Test Definition: INFXR

Infliximab QN with Reflex to Ab, S

Overview

Useful For

Trough level quantitation for evaluation of patients undergoing therapy with infliximab, infliximab-dyyb, infliximab-abda or infliximab-axxq

Testing Algorithm

Infliximab will be performed by liquid chromatography-tandem mass spectrometry on all specimens. When infliximab results are below 5.1 mcg/mL, testing for antibodies to infliximab will be performed at an additional charge.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
INFX	Infliximab, S	No	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
INXAB	Infliximab Ab, S	No	No

Method Name

INFXR, INFX: Selective Reaction Monitoring Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

INXAB: Electrochemiluminescent Bridging Immunoassay with Acid Dissociation

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Specimen Required

Patient Preparation:

1. Draw blood immediately before next scheduled dose (trough specimen).

2. **For 12 hours before specimen collection do not** take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.

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

Specimen Volume: 1 mL

Collection Instructions: Centrifuge within 2 hours of collection.

Forms

If not ordering electronically, complete, print, and send 1 of the following with specimen:

-Gastroenterology and Hepatology Client Test Request (T728)

-Therapeutics Test Request (T831)

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject

Specimen Minimum Volume

0.5 mL

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Infliximab is a chimeric immunoglobulin (IgG1 kappa) targeting tumor necrosis factor-alpha (TNF-a), and it is currently US Food and Drug Administration (FDA)-approved for the treatment of multiple inflammatory conditions. Infliximab binds to soluble TNF-a and transmembrane homotrimers, which are found on the surface of macrophages and T cells, with similar affinity. Infliximab has the ability to mediate complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity, which leads to the lysis of target cells.

The reference product for infliximab is Remicade (Janssen) and as of November 2021, four biosimilar products are FDA-approved: Renflexis, Inflectra, Ixifi and Avsola.

A biosimilar product is a biological product that it is highly similar to an FDA-approved biological product, known as the

reference product, but manufactured by a different company. No clinically meaningful differences in terms of safety and effectiveness from the reference product are present. Only minor differences in clinically inactive components are allowable in biosimilar products. In contrast to generic medications, a prescription of biosimilars needs to come from the ordering physician and not the dispensing pharmacy (pharmacies cannot substitute a biosimilar for another medication; a separate prescription is required).

This assay has been verified to measure antibodies to infliximab (Remicade, Janssen Pharmaceuticals), and the biosimilars infliximab-dyyb (Inflectra, Pfizer Inc), infliximab-abda (Renflexis, Organon), and infliximab-axxq (Avsola, Amgen) with no analytical differences between the detection of ATI for the four drugs. Inflectra, Renflexis, and Avsola have the same primary amino acid sequence. Therefore, "infliximab" will be used to refer to both the reference product and the biosimilar products interchangeably. This test cannot distinguish between Remicade and the infliximab biosimilar products.

Infliximab pharmacokinetic properties may vary with disease and clearance is affected by concomitant use of immunosuppressants, high concentrations of TNF-a and C-reactive proteins,(1,2) low albumin concentrations, high body mass index, and presence of antibodies to infliximab (ATI), also known as human antichimeric antibodies (HACA).(3) Male patients seem to clear infliximab faster than female patients.(3)

Several studies have demonstrated that infliximab quantitation in the setting of loss of response to therapy can aid in patient management, as trough concentrations defined as therapeutic have been associated with superior clinical response and improved prognosis.(4-6)

Evaluation of infliximab concentrations may be of value for all inflammatory diseases for which it is prescribed. Primary indications for testing of infliximab include loss of response, partial response on initiation of therapy, autoimmune or hypersensitivity reactions, primary nonresponse, reintroduction after drug holiday, endoscopic/computed tomography enterography recurrence (in inflammatory bowel disease), and acute infusion reactions.

Measurement of infliximab concentrations is indicated at trough, immediately prior to the next scheduled infusion. Low trough concentrations may be correlated with loss of response to infliximab. Assessment of antibodies to infliximab is suggested when infliximab quantitation at trough is 5.0 mcg/mL or less. Infliximab concentrations tend to reach steady state and stabilize after 14 weeks (approximately 100 days). Quantitation of peak infliximab concentrations is strongly discouraged.

Reference Values

INFLIXIMAB QUANTITATION:

Limit of quantitation is 1.0 mcg/mL. Therapeutic ranges are disease specific.

Pediatric reference ranges are not established.

INFLIXIMAB ANTIBODIES

Absence of antibodies to infliximab (ATI) is defined as <50 U/mL

Presence of ATI is reported as positive when concentrations are > or =50 U/mL

Interpretation

Low trough concentrations may be correlated with loss of response to infliximab. For infliximab trough concentrations 5.0 mcg/mL or less, testing for antibodies to infliximab (ATI) is suggested.

For infliximab trough concentrations above 5.0 mcg/mL, the presence of ATI is unlikely; patients experiencing loss of response to infliximab may benefit from treatment with a different pharmaceutical agent.

Results above 35 mcg/mL are suggestive of a blood draw at a time-point in treatment other than trough.

Cautions

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

During the initial induction phase of treatment (weeks 0, 2, and 6), steady-state has not been achieved and concentrations of infliximab may vary significantly between infusions.(3)

Therapeutic concentrations of infliximab may vary according to the disease (eg, Crohn disease versus ulcerative colitis versus rheumatoid arthritis).

The American Gastroenterology Association established thresholds associated with positive outcomes for adults with active inflammatory bowel disease based on several clinical studies.(7)

Samples containing more than 12.5 ng/mL biotin (vitamin B7) may interfere (in the form of depressed signal) with INXAB / Infliximab Antibodies, Serum.

For antibodies-to-infliximab (ATI), pediatric and adult reference ranges were validated, and the presence of an ATI is established as greater than or equal to 50 U/mL by our bridging electrochemiluminescent/acid dissociation method.

The presence of endogenous infliximab is a recognized interference in most ATI methods. This assay includes an acid dissociation step, which partially mitigates this interference. Tolerance up to 12.5 mcg/mL infliximab has been documented, although this is also determined by the titer of the ATI present in the patient sample.

Clinical Reference

1. Colombel JF, Sandborn WJ, Reinisch W, et al: Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010 Apr;362(15):1383-1395. doi: 10.1056/NEJMoa0904492

2. Jurgens M, Mahachie John JM, Cleynen I, et al: Levels of C-reactive protein are associated with response to infliximab therapy in patients with Crohn's disease. Clin Gastroenterol Hepatol. 2011 May;9(5):421-427.e1. doi: 10.1016/j.cgh.2011.02.008

3. Ordas I, Mould DR, Feagan BG, Sandborn WJ: Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin Pharmacol Ther. 2012 Apr;91(4):635-646. doi: 10.1038/clpt.2011.328

4. Afif W, Loftus EV Jr, Faubion WA, et al: Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010 May;105(5):1133-1139. doi: 10.1038/ajg.2010.9

5. Imaeda H, Bamba S, Takahashi K, et al: Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. J Gastroenterol. 2014 Apr;49(4):674-682. doi: 10.1007/s00535-013-0829-7

6. Steenholdt C, Bendtzen K, Brynskov J, et al: Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. Scand J Gastroenterol. 2011 Mar;46(3):310-318. doi: 10.3109/00365521.2010.536254

7. Feuerstein JD, Nguyen GC, Kupfer SS, et al: American Gastroenterological Association Institute guideline on therapeutic drug monitoring in inflammatory bowel disease. Gastroenterology. 2017 Sep;153(3):827-834. doi: 10.1053/j.gastro.2017.07.032

8. Silva-Ferreira F, Afonso J, Pinto-Lopes P, Magro F: A systematic review on infliximab and adalimumab drug monitoring: Levels, clinical outcomes and assays. Inflamm Bowel Dis. 2016 Sep;22(9):2289-2301. doi: 10.1097/MIB.0000000000000855

Performance

Method Description

Infliximab Quantitation:

This test is performed using liquid-chromatography and tandem mass spectrometry. Preanalytical sample preparation includes a trypsin digestion; unique clonotypic peptides from the light chain of the infliximab chimeric structure (IgG1 kappa) are monitored.(Willrich MAV, Murray DL, Barnidge DR, et al: Quantitation of infliximab using clonotypic peptides and selective reaction monitoring by LC-MS/MS. Int Immunopharmacol. 2015 Sep;28(1):513-520. doi: 10.1016/j.intimp.2015.07.007)

Infliximab Antibodies:

This lab developed immunoassay is designed to measure antibodies-to-infliximab (ATI) in human serum by means of electrochemiluminescence (ECL) on the MesoScale Discovery (MSD) platform. The assay uses a "bridging" format in which the ATI forms a link between biotin labeled infliximab and SULFO-Tag labeled infliximab. The biotin binds to a streptavidin (SA) coated surface and the SULFO-Tag creates a signal in the presence of a conjugate following application of an electric current via the MSD QuickPlex SA120. During sample preparation, serum is mixed with acetic acid to break the infliximab/ATI complex. Biotinylated and SULFO-Tagged infliximab are then added together with buffer containing Tris-HCL pH 10 to neutralize the pH and bind with ATI that is present in the sample. After the incubation with the labeled drug, the calibrators, controls, and samples are added to a SA plate that has been blocked with a solution of bovine serum albumin. The biotinylated infliximab then binds to the SA plate. After an incubation period, the SA plate is washed, and MSD read buffer is added. Immediately after the addition of read buffer, the plate is analyzed on the MSD QuickPlex SQ120. The read buffer provides an appropriate chemical environment for ECL when a voltage is applied to the electrodes on the plate by the MSD QuickPlex SQ120. This voltage causes bound SULFO-Tagged infliximab to emit measurable light. The MSD QuickPlex SQ120 measures the intensity of emitted light and correlates it to a set of standards with known concentrations of ATI by means of a 4-point logistics curve fitting method.(Willrich MAV, Lazar-Molnar E, Snyder MR, Delgado JC. Comparison of clinical laboratory assays for measuring serum infliximab and antibodies to infliximab. J Appl Lab Med. 2018 May;2(6):893-903. doi: 10.1373/jalm.2017.024869)

PDF Report

No

Specimen Retention Time

2 weeks

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

80230

82397-(if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
INFXR	Infliximab QN with Reflex to Ab, S	39803-2

Result ID	Reporting Name	LOINC®
63000	Infliximab, S	39803-2
36847	Interpretation	59462-2