MAYO CLINIC LABORATORIES

Test Definition: ADALP

Adalimumab Quantitative with Antibody, Serum

Overview

Useful For

Therapeutic drug monitoring of adalimumab concentration and antibody levels

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
QNADL	Adalimumab QN, S	Yes, (ADALX)	Yes
ABADL	Adalimumab Ab, S	No	Yes
INTAD	Adalimumab Interpretation	No	Yes

Testing Algorithm

For more information see Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm.

Special Instructions

- Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm

Highlights

Adalimumab (brand names Amjevita and Humira) is a fully human therapeutic monoclonal antibody targeting tumor necrosis factor alpha, a proinflammatory cytokine that is upregulated in several autoimmune inflammatory states.

Testing for adalimumab concentration and the presence of anti-adalimumab antibodies is helpful to adjust therapeutic strategies for patients starting therapy (proactive monitoring) and dosing or treatment strategy when partial response or loss of response to therapy is observed, manifested as recurrence of symptoms.

Method Name

Enzyme Linked Immunosorbent Assay (ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

If there is a known justification for performing **both** quantitation and antibody levels, this is the correct test to order. If

there is not a known reason to perform the antibody levels component, consider ADALX / Adalimumab Quantitative with Reflex to Antibody, Serum. ADALX testing begins with adalimumab quantitation and only performs testing for antibodies when the quantitation results are 8.0 mcg/mL or less.

Specimen Required

Patient Preparation: For 12 hours before specimen collection, patient **should not** take multivitamins or dietary supplements (eg, hair, skin, and nail supplements) containing biotin (vitamin B7).
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1.0 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial

Forms

If not ordering electronically, complete, print, and send a Gastroenterology and Hepatology Test Request (T728) with the specimen.

Specimen Minimum Volume

0.7 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK
Heat-treated specimens	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	28 days	
	Frozen	28 days	

Clinical & Interpretive

Clinical Information

Adalimumab, sold under the brand names Amjevita and Humira, is a US Food and Drug Administration-approved medication used to treat rheumatoid arthritis, psoriatic arthritis, Crohn disease, ulcerative colitis, and chronic psoriasis, among others. It is usually self-administered as a subcutaneous injection every other week at a fixed dose of 40 mg in

adults, although dosing can vary. Adalimumab is a tumor necrosis factor (TNF)-inhibiting, antiinflammatory, biologic medication. TNF-alpha normally binds to TNF-alpha receptors, leading to the inflammatory response of autoimmune diseases. By binding to TNF-alpha, adalimumab can reduce the inflammatory response. Because TNF-alpha is also part of the immune system that protects the body from infection, treatment with adalimumab may increase the risk of infections. Treatment with adalimumab is effective in reducing disease activity, offers significant benefits in quality of life, and may have the potential to slow or halt the progression of the disease when given early. However, over 30% of patients fail to respond to anti-TNF-alpha therapy, and approximately 60% of patients who responded initially lose the response over time and require either drug dose-escalation or a switch to an alternative therapy in order to maintain response.(1)

Reasons for primary loss of response may include disease processes mediated by proinflammatory molecules other than TNF. Secondary loss of response, on the other hand, is associated with low serum albumin, high body-mass index, the degree of systemic inflammation and development of an immune response to therapy, or immunogenicity.(2,3) Antidrug antibody formation may increase drug clearance in treated patients or neutralize the drug effect, thereby potentially contributing to the loss of response. Antidrug antibodies could also cause adverse events such as serum sickness and hypersensitivity reactions.(4) Currently, adalimumab quantitation is commonly performed in conjunction with immunogenicity assessment for antibodies to adalimumab (ATA). Most often, this testing is ordered in patients on therapy who are experiencing partial or complete loss of response but can also be performed at any stage during therapy, whether patients are responding well to the therapy or not.

There is positive correlation between the concentration of serum biologic drug concentration and favorable therapeutic outcome; whereas low or undetectable drug concentrations are associated with immunogenicity and treatment failure. Thus, therapeutic drug monitoring of TNF inhibitors and antidrug antibody is a useful tool for optimizing the use of these medications and maximize their effectiveness.(5) In addition, TNF inhibitor therapies are expensive and adverse events include greater risk for infections, such as reactivation of latent tuberculosis or hepatitis B; infusion or injection site reactions; cutaneous reactions; and reports of hepatoxicity, demyelinating disease, and higher incidence of mortality and hospitalization in patients with heart failure have been documented.

This assay has been verified to measure the reference product adalimumab (Humira, AbbVie) and the biosimilar adalimumab-atto (Amjevita, Amgen) with no analytical differences in the quantitation of the medications. Humira and Amjevita have the same primary amino acid sequence. Therefore, adalimumab will be used to refer to both the reference product and the biosimilar product interchangeably. This test cannot distinguish between Humira and the adalimumab biosimilar product.

Reference Values

ADALIMUMAB QUANTITATIVE:
Limit of quantitation is 0.8 mcg/mL. Optimal therapeutic ranges are disease specific.

ADALIMUMAB ANTIBODY:
<14.0 AU/mL

Interpretation

Adalimumab quantitation is generally performed in conjunction with immunogenicity assessment for antibodies to adalimumab (ATAs). Most often, this testing is ordered for patients with inflammatory bowel disease (IBD) who are on

adalimumab therapy and who are experiencing loss of response (reactive monitoring),(6), but the testing may be ordered for anyone on adalimumab-even when treatment is going well (proactive monitoring).(7-9)

Results from adalimumab and ATAs testing play an important role in patient management. In the setting of loss of response to adalimumab therapy for adults with active IBD, a clinical decision tool from the American Gastroenterology Association (6,10,11) suggests the following scenarios for a blood draw that occurred at trough, ie, immediately before the next injected dose:
-For patients who have undetectable or low concentrations of adalimumab (<8 mcg/mL) but no detectable ATAs, the patient care team may choose to increase the dose of adalimumab in an attempt to increase the amount of the drug in circulation.
-If the patient has subtherapeutic adalimumab concentrations (<8 mcg/mL) in the presence of an ATA, the patient care team may switch the patient to another tumor necrosis factor inhibitor.
-For patients with increased trough concentrations of adalimumab (therapeutic or greater), whether an ATA is present or not, it may be necessary to switch the patient to a therapy with a different mechanism of action such as the anti-alpha 4-beta-7-integrin antibody vedolizumab or the IL12/IL23 antibody ustekinumab.
-Low trough concentrations may be correlated with loss of response to adalimumab.

Adalimumab concentration results above 35 mcg/mL are suggestive of a blood draw at a timepoint in treatment other than trough.

Test interpretation relies on clinical presentation and may differ from the statements above, which were designed for adults with IBD experiencing loss of response. For individuals on adalimumab therapy for other conditions such as rheumatoid arthritis, or pediatric patient populations or proactive monitoring, drug concentration therapeutic targets and patient management decision may be individualized. When both the drug quantitation and anti-drug-antibodies are ordered, an interpretive guide is offered below.

Adalimumab quantitation, mcg/mL	ATA, AU/mL	Comment
<8	Negative	Absence of detectable antibody-to-adalimumab (ATA). Low concentration of adalimumab (ADL) may be attributable to other parameters related to adalimumab clearance.
<8	Positive	Presence of antibody-to-adalimumab (ATA) detected, which correlates with low concentration of adalimumab (ADL). ATAs may be associated with increased clearance and lower circulating concentrations of ADL.
8.1-15	Negative	Absence of detectable antibody-to-adalimumab (ATA) At this concentration of adalimumab (ADL), a low-titer (50-150 AU/mL) or moderate titer (150-500 AU/mL) ATA cannot be excluded. However, the presence of a high-titer ATA (> or =500 U/mL) is unlikely.

		If there is clinical suspicion for a low-titer ATA, suggest submission of a new sample obtained at trough. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
	Low or moderate positive (14-499)	Presence of antibody-to-adalimumab (ATA) detected. At this concentration of adalimumab (ADL), the detected titer of the ATA may be modestly underestimated. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
	High positive (> or =500)	Presence of antibody-to-adalimumab (ATA) detected. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
>15	Negative	At this concentration of adalimumab (ADL), a low (50-150 AU/mL) or moderate titer (150-500 AU/mL) ATA cannot be excluded. The presence of a high-titer ATA (> or =500 U/mL) is unlikely but cannot be completely excluded. If there is clinical suspicion for an ATA, suggest submission of a new sample obtained at trough, preferably during the maintenance phase of therapy. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
	Low positive (14-149)	Presence of antibody-to-adalimumab (ATA) detected. At this concentration of adalimumab (ADL), the detected titer of the ATA is likely underestimated. Suggest submission of a new sample obtained at trough, preferably during the maintenance phase of therapy. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
	Moderate positive	Presence of antibody-to-adalimumab (ATA) detected. At this concentration of adalimumab (ADL), the detected titer of the ATA

	(150-499 U/mL)	may be underestimated. Suggest submission of a new sample obtained at trough, preferably during the maintenance phase of therapy. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.
	High positive (> or =500)	Presence of antibody-to-adalimumab (ATA) detected. This test has demonstrated drug tolerance up to 40 mcg/mL for ATAs > or =500 AU/mL, up to 15 mcg/mL for ATAs between 150-500, and up to 8 mcg/mL ADL for ATAs between 50-150 AU/mL.

Cautions

Tumor necrosis factor (TNF) measurement is not the analyte of choice for monitoring therapy with TNF inhibitors (such as adalimumab or infliximab) since TNF testing would not distinguish between free TNF and TNF bound to the monoclonal antibody, either in the extracellular or membrane-bound form of the cytokine.

Toxicity effects other than acute hypersensitivity infusion reactions have not been described nor correlated with high adalimumab concentrations.

Optimal therapeutic concentrations of adalimumab may vary according to the disease.(12-14) For adults with active inflammatory bowel disease, a concentration of 7.5 mcg/mL or greater is considered therapeutic.(6)

For patients taking biotin supplements, it is recommended to wait at least 12 hours after the last ingestion of biotin to collect a blood sample for this test.

Clinical Reference

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Performance

Method Description

Adalimumab Quantitation:
The adalimumab enzyme-linked immunosorbent assay (ELISA) is designed to determine the quantity of free adalimumab (therapeutic antibody against tumor necrosis factor-alpha: TNF-alpha) in serum samples. In a first incubation step, the free adalimumab from the sample is bound to the specific monoclonal anti-adalimumab antibody coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, peroxidase-labeled antibody is added. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of free adalimumab in the sample. A dose response curve of the absorbance unit (optical density: OD) verses concentration is generated, using the values obtained from standard. The concentrations of free adalimumab in the samples are determined directly from this curve.(Unpublished Mayo method)

Antibodies to Adalimumab:
An ELISA is used to determine the presence of antibodies against TNF-alpha blocker adalimumab (Amjevita and Humira). During sample preparation, the antibodies-to-adalimumab (ATA) are separated from the therapeutic antibody adalimumab using an acid dissociation to acquire free ATA. By adding the peroxidase conjugate (POD-therapeutic antibody adalimumab) and the tracer (biotinylated therapeutic antibody adalimumab), the unlabeled therapeutic antibodies are replaced, and the labeled antibodies can form a complex with the ATA. This complex binds via biotin to the streptavidin-coated microtiter plate. It is detected via the peroxidase conjugate with the peroxidase converting the

substrate, TMB, to a blue product. The enzymatic reaction is stopped by adding an acidic solution. The samples convert from blue to yellow. The color change is measured in a photometer at 450 nm. The interpretation is made using the cut-off control.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday, Wednesday, Friday

Report Available
3 to 6 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
80145
83520

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ADALP	Adalimumab QN with Antibodies, S	99781-7

Result ID	Test Result Name	Result LOINC® Value
QNADL	Adalimumab QN, S	86894-3
ABADL	Adalimumab Ab, S	90779-0
INTAD	Adalimumab Interpretation	77202-0