Neisserial meningitis. The use of the CH50 and AH50 assays allow identification of the specific pathway abnormality.

Functional testing for complement pathways activity is indicated in the study of complement components deficiency, where testing serves as a first-tier screening, or in the study of complement dysregulation. Complement dysregulation is a general grouping of complement conditions where there is loss of control of the complement cascade with

over-activation. In several cases, the complement system will attack the host and the over-activation of the complement cascade may cause disease.

Over-activation of the alternative pathway usually presents with renal function impairment, in rare conditions such as atypical hemolytic uremic syndrome and C3 glomerulopathies (dense deposit disease and C3 glomerulonephritis).

The use of complement inhibitor therapies such as eculizumab and ravulizumab will result in the blocking of C5. C5 is necessary for the AH50 test to progress until the formation of the MAC. Hence, in the presence of eculizumab or ravulizumab, AH50 results will be decreased or undetectable.

Reference Values

> or =46% normal

Interpretation

Absent complement alternative pathway (AH50) in the presence of a normal total hemolytic complement (CH50) suggests an alternative pathway component deficiency.

Normal AH50 with absent CH50 suggests an early (C1, C2, C4) classic pathway deficiency.

Absent AH50 and CH50 suggests a late (C3, C5, C6, C7, C8, C9) component deficiency or complement consumption.

Absent AH50 and CH50 in the presence of a normal C3 and C4 suggests a late (C5, C6, C7, C8, C9) component deficiency.

Normal CH50 and AH50 in the presence of recurrent infection and continued suspicion of complement deficiency, suggest testing for lectin pathway function.

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 preanalytic 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. Frank MM. Medical intelligence current concepts: complement in the pathophysiology of human disease. N Engl J Med. 1987;316(24):1525-1530. doi:10.1056/NEJM198706113162407
2. Thurman JM, Holers VM. Brief reviews: the central role of the alternative complement pathway in human disease. J Immunol. 2006;176(3):1305-1310. doi:10.4049/jimmunol.176.3.1305
3. Frank MM. Complement deficiencies. Pediatr Clin North Am. 2000;47(6):1339-1354. doi:10.1016/s0031-3955(05)70274-1
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

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)

PDF Report

No

Day(s) Performed

Varies

Report Available

3 to 5 days

Specimen Retention Time

14 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Test Definition: AH50

Alternative Complement Pathway, Functional, Serum

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

86161

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AH50	Alternative Complement Path Func, S	74520-8

Result ID	Test Result Name	Result LOINC® Value
88676	Alternative Complement Path Func, S	74520-8