Theradiag

LISA TRACKER Duo Trastuzumab

REF LTTR 005

2 x 48

42 determinations

English

DEFINITION

LISA-TRACKER Duo Trastuzumab (Theradiag) is an enzyme linked immunoassay (ELISA) for the quantitative determination of Trastuzumab (anti-HER2) and anti-Trastuzumab antibodies in human serum samples. These tests can be separatly or simultaneously done by following the standardized assay protocol.

DIAGNOSTIC VALUE

Trastuzumab is a humanized monoclonal antibody that produces proliferation cell inhibition by inhibiting HER2 (Human epidermal growth factor receptor-2). It is indicated for the treatment of metastatic breast cancer and metastatic gastric cancer whose tumors overexpress HER2. It permits to stop the action of the HER2 responsible for the cell proliferation. During the treatment, some patients can develop antibodies against Trastuzumab.

LISA-TRACKER Duo Trastuzumab (Theradiag) allows the detection of 2 parameters: Trastuzumab and anti-Trastuzumab 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 Trastuzumab

HER2 is coated onto a polystyrene microtiter plate (6 strips of 8 wells).

- First, the diluted sample is added to the HER2 coated well, which allows to bind. After incubation, unbound proteins are removed by washing.
- Biotinylated HER2 is added. After incubation, unbound biotinylated HER2 are removed by washing.
- Then horseradish peroxydase labelled streptavidin is added. The streptavidin binds to the complex formed « HER2 / Trastuzumab / Biotinylated HER2 » . 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' tetramethylbenzidin). The colour intensity is proportional to the amount of Trastuzumab.
- Adding H_2SO_4 allows to stop the enzymatic reaction.
- After stopping the reaction by H_2SO_4, the optical density is read by a spectrophotometer at 450nm.

A range of calibration allows to define the quantity of Trastuzumab of each patient sample expressed in µg/mL.

B. Dosage of anti-Trastuzumab

The Trastuzumab 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 Trastuzumab is added. After incubation, unbound antibodies are removed by washing.
- Then horseradish peroxydase labelled streptavidin is added. The streptavidin binds to the complex formed with biotinylated Trastuzumab. 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' tetramethylbenzidin). The colour intensity is proportional to the amount of anti-Trastuzumab antibodies.
- Adding H_2SO_4 allows to stop the enzymatic reaction.
- After stopping the reaction by H_2SO_4, the optical density is read by a spectrophotometer at 450nm.

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

REAGENTS

3 reagent families :

Color	Trastuzumab reagents	anti-Trastuzumab antibodies reagents	Common reagents
cap of vials	**Blue**	**white**	**Green, White, Black or Purple**
microwells	**Blue**	**colourless**	**-**

A) Specific reagents for the Trastuzumab determination

Strips of individual breakaway blue wells coated with HER2. **MP**	6 strips
5 vials of « Trastuzumab » Standards, (µg/mL). Ready to use. The vials can be reused several times. ***The quantity of Trastuzumab is indicated on the vial label.*** ***Blue caps*** **TRA CAL n**	5 x 1,5mL
«Positive control - Trastuzumab», (µg/mL). To dilute. The vials can be reused several times. ***The quantity of Trastuzumab is indicated on the vial label.*** ***Blue cap*** **TRA CONTROL +**	1 x 250µL
Biotinylated HER2 vial. Ready to use. ***Blue cap*** **TRA Ab BIOT**	1 x 7,5mL

B) Specific reagents for the anti-Trastuzumab antibodies determination

Strips of individual breakaway colourless wells coated with Trastuzumab. **MP**	6 strips
5 vials of « anti-Trastuzumab » Standards, (ng/mL). Ready to use. The vials can be reused several times. ***The quantity of anti-Trastuzumab is indicated on the vial label.*** ***White caps*** **A-TRA CAL n**	5 x 1,5mL
« Positive control – anti-Trastuzumab», (ng/mL). To dilute. The vial can be reused several times. ***The quantity of anti-Trastuzumab is indicated on the vial label.*** ***White cap*** **A-TRA CONTROL +**	1 x 1mL
Biotinylated antibody vial. Ready to use. ***White cap*** **A-TRA Ab BIOT**	1 x 7,5mL

C) Common reagents

HRP labelled Streptavidin. Ready to use. ***Green cap*** **CONJ HRP**	1 x 12mL
Phosphate-Tween Buffer pH 7,2 (10x) – To reconstitute with distilled water. ***White cap*** **BUF WASH 10x**	1 x 100mL
Substrate (TMB). Ready to use. ***Black cap*** **SUBS TMB**	1 x 12mL
Stop solution - H_2SO_4 (0.25 N). Ready to use. ***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 prepare 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

- Trastuzumab determination

- Dilute to 1/1001 in TDL

Ex : 5µL sample + 5mL TDL

- Vortex vigorously.

- Anti-Trastuzumab determination

- Dilute to 1/2 in TDL

Ex : 130µL sample + 130µL TDL

- Vortex vigorously.

b. Positive controls

- Trastuzumab determination

- Dilute to 1/1001 in TDL

Ex : 5µL positive control + 5mL TDL

- Vortex vigorously.

- anti-Trastuzumab determination

- Dilute to 1/2 in TDL

Ex : 130µL positive control + 130µL TDL

- Vortex vigorously.

3. Use of ready-to-use biotinylated HER2 or 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 Trastuzumab (Theradiag) 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 Trastuzumab (Theradiag) has been optimized for the use as describe in this procedure. Do not substitute other manufacter'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 Trastuzumab then dosage of anti-Trastuzumab.

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 HER2 or biotinylated antibody

Add 100µL of **specific** biotinylated HER2 or biotinylated antibody 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 H_2SO_4 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 Trastuzumab

- 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 *polynomial standard curve or a 4PL* 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 Trastuzumab 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-Trastuzumab

- 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 *polynomial standard curve* with 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-Trastuzumab 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 samples of healthy patient samples.

Limit of detection Trastuzumab	Limit of detection anti-Trastuzumab
10 µg/mL 99th percentile	10 ng/mL >99th percentile

Assay range

Trastuzumab	anti-Trastuzumab
10 µg/mL - 200 µg/mL	10 ng/ml - 120 ng/mL

Interfering Substances Study

LISA-TRACKER Duo Trastuzumab (Theradiag) 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

	Intra-run (30 tests in a same assay)		Inter-runs (9 different assays)	
Parameters	**Mean**	**CV (%)**	**Mean**	**CV (%)**
Trastuzumab (µg/mL)	39	13,8 %	46	11,5 %
	100	7,9 %	103	9,5 %
	140	9,7 %	157	11,7 %
anti-Trastuzumab (ng/mL)	30	12,8 %	35	17,2 %
	40	7,7 %	50	13,9 %
	77	7,4 %	77	11,7 %

LIMITS

The dosage of Trastuzumab shows cross reactivity with serums of patients treated with the Pertuzumab which is another anti-HER2 antibody.

REFERENCES

Baselga J. et al., Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer, J. Clin. Oncol., 1996, 14, p.737-744.

Baselga J. et al., Pertuzumab plus Trastuzumab plus Docetaxel for metastatic Breast Cancer, The New England Journal of Medicine, 2012, 366, p.109-119.

Boekhout A. et al. Trastuzumab, the oncologist, 2011, 16, p.800-810.

Damen C. et al., Development and validation of an enzyme-linked immunosorbent assay for the quantification of Trastuzumab in human serum and plasma, Analytical Biochemistry, 2009, 391, p.114-120.

Goldenberg MM et al., Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer, Clin. Ther., 1999, 21, p.309-318.

Jamieson D. et al., Development and validation of cell-based ELISA for the quantification of Trastuzumab in human plasma, Journal of immunological methods, 2009, 345, p. 106-111.

Levêque et al., Clinical Pharmacology of Trastuzumab, 2008, 3, 51-55.

Maple L. et al., Development and validation of ELISA for Herceptin detection in human serum, Journal of immunological methods, 2004, 295, p. 169-182.

Molina MA et al., Trastuzumab (Herceptin), a humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells, Cancer Research , 2001, 61, p. 4744-4749.

Pegram MD et al., HER-2/neu as a predictive marker of response to breast cancer therapy, Breast Cancer Res Treat., 1998, 52, p.65-77.

Sarup JC et al., Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth, Growth Reg., 1991, 1, p.72-82.

Slamon DJ. Et al., Human Breast Cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, Science, 1987, 235, p.177-182.

SUMMARY OF METHOD

A) Sample Dilution

Trastuzumab	anti-Trastuzumab
1/1001	1/2

B) Positive Control Dilution

Trastuzumab	anti-Trastuzumab
1/1001	1/2

C) Procedure

Reagents	Procedure
Standards	100µL / wells
Diluted positive controls	
Diluted samples	
Incubation	1 h at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / wells
Biotinylated HER2 or biotinylated antibody	100µL / wells **(specific reagents)**
Incubation	1 h at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / wells
HRP-Streptavidin	100µL / wells
Incubation	30 minutes at room temperature
Washing*	Wash 3 times with TDL buffer : 3 x 300µL / wells
Substrate (TMB)	100µL / wells
Incubation	15 minutes at room temperature. Protect from light.
Stop solution	100µL / wells

* 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 Trastuzumab and anti-Trastuzumab

	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 38	**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

Trastuzumab assay

anti-Trastuzumab assay

b. 2 sera for Trastuzumab and anti-Trastuzumab

	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										

Trastuzumab assay | anti-Trastuzumab assay

c. 26 sera for anti-Trastuzumab determination

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Standard 5**	Sera 3	Sera 11	Sera 19								
B	**Standard 4**	Sera 4	Sera 12	Sera 20								
C	**Standard 3**	Sera 5	Sera 13	Sera 21								
D	**Standard 2**	Sera 6	Sera 14	Sera 22								
E	**Standard 1**	Sera 7	Sera 15	Sera 23								
F	**C+**	Sera 8	Sera 16	Sera 24								
G	Sera 1	Sera 9	Sera 17	Sera 25								
H	Sera 2	Sera 10	Sera 18	Sera 26								

anti-Trastuzumab assay

SYMBOLS USED

CE	EC Declaration of Conformity	▽	Number of test
	ELISA Test		Consult Instructions
REF	Catalogue number		Temperature limitation
LOT	Lot Number		Biological hazard
	Expiry Date	CONT NaN_3	Contains sodium azide
IVD	*In vitro* Diagnostic device	RCNS	Reconsitute with
	Manufacturer		Warning

Theradiag

IVD

CE

14 rue Ambroise Croizat
CS 90136 CROISSY BEAUBOURG
77435 MARNE LA VALLEE CX2
France

Tel : +33 (0)1 64 62 10 12
Fax : +33 (0)1 64 62 09 66

E-mail : support@theradiag.com
Internet : www.theradiag.com