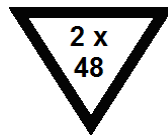


LISA TRACKER

Duo Tocilizumab

REF LTT 005

English
42 determinations

DEFINITION

LISA-TRACKER Duo Tocilizumab (Theradiag) is an enzyme linked immunoassay (ELISA) for the quantitative determination of Tocilizumab (anti-IL6-R) and anti-Tocilizumab antibodies in human serum samples. These tests can be separately or simultaneously done by following the standardized assay protocols.

DESCRIPTION AND DIAGNOSTIC VALUE

Tocilizumab is indicated for the treatment of patient with moderate to severe rheumatoid arthritis showing inadequate response to conventional disease-modifying anti-rheumatic drugs (DMARDs) and anti-TNF α agents.

Tocilizumab is a humanized monoclonal antibody (IgG1 isotype) directed against human IL6 receptor (IL6-R). During the course of the treatment, patients treated with Tocilizumab can develop antibodies directed against Tocilizumab.

LISA-TRACKER Duo Tocilizumab (Theradiag) allows the detection of 2 parameters: Tocilizumab and anti-Tocilizumab antibodies. This kit allows the physician to monitor the level of these 2 parameters in patient sera.

SAMPLES COLLECTION AND HANDLING

- The test should be performed on serum or on plasma.
- Lipemic sera should be avoided, as well as samples which have been frozen and defrosted more than once.
- To avoid any non-specific binding, samples which have been frozen for more than 6 months or which are cloudy, should be centrifuged and filtered.

ASSAY PRINCIPLE

A. Dosage of Tocilizumab

IL6-R is coated onto a polystyrene microtiter plate (6 strips of 8 wells).

- First, the diluted sample is added to the IL6-R coated well, which allows to bind. After incubation, unbound proteins are removed by washing.
- Anti-human IgG biotinylated antibodies is added. After incubation, unbound antibodies are removed by washing.
- Then horseradish peroxidase labelled streptavidin is added. The streptavidin binds to the complex formed with biotinylated anti-IgG antibodies. After incubation, the wells are washed again to eliminate any excess of conjugate.

- The bound enzyme is revealed by addition of substrate TMB (3,3',5,5' tetramethylbenzidine). The colour intensity is proportional to the amount of Tocilizumab.
- Adding H₂SO₄ (0.25M) allows to stop the enzymatic reaction.
- After stopping the reaction by H₂SO₄ (0.25M), the optical density is read by a spectrophotometer at 450nm.

A range of calibration allows to define the quantity of Tocilizumab of each patient samples expressed in μ g/mL.

B. Dosage of anti-Tocilizumab

Tocilizumab is coated onto a polystyrene microtiter plate (6 strips of 8 wells).

- First, the diluted sample is added to the antibody coated well, which allows to bind. After incubation, unbound proteins are removed by washing.
- Biotinylated Tocilizumab is added. After incubation, unbound antibodies are removed by washing.
- Then horseradish peroxidase labelled streptavidin is added. The streptavidin binds to the complex formed with biotinylated Tocilizumab. After incubation, the wells are washed again to eliminate any excess of conjugate.
- The bound enzyme is revealed by addition of substrate TMB (3,3',5,5' tetramethylbenzidine). The colour intensity is proportional to the amount of anti-Tocilizumab antibodies.
- Adding H₂SO₄ allows to stop the enzymatic reaction.
- After stopping the reaction by H₂SO₄, the optical density is read by a spectrophotometer at 450nm.

A range of calibration allows to define the quantity of anti-Tocilizumab antibodies of each patient samples expressed in ng/mL.

REAGENTS

3 reagent families :

Color	Tocilizumab reagents	anti-Tocilizumab antibodies reagents	Common reagents
cap of vials	Blue	White	Green, White, Black or Purple
microwells	Blue	White	-

A) Specific reagents for Tocilizumab determination

Strips of individual breakaway blue wells coated with IL6-R. MP	6 strips
5 vials of « Tocilizumab » Standards, (µg/mL). <u>Ready to use.</u> The vials can be reused several times. The quantity of Tocilizumab is indicated on the vial label. Blue caps TOC CAL n	5 x 1.5mL
«Positive control - Tocilizumab», (µg/mL). <u>To dilute.</u> The vials can be reused several times. The quantity of Tocilizumab is indicated on the vial label. Blue cap TOC CONTROL +	1 x 250µL
Biotinylated antibody vial. <u>Ready to use.</u> Blue cap TOC Ab BIOT	1 x 7.5mL

B) Specific reagents for anti-Tocilizumab antibodies determination

Strips of individual breakaway white wells coated with Tocilizumab. MP	6 strips
5 vials of « anti-Tocilizumab » Standards, (ng/mL). <u>Ready to use.</u> The vials can be reused several times. The quantity of anti-Tocilizumab is indicated on the vial label. White caps A-TOC CAL n	5 x 1.5mL
« Positive control – anti-Tocilizumab», (ng/mL). <u>To dilute.</u> The vial can be reused several times. The quantity of anti-Tocilizumab is indicated on the vial label. White cap A-TOC CONTROL +	1 x 1mL
Biotinylated antibody vial. <u>Ready to use.</u> White cap A-TOC Ab BIOT	1 x 7.5mL

C) Common reagents

HRP labelled Streptavidin. <u>Ready to use.</u> Green cap CONJ HRP	1 x 12mL
Phosphate-Tween Buffer pH 7,2 (10x) – <u>To reconstitute with distilled water.</u> White cap BUF WASH 10x	1 x 100mL
Substrate (TMB). <u>Ready to use.</u> Black cap SUBS TMB	1 x 12mL
Stop solution - H ₂ SO ₄ (0.25 N). <u>Ready to use.</u> Purple cap SOLN STOP	1 x 15mL

MATERIAL REQUIRED BUT NOT PROVIDED

- distilled water
- precision pipettes
- microplate spectrophotometer with 450 nm filter
- 8 channel pipettes

STABILITY AND STORAGE

- Store reagents and micro-wells at +2°C/+8°C in their own package.
- Do not use kits beyond the expiration date.
- Store unused strips into their plastic bag with the desiccant.
- Store all components immediately after use again at +2°C/+8°C.

SETUP

Except the TDL, which can be prepared in advance, all reagents must be prepared extemporaneously.

1. Dilution and Wash buffer (TDL)

- Dilute concentrated Phosphate-Tween Buffer 1/10 in distilled water.

BUF **WASH** **10x**

- Shelf life : 3 months at +2°C/+8°C (avoid to use if signs of contamination or other visible changes occur).

NB. If there are crystals in the concentrated solution, warm the bottle up to +37°C for 15 minutes before use.

2. Preparation of samples and positive controls

a. Samples

- Tocilizumab determination

- Dilute to 1/1001 in TDL. Vortex vigorously.
Ex : 3µL sample + 3mL TDL

If micropipette is not available to take 3µL, a serial dilution of the sample should be done. In a first time make a 1/101 dilution of the sample (10µL + 1ml of TDL). In a second time, make a 1/10 dilution of the 1/101 dilution (100µL of the 1/101 dilution + 900µL TDL). Vortex vigorously.

- Anti-Tocilizumab determination

- Dilute to 1/2 in TDL
Ex : 100µL sample + 100µL TDL
- Vortex vigorously.

b. Positive controls

- Tocilizumab determination

- Dilute to 1/1001 in TDL
Ex : 3µL positive control + 3mL TDL

If micropipette is not available to take 3µL, a serial dilution of the sample should be done. In a first time make a 1/101 dilution of the sample (10µL + 1ml of TDL). In a second time, make a 1/10 dilution of the 1/101 dilution (100µL of the 1/101 dilution + 900µL TDL).
- Vortex vigorously.

- anti-Tocilizumab determination

- Dilute to 1/2 in TDL
Ex : 100µL positive control + 100µL TDL
- Vortex vigorously.

3. Use of ready-to-use biotinylated antibody.

- Estimate the amount required for handling and transfer to a tube.

4. Use of ready-to-use HRP Streptavidin conjugate.

- Estimate the amount required for handling and transfer to a tube.

5. Use of ready-to-use substrate.

- Estimate the amount required for handling and transfer to a dark tube.

PRECAUTIONS

- Unpack all reagents in order to let them warm at room temperature (+18°C/+25°C) at least half an hour before starting the test.


⚠ The temperature of the reagents can impact the final result.


- Check that all plates are well drained after each wash.


- Avoid to use reagents if signs of contamination or other visible changes occur.

- *Human sources for the preparation of standards and controls have been tested and found negative for antibody to HIV 1 and 2, antibody to hepatitis C virus and hepatitis B virus antigen. Nevertheless, no test can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent. Therefore, the reagents should be handled as potentially infective materials.*

Reagents in solution (except for substrate buffer and stop solution) contain <0.1% of sodium azide and <0.6% of ProClin® 300. Do not eat and avoid contact with skin and eyes. Azide can form explosive mixtures in copper or lead piping. Rinse thoroughly after flushing.

-  At this concentration, ProClin® 300 is irritating to eyes and skin, and may be detrimental if enough quantity is ingested. It is a skin sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

- **LISA-TRACKER Duo Tocilizumab** () has been developed according CE directives 67/548/EEC and 1999/45/EC relating to the classification, packaging and labeling of dangerous preparations.

- **LISA-TRACKER Duo Tocilizumab** () has been optimized for the use as describe in this procedure. Do not substitute other manufacturer's reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Close adherence to the test procedure will assure optimal performance.

METHOD

1. Preparing the test

Use the work sheet to note the sample locations.

Set out:

- 5 "standard" wells
- 1 well for positive control
- 1 well for each sample

For a simultaneous testing of the 2 parameters, detach the exact number of wells needed. Return unused wells to plastic pouch provided in the kit, with the desiccant bag.

Remark :

If a dispensing/diluting device is used, place the specific wells in the following order : dosage of Tocilizumab then dosage of anti-Tocilizumab.

2. Samples, positive controls and standards incubation

Add 100 µL of standards, diluted controls or samples.

Incubate for 60 minutes at room temperature (+18°C/+25°C).

Wash step:

Remove the content of the wells by rapid inversion.

Wash 3 times with 300µL of TDL buffer.

Dry the microplate by tapping it gently on an absorbent paper to eliminate the excess of liquid.

3. Incubation of biotinylated antibodies

Add 100µL of **specific** biotinylated antibodies in identified wells.

Incubate for 60 minutes at room temperature (+18°C/+25°C).

Wash step:

Remove the content of the wells by rapid inversion.

Wash 3 times with 300µL of dilution and washing buffer.

Dry the microplate by tapping it gently on an absorbent paper to eliminate the excess of liquid.

4. Incubation of Conjugate

Add 100µL of conjugate.

Incubate for 30 minutes at room temperature (+18°C/+25°C).

Wash step:

Remove the content of the wells by rapid inversion.

Wash 3 times with 300µL of dilution and washing buffer.

Dry the microplate by tapping it gently on an absorbent paper to eliminate the excess of liquid.

5. Incubation of Substrate

Add 100µL substrate into each well.

Incubate for 15 minutes at room temperature (+18°C/+25°C), in the dark.

6. Stop of the reaction

Add 100µL of H₂SO₄ to each well.

7. Reading

Read the optical density of each well at 450nm within 30 minutes after stopping reaction.

RESULTS AND INTERPRETATION

A. Dosage of Tocilizumab

- The OD of the standard 1 should be at least 0.8.
- The positive control value should be comprised into the range indicated on the vial label.
- Trace a *degree 4 polynomial standard curve or a 4PL standard curve*, with plotting the units of the 5 standard points (µg/mL) along the abscissa (X axis) and the corresponding OD values along the ordinate (Y axis).
- The Tocilizumab value can be directly read on the curve.
- Samples with values greater than that of standard 1 may be diluted to obtain a more precise result. The number of units should be multiplied by the selected dilution.

B. Dosage of anti-Tocilizumab

- The OD of the standard 1 should be at least 0.8.
- The positive control value should be comprised into the range indicated on the vial label.
- Trace the standard curve (polynomial curve), plotting the units of the 5 standard points (ng/mL) along the abscissa (X axis) and the corresponding OD values along the ordinate (Y axis).
- The anti-Tocilizumab value can be directly read on the curve.
- Samples with values greater than that of standard 1 may be diluted to obtain a more precise result. The number of units should be multiplied by the selected dilution.

CHARACTERISTICS AND PERFORMANCE OF THE TEST

Limits of detection / threshold values


Estimated on 150 healthy patient samples.

Limit of detection Tocilizumab	Limit of detection Anti- Tocilizumab
1 µg/mL > 99 th percentile	5 ng/mL > 99 th percentile

Assay range

Tocilizumab	Anti-Tocilizumab
1 µg/mL - 50 µg/mL	5 ng/mL - 100 ng/mL

Interfering Substances Study

LISA-TRACKER Duo Tocilizumab () was evaluated to assess potential cross reactivity to other antibodies and interference from serum components (cryoglobulins, rheumatoid factors, heterophilic antibodies, high levels of triglycerides, bilirubin, IgG and/or IgM, and C1q proteins, autoantibodies). No interference was detected.

Precision

Parameters	Intra-run (4 tests in a same assay)		Inter-runs (4 tests 4 different assays)	
	Mean	CV (%)	Mean	CV (%)
Tocilizumab (µg/mL)	44.9	3.6	46.1	4.9
	19.0	3.0	22.0	9.7
	11.9	5.4	14.7	14.8
	4.4	4.9	5.1	13.9
Anti- Tocilizumab (ng/mL)	43	3.1	52	13.5
	21	1.3	24	12.9
	11	2.9	12	13.9

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SUMMARY OF METHOD

A) Sample Dilution

Tocilizumab	Anti-Tocilizumab
1/1001	1/2

B) Positive Control Dilution

Tocilizumab	Anti-Tocilizumab
1/1001	1/2

C) Procedure

Reagents	Procedure
Standards	100µL / well
Diluted positive controls	
Diluted samples	
Incubation	1 h at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / well
Biotinylated antibodies	100µL / wells (specific reagents)
Incubation	1 h at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / well
HRP-Streptavidin	100µL / well
Incubation	30 minutes at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / well
Substrate (TMB)	100µL / well
Incubation	15 minutes at room temperature. Protect from light.
Stop solution	100µL / well

* Dry the microplate by tapping it gently on a towel to eliminate the excess of liquid.

D) Configuration of the assays

a. 42 sera for Tocilizumab and anti-Tocilizumab determinations

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 5	Sera 3	Sera 11	Sera 19	Sera 27	Sera 35	Standard 5	Sera 3	Sera 11	Sera 19	Sera 27	Sera 35
B	Standard 4	Sera 4	Sera 12	Sera 20	Sera 28	Sera 36	Standard 4	Sera 4	Sera 12	Sera 20	Sera 28	Sera 36
C	Standard 3	Sera 5	Sera 13	Sera 21	Sera 29	Sera 37	Standard 3	Sera 5	Sera 13	Sera 21	Sera 29	Sera 37
D	Standard 2	Sera 6	Sera 14	Sera 22	Sera 30	Sera 30	Standard 2	Sera 6	Sera 14	Sera 22	Sera 30	Sera 30
E	Standard 1	Sera 7	Sera 15	Sera 23	Sera 31	Sera 39	Standard 1	Sera 7	Sera 15	Sera 23	Sera 31	Sera 39
F	C+	Sera 8	Sera 16	Sera 24	Sera 32	Sera 40	C+	Sera 8	Sera 16	Sera 24	Sera 32	Sera 40
G	Sera 1	Sera 9	Sera 17	Sera 25	Sera 33	Sera 41	Sera 1	Sera 9	Sera 17	Sera 25	Sera 33	Sera 41
H	Sera 2	Sera 10	Sera 18	Sera 26	Sera 34	Sera 42	Sera 2	Sera 10	Sera 18	Sera 26	Sera 34	Sera 42

Tocilizumab
assay

anti-
Tocilizumab
assay

b. 2 sera for Tocilizumab and anti-Tocilizumab determinations

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 5	Standard 5										
B	Standard 4	Standard 4										
C	Standard 3	Standard 3										
D	Standard 2	Standard 2										
E	Standard 1	Standard 1										
F	C+	C+										
G	Sera 1	Sera 1										
H	Sera 2	Sera 2										

Tocilizumab assay	anti-Tocilizumab assay
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SYMBOLS USED



EC Declaration of conformity



ELISA Test



Catalogue number



Lot Number



Expiry Date



In Vitro Diagnostic Device



Manufacturer



Number of test



Consult Instructions



Temperature limitation



Biological hazard



Contains sodium azide



Reconstitute with



Warning



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