Toxotest IgG

ELISA (+Avidity)

Enzyme immunosorbent assay (ELISA) for the detection of anti-*Toxoplasma gondii* IgG antibodies and avidity

SUMMARY

Toxoplasmosis is an infectious disease of humans and animals, of universal distribution, whose etiologic agent is an obligate intracellular parasite, *Toxoplasma gondii*.

It is a generally benign and asymptomatic disease in immunocompetent individuals, becoming a serious complication in immunocompromised patients.

When the infection is acquired during pregnancy, there is a high risk of fetal infection and might cause abortions or serious injury. The fetal injuries are more severe as earlier in the pregnancy is the maternal infection.

If anti-T*oxoplasma gondii* IgG and IgM, are increased during testing, it is unlikely to define whether it is an acute infection, because IgM can result from an infection that takes months. As the strength of the antigen-antibody IgG bond increases during the course of an infection, determination of this parameter (called avidity) may be directly correlated to the time of infection. However, clinical data are definitive for diagnosing whether an infection is recent or chronic.

PRINCIPLE

The diluted sample is placed in the microtitration plate whose wells are coated with *T. gondii* antigens. If the sample contains specific antibodies, these antigens will form complexes with the antigens and remain bound to the solid phase. The unbound fraction is removed by washing, then the conjugate is added which specifically reacts with immunocaptured anti-*T.gondii* antibodies. The unbound conjugate is removed by washing. The presence of peroxidase bound to the complex is revealed by addition of tetramethylbenzidine, the chromogenic substrate. Reactive samples develop a light- blue color. The enzymatic reaction is stopped by adding sulfuric acid to produce a shift from light-blue to yellow. The color intensity measured in spectrophotometer at 450 and 405 nm is directly proportional to the concentration of anti-*Toxoplasma gondii* IgG in calibrators and samples.

When avidity is determined, it is possible to distinguish low-affinity antibodies produced in an early stage of the disease from those with a higher binding affinity to reflect pre-existing immunity. To this end the sample is placed in contact with a Dissociation Reagent capable of dissociating low-avidity bonds of the antibodies bound to the antigen in the plate. By reactivity comparison with antibodies not treated with the Dissociation Reagent, it is possible to discriminate both types of antibodies (low or high avidity).

PROVIDED REAGENTS

Coated microtitration plate: wells coated with *T. gondii* antigens.

Sample Diluent: saline buffer with surfactant. Violet color.

Conjugate Concentrate: monoclonal anti-human IgG conjugated to peroxidase (10x). Red color.

Conjugate Diluent: saline buffer with proteins.

Substrate: tetramethylbenzidine and hydrogen peroxide solution.

Stopper: 2 N sulfuric acid

Concentrated Wash Buffer: saline buffer with surfactant (25x). Green color.

Calibrators 1-3: anti-*Toxoplasma gondii* IgG in matrix serum with concentrations: 15, 60 and 240 IU/ml. The calibrators have been adjusted according to the 2nd International Standard (SIS), 1980.

Negative Control: non-reactive protein solution for anti-*Toxoplasma gondii* IgG.

Dissociation Reagent: 6 M urea solution

NON-PROVIDED REAGENTS

Distilled or deionized water

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring the stated volumes
- Incubator at 37ºC
- Timer or stopwatch
- Microtitration washing system (manual or automatic)
- Spectrophotometer for microtitration plate reading

WARNINGS

To obtain correct and reproducible results:

- Do not exchange reagents from different lots.
- Avoid touching the sides of the wells with the tips.
- The microtitration plate should be incubated in incubator. Do not use water bath.
- Ensure that the reagents are at room temperature before testing.
- Use perfectly clean distilled or deionized water.
- Use clean material free from metals or oxidizing agents.
- Avoid conjugate contamination with sprays, saliva, etc.
- Avoid changing time and temperature in the assay technique.

To prevent personal and environmental contamination:

- The reagents are for "in vitro" diagnostic use.
- All patient samples should be handled as if capable of transmitting infection.
- The controls have been tested for surface antigen of hepatitis B (HBsAg) and antibodies against human immunodeficiency virus (HIV) and hepatitis C (HCV), found non-reactive. However, it is recommended to be handled with the precautions required for potentially infectious samples.

- Avoid contact of sulfuric acid (Stopper) with the skin and mucous membranes. If this occurs, rinse thoroughly. H315 + H320: Causes skin and eye irritation. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P280: Wear protective gloves/protective clothing/eye protection/face protection.
- Avoid spilling liquids and aerosol formation.
- The liquid waste may be disinfected with sodium hypochlorite (final concentration 5%) for at least 60 minutes.
- Do not pipette by mouth. Use disposable gloves and eye protection when handling samples and test reagents.
- All reagents and samples should be discarded according to current regulations.

REAGENT PREPARATION

Wash Buffer: at low temperature concentrated reagent components may precipitate. In that case, bring the solution to 37ºC until complete dissolution. To obtain the Wash buffer ready for use, dilute one part Concentrated Wash Buffer (25x) with 24 parts distilled or deionized water. E.g.: 20 ml with 480 ml for 1 microtitration plate.

Conjugate: dilute one part Concentrated Conjugate (10x) with 9 parts Conjugate Diluent (e.g.: refer to the following table for required volume of Conjugate Diluent and Conjugate Concentrate):

Nº of Wells	Concentrated Conjugate	Conjugate Diluent
8	100 µl	0,9 ml
16	200 µl	1,8 ml
24	300 µl	2,7 ml
32	400 µl	3,6 ml
96	1200 µl	10,8 ml

Coated microtitration plate, Sample Diluent, Conjugate Diluent, Substrate, Stopper, Dissociation Reagent, Calibrators and Negative Control: ready to use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not freeze.

Concentrated Wash Buffer and Stopper: may be stored at 2-25ºC.

Wash Buffer (1x): once diluted it is stable for 3 months at 2-25ºC.

Conjugate: once diluted it is stable for 6 hours at 2-25ºC.

Coated microtitration plate: do not open the pouch until use, or before it has reached room temperature. The unused strips should be stored at 2-10 °C in tightly closed pouch with desiccant. The strips preserved in such conditions may be used within 4 months until the expiration date on the box.

SAMPLE

Serum or plasma

a) Sample collection: obtain in the usual way.

b) Additives: not required for serum. For plasma samples heparin, citrate or EDTA may be used as anticoagulant.

c) Known interfering substances: no interference was observed with samples containing up to 30 mg/dl bilirubin, 50 mg/dl ascorbic acid, 1500 mg/dl triglycerides or 300 mg/dL hemoglobin. Samples containing particulate matter should be clarified by centrifugation.

d) Stability and storage instructions: sample should be stored refrigerated (2-10ºC). If the test is not performed within 72 hours samples should be frozen at -20ºC. It is unadvisable to perform multiple cycles of freezing and thawing. If using frozen samples, they must be homogenized and centrifuged before use.

Heat inactivation may affect the result.

Do not use samples with microbial contamination.

TEST PROCEDURE

1- Bring the reagents and samples to room temperature before starting the test.

2- Place in the strip holder the number of wells required for the number of determinations to be made

3- Dilute the sample 1:101 with Sample Diluent, placing 10 ul and 1000 ul Sample Diluent (test tube).

Calibrators and Negative Controls should not be diluted.

4- Add 100 ul Controls or Calibrators and diluted samples.

5- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 30 ± 2 minutes at 37 ± 1ºC. In parallel, prepare the diluted conjugate (see table in REAGENT PREPARATION).

6- After incubation, completely remove the liquid from each well. Wash 5 times according to the washing instructions (see WASHING PROCEDURE).

7- Add 100 ul conjugate. Homogenize by gentle tapping the sides for 5 seconds.

To prevent evaporation cover the microtitration plate with adhesive tape.

8- Incubate for 30 ± 2 minutes at 37 ± 1ºC.

9- Wash 5 times according to washing instructions.

10- Dispense 100 ul Substrate. Homogenize by gentle tapping the sides for 5 seconds.

11- Incubate for 30 ± 2 minutes at room temperature (18-25ºC), protected from light.

12- Add 100 ul Stopper

13- Read absorbance in bichromatic spectrophotometer at 450/620-650 nm or 450 nm.

For higher absorbance values (above the maximum reading value of the spectrophotometer used) read at 405/620-650 nm. This allows a greater curve range.

STABILITY OF FINAL REACTION

Reaction color is stable for 10 minutes, so the results should be read within that period.

WASHING PROCEDURE

Remove the liquid from the wells by aspiration or rotation. The wells are washed with 350 ul diluted wash buffer. Avoid liquid overflow. The wash solution should be in contact with the wells for 30 to 60 seconds.

Ensure that after the last wash there is no residual liquid. Perform double aspiration to remove excess buffer. The plate may also be inverted on absorbent paper and tapped several times. Otherwise erroneous results may be obtained.

Note: the washing procedure is critical to the test result. If wash buffer remains in the well or if wells are not completely filled, erroneous results will be obtained. Do not allow the wells to dry during the procedure.

SUMMARY OF THE PROCEDURE

STAGE	PROCEDURE
Sample dilution	10 µl sample in 1000 µl Sample Diluent
Samples	Add 100 ul diluted sample, NC, and Calibrators
Incubation	Incubate for 30 ± 2 minutes at 37ºC ± 1ºC
Washing step	Wash each well with 350 µl wash buffer (5 times)
Conjugate dilution	Conjugate preparation (1x)
Conjugate	Add 100 µl diluted Conjugate
Incubation	Incubate for 30 ± 2 minutes at 37ºC ± 1ºC
Washing step	Idem previous washing step
Substrate	Add 100 ul Substrate
Incubation	For 30 ± 2 minutes at 18-25ºC
Stop	Add 100 ul Stopper
Reading	Read in spectrophotometer

CALCULATION

Qualitative assay

Consider the optical density of the Negative Control and Calibrator 15 IU/ml (considered as cut-off). The presence or absence of IgG antibodies to *Toxoplasma gondii* is defined by comparison with the absorbance of the Cut-off Calibrator. Samples with optical density lower than the 15 IU/ml Calibrator are considered non-reactive for anti-*Toxoplasma gondii* antibodies. Samples with higher optical density than the Cut-off Calibrator are considered reactive for anti-*Toxoplasma gondii* antibodies.

Quantitative Assay

The Negative Control is considered as the first point of the calibration curve (0 IU/ml).

Draw the calibration curve by placing the calibrator concentration on the x-axis and the absorbance obtained for each calibrator on the y-axis. Concentrations in IU/ml for each sample are obtained by interpolation of the absorbances for each standard.

Samples with IgG values below 15 IU/ml are considered non-reactive for IgG antibodies to *Toxoplasma gondii*.

Samples with IgG values above 15 IU/mL are considered reactive for IgG antibodies to *Toxoplasma gondii*.

Calculation example

The following values must be considered only as an example and not be used instead of experimental data

Description	Absorbance (450-620 nm)	Anti-IgG anti-*Toxoplasma gondii*	Absorbance (405-620 nm)
Negative Control	0,010	0 IU/ml	0,011
Calibrator 1	0,325	15 IU/ml	0,108
Calibrator 2	1,502	60 IU/ml	0,445
Calibrator 3	3,351	240 IU/ml	0,982
Sample	1,230	50 IU/ml	0,408

Interpolating the dosed sample on the calibration curve a titer of 50 IU/ml anti-*Toxoplasma gondii* IgG is obtained.

ASSAY VALIDATION CRITERIA

The test is considered valid if the following conditions are simultaneously met (reading at 450-620 nm):

1- Negative Control absorbance ≤ 0.100.
2- Calibrator 1 absorbance (15 IU/ml)/Negative Control absorbance > 6
3- Calibrator 3 absorbance (240 IU/ml)/Calibrator absorbance 15 IU/ml > 6

If one of these conditions is not met, repeat the test.
Remember that the readings obtained will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

Nonreactive samples: considered negative for anti-*Toxoplasma gondii* IgG.
Reactive or indeterminate samples: considered positive for anti-*Toxoplasma gondii* IgG.
Consecutive samples of the same patient may be compared only if they are tested in the same assay. In this case absorbance increased by 60% in the second sample may be considered as a significant indication of a recent or ongoing infection. In that case, IgM must be specifically tested.

All samples initially reactive should be repeated twice. If one or both replicates test positive, it must be considered reactive.

PROCEDURE LIMITATIONS

See Known Interfering Substances under Sample.

PROCEDURE FOR AVIDITY DETERMINATION

Pipette samples, Calibrator 2 (Control) and sample blank in duplicate.

1- Bring the reagents and samples to room temperature before starting the test.
2- Place in the strip holder, the number of wells required for the number of determinations to be made, including two wells for the control.

3- Dilute the sample 1:101 with Sample Diluent, placing 10 ul and 1000 ul Sample Diluent (test tube).
Calibrator must not be diluted.
4- Pipette 100 ul Calibrator 2, diluted samples and blank (Sample Diluent) in two parallel wells:
In the A1/A2 wells place Calibrator 2 (Control run).
In the B1/B2 wells place Sample Diluent (Blank run).
In C1/C2 wells place the diluted sample
In D1/D2 wells place the second diluted sample, etc.
5- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 30 ± 2 minutes at 37 ± 1ºC.
6- After incubation, completely remove the liquid from each well. Wash 5 times according to washing instructions (see Washing Procedure).
7- Add 100 ul Dissociation Reagent in A1, B1, C1, D1 wells etc.
Add 100 ul Sample Diluent in A2, B2, C2, D2 wells etc.
Homogenize with small taps for 5 seconds.
8- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 30 ± 2 minutes at 37 ± 1ºC.
In parallel, prepare the diluted conjugate (see Table).
9- After incubation, completely remove the liquid from each well. Wash 5 times according to washing instructions (see Washing Procedure).
10- Add 100 ul Conjugate and homogenize gently taping the sides for 5 seconds.
To prevent evaporation cover the microtitration plate with adhesive tape.
11- Incubate for 30 ± 2 minutes at 37 ± 1ºC.
12- Wash 5 times according to the washing instructions.
13- Dispense 100 ul Substrate.
Homogenize gently by taping the sides for 5 seconds.
14- Incubate for 30 ± 2 minutes at room temperature (18-25ºC), protected from light.
15- Add 100 ul Stopper
16- Read absorbance bichromatically in spectrophotometer at 450/620-650 nm or 450 nm.
For higher absorbance values (above the maximum reading of the spectrophotometer used) read at 405/620-650 nm. This allows a greater curve range.

STABILITY OF FINAL REACTION MIXTURE
The color reaction is stable for 10 minutes.

WASHING PROCEDURE
See the above Washing Procedure.
Note: The washing procedure is critical to the test result. If the wash buffer remains in the well or the wells are not completely filled, erroneous results will be obtained. Do not allow the wells to dry during the procedure. Automatic washers should be rinsed with distilled or deionized water later in the day to avoid obstructions due to salts in the wash buffer.

SUMMARY OF THE PROCEDURE

STAGE	PROCEDURE
Sample dilution	10 µl sample in 1000 µl sample diluent
Samples	Add 100 µl Diluted sample in duplicate, Control, Blank
Incubation	Incubate for 30 ± 2 minutes at 37ºC ± 1ºC
Washing step	Wash each well with 350 µl wash buffer (5 times)
Avidity	Add 100 µl dissociation reagent to 1 row of wells Add 100 µl Sample Diluent to the other row
Incubation	Incubate for 30 ± 2 minutes at 37ºC ± 1ºC
Washing step	Idem to the previous washing step
Conjugate dilution	Conjugate preparation (1x)
Conjugate	Add 100 µl diluted Conjugate
Incubation	Cover the wells and incubate for 30 ± 2 minutes at 37ºC ± 1ºC
Washing step	Idem to the previous washing step
Substrate	Add 100 µl Substrate
Incubation	For 30 ± 2 minutes at 18-25ºC
Stop	Add 100 µl de Stopper
Reading	Read in spectrophotometer

CALCULATION OF RESULTS
Verify that for each sample the optical density of the wells incubated with the Sample Diluent is over 0.700 OD or has a concentration of more than 30 IU/ml. Otherwise, the sample does not have enough IgG concentration to evaluate avidity. If the reading is above 0.700 OD or more than 30 IU/ml, calculate the avidity. In cases of readings above 3.000 at 450 nm, read at 405 nm and perform calculations using this data.
Calculate for each patient and control the ratio between the absorbance (OD) of the well treated with Dissociation Reagent and the absorbance (OD) of the wells incubated with the Sample Diluent, multiplied by 100.

$$\frac{\text{OD with Dissociation Reagent}}{\text{OD with Sample Diluent}} \times 100 = \text{Avidity (\%)}$$

Calculation example
The following values must be considered only as an example and not used instead of the experimental data:

Description	**Absorbance** (450- 620 nm) **With Dissociation Reagent**	**Absorbance** (450- 620 nm) **With Sample Diluent**	Avidity (%)
Control	0,395	1,100	36%
Sample 1	1.520	1.780	85.4%
Sample 2	0.325	1.860	17.5%
Sample 3	0.029	1.450	2.0%

ASSAY VALIDATION CRITERIA

The test is considered valid if the following conditions are simultaneously met (with reading at 450-620 nm):

1- Control must have a higher reading than 0.700 OD
2- The % avidity of the Control should be greater than 15%.

If one of these conditions is not fulfilled, repeat the test. Remember that the readings obtained will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

Percentage	Avidity	Interpretation
> 30%	Presence of anti-*T gondii* IgG high avidity	Past infection
Between 15 - 30%	Presence of anti-*T gondii* IgG mean avidity (gray area)	It cannot be determined whether it is recent or past infection. Collect a new sample after 2 weeks.
< 15%	Presence of anti-*T gondii* IgG low avidity	Recent infection

Important: the results must be validated with clinical evaluations and other diagnostic tests. Low avidity results cannot be used to diagnose acute toxoplasmosis. High avidity results refer to an infection of more than 4 months. If avidity results are not consistent with the clinical evidence, we suggest additional testing to confirm the result. For diagnostic purposes, the results should be used in conjunction with other data (IgG, IgM, clinical evidence, etc.)

SPECIFIC PERFORMANCE CHARACTERISTICS FOR TOXOTEST IgG ELISA

Sensitivity

Clinical Sensitivity in Performance Panels
In a study performed on an international commercial panel, the following results were obtained:
PTT 201 (Anti-*T gondii* Performance Panel, BBI, USA): 22 out of 22 reactive samples were detected.
PTT202 (Anti-*T gondii* Performance Panel, BBI, USA): 20 out of 20 reactive samples were detected.

Clinical sensitivity

In a study of (202) samples with IgG antibodies to *T. gondii* confirmed by different methods, 98.5% were found reactive with the Toxotest IgG ELISA kit (+ Avidity).

Analytical Sensitivity

The analytical sensitivity or detection limit of the system, i.e. the minimum amount of the specific analyte detectable by the assay is 0.6 IU/ml.

Specificity

In a study of 439 samples with less than 15 IU/ml of IgG antibodies to *T. gondii* 3 different health centers, a specificity of 99.1% was found.
The possible occurrence of crossed reactivity was studied testing samples from 97 individuals negative for Toxoplasma antibodies but with different clinical conditions that may cause unspecific reactions with the Toxotest IgG ELISA (+ Avidity) assay. These conditions include patients with autoimmune diseases (rheumatoid factor, anti-nucleus, etc.) or infectious diseases other than Toxoplasmosis (Chagas, HIV, HTLV, hepatitis C, hepatitis B, syphilis, etc.). For this population the specificity was 100%, being the false positive a sample with a high rheumatoid factor.

Accuracy

The accuracy of the test was evaluated following the EP15A protocol recommended by the CLSI. The tests were performed on samples with different reactivity levels. A daily assay was performed to evaluate each sample in quadruplicate during 5 days.

	Mean UI/ml	Intra-assay		Total	
		S	CV	S	CV
Sample 1	14,41	1,570	10,87%	2,543	17,61%
Sample 2	24,68	1,144	4,64%	1,616	6,55%
Sample 3	39,81	1,693	4,25%	3,220	8,09%
Sample 4	109,21	8,049	7,37%	7,850	7,19%

n=5

SPECIFIC PERFORMANCE CHARACTERISTICS FOR AVIDITY

Specificity

The diagnostic specificity was evaluated using a panel of 100 samples with chronic or past infection clinically tested and found to be 97%.

Sensitivity

The diagnostic sensitivity was evaluated by a panel of 42 samples with primary infection, resulting equal to 100%.

Accuracy

The accuracy of the test was evaluated following the EP15A protocol recommended by the CLSI. The tests were carried out with samples from different levels anti-*Toxoplasma gondii* IgG avidity. A daily assay was performed to evaluate each sample in quadruplicate during 5 days.

	Mean Avidity (%)	Intra-assay		Total	
		S	CV	S	CV
Sample 1	6,3	0,59	9,38%	0,941	14,96%
Sample 2	54,9	4,95	8,50%	5,033	8,65%
Sample 3	26,5	2,257	8,71%	2,279	8,80%

n=5

WIENER LAB. PROVIDES

- Kit for 96 determinations (Code 1743252).

REFERENCES

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- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, EP17-A (2004)
- User Demostration of Performance for Precision and Accuracy – Approved Guideline EP15-A (2001).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.

SYMBOLS EXPLANATION

Policubeta | Sensib. Coated microtitration plate	**Diluyente | Muestra** Sample Diluent
Conjugado | Conc. Concentrated Conjugate	**Conjugado | Diluy.** Conjugate Diluent
Revelador Substrate	**Buf. Lavado | Conc.** Concentrated Wash Buffer
Calibr | Calibrator 1-3	**Control | -** Negative Control
Stopper Stopper	**Reactivo | Dis.** Dissociating Reagent

SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
	Harmful
	Corrosive / Caustic
	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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