

TOXO IgG

**Enzyme Immunoassay for the
quantitative/qualitative determination of
IgG antibodies to Toxoplasma gondii
in human serum and plasma**

- for "in vitro" diagnostic use only -



DIA.PRO

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Code: TOXOG.CE
96 Tests

TOXO IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to *Toxoplasma gondii* in plasma and sera.

For "in vitro" diagnostic use only.

B. INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man. The detection of IgM antibodies to *T.gondii* is particularly helpful for the diagnosis of acute infections in "risk" individuals, in association with AIDS, organ transplantation and pregnancy. As most of *T.gondii* infections are mild or asymptomatic in otherwise healthy individuals, the detection of *T.gondii* specific IgM antibodies, in absence of detectable specific IgG, has become important for the monitoring of acute infections in pregnant women, as the parasite can lead to severe birth defects. Moreover, as *T.gondii* infections are most severe in immunocompromised patients, where the disease can be fatal, acute infections due to this parasite have to be distinguished from other disorders.

Recently developed IgM capture assays provide the clinician with a helpful and reliable test, not affected by the rheumatoid factor as it happens to be in classic sandwich tests.

C. PRINCIPLE OF THE TEST

Microplates are coated with native *T. gondii* antigens, highly purified by sucrose gradient centrifugation and inactivated.

The solid phase is first treated with the diluted sample and IgG to *T. gondii* are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti *Toxoplasma gondii* IgG are detected by the addition of polyclonal specific anti human IgG antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti *Toxoplasma gondii* IgG antibodies present in the sample. A Calibration Curve, calibrated against the W.H.O 3rd international standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. : Microplate: MICROPLATE

12 strips x 8 microwells coated with purified and gamma-irradiation inactivated *Toxoplasma gondii* in presence of bovine proteins.

Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N°....

Ready to use and colour coded, calibrated against the 3rd international standard produced by the World Health Organization (WHO). The calibration curve range is as follows:

4ml CAL 1 = 0 WHO IU/ml

4ml CAL 2 = 50 WHO IU/ml

2ml CAL 3 = 100 WHO IU/ml

2ml CAL 4 = 250 WHO IU/ml

2ml CAL 5 = 500 WHO IU/ml

4ml CAL 6 = 1000 WHO IU/ml.

It contains Toxo IgG positive plasma titrated against WHO 3rd international standard code TOXM, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 2% casein, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL

n° 1 vial - Lyophilized. To be dissolved with the volume of EIA grade water reported on the label. It contains fetal bovine serum, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives and human plasma positive to *T.gondii* calibrated at 250 IU/ml +/-10%, whose content is calibrated on 3rd international standard produced by the World Health Organization (WHO - TOXM).

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

4. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. Enzyme conjugate : CONJ

2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. Coded with 0.01% red alimentary dye

6. Chromogen/Substrate: SUBS TMB

1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide or H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H2SO4 0.3 M

1x15ml/vialIt contains 0.3 M H₂SO₄ solution.

Attention !: Irritant (Xi R36/38; S2/26/30)

8. Specimen Diluent: DILSPE

2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not point out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or

microbial filaments and bodies should be discarded as they could give rise to false results.

4. Sera and plasma can be stored at +2..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.
6. Samples whose anti-T.gondii IgG antibody concentration is expected to be higher than 1000 IU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 IU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 µl of each specimen with 450 µl of Cal 0 (1:10). Then 50 µl of the 1:10 dilution are diluted with 450 µl of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing. In this case, call Dia.Pro's customer service. Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

Note: *The control after dissolution is not stable. Store frozen in aliquots at -20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: *Once diluted, the wash solution is stable for 1 week at +2..8°C.*

Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent

Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Legenda: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm 5\%$.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 2.0 ; repeatability $\geq 1\%$. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

The kit may be used for quantitative and qualitative determinations as well.

M.1 Quantitative analysis

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 μ l Sample Diluent and then 10 μ l sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 μ l samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 μ l Sample Diluent + 10 μ l sample) into a second dilution platform. Make then the instrument aspirate first 100 μ l Sample Diluent, then 10 μ l liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 μ l calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st and 2nd wells (positions A1 and B1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st and the 2nd blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important notes:

1. Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.
2. Mix thoroughly the Enzyme Conjugate on vortex before its use !!!
7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: Do not expose to strong direct illumination. High background might be generated.

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

M.2 QUALITATIVE ANALYSIS

If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st well (positions A1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 IU/ml and 100 µl of Calibrator 50 IU/ml in duplicate, and 100 µl of Calibrator 1000 IU/ml in single. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important notes:

1. Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.
2. Mix thoroughly the Enzyme Conjugate on vortex before its use !!!
7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.
10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

N. ASSAY SCHEME

Method	Operations
Calibrators & Control	100 µl
Samples diluted 1:101	100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS	S 7									
H	CAL3	CS	S 8									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators CS = Control Serum S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC. Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Calibrator 0 IU/ml (CAL1)	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 50 IU/ml	OD450nm > OD450nm CAL1 + 0.100
Calibrator 1000 IU/ml	OD450nm > 1.000
Control Serum	250 WHO IU/ml +/-10%

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
Calibrator 0 IU/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one);

	4. that no contamination of the negative calibrator or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator 50 IU/ml OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 1000 IU/ml < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: a) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. b) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

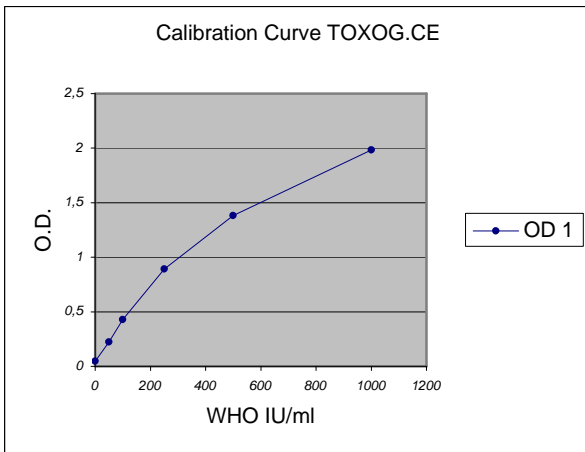
P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti Toxoplasma gondii IgG antibody in samples.

An example of Calibration curve is reported in this page.



Important Notes:

Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 50 IU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
 Mean Value: 0.022 OD450nm
 Lower than 0.150 – Accepted
 Calibrator 50 IU/ml: 0.250 – 0.270 OD450nm
 Mean Value: 0.260 OD450nm
 Higher than Cal 0 + 0.100 – Accepted
 Calibrator 1000 IU/ml: 2.845 OD450nm
 Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Toxoplasma gondii due to the risk of severe neonatal malformations. The cut-off of the device has been set at 50 IU/ml, and not lower as some other devices present on the market do, in order to assure the highest diagnostic value to the test, in particular when the assay is applied in pregnancy monitoring. Upon infection, in fact, a part from the very first time of seroconversion, patients develop a strong immunological response to Toxoplasma gondii, far exceeding 50 IU/ml. Low titer antibodies (below 50 IU/ml) mostly show low avidity to the infective agent and may represent a diagnostic marker of a recent infection, in combination with IgM. Pregnant women, with antibodies concentrations below 50 IU/ml are by the device considered negative in order to make the clinician consider them “risk” patients and follow them up for both IgG and IgM along pregnancy. Samples with a concentration higher than 50 WHO IU/ml are considered positive for anti Toxoplasma gondii IgG antibody, surely able to provide immunity against the infection. This titer is considered the lowest concentration of IgG to provide an effective immunological protection against a second infection of Toxoplasma gondii by NCCLS, USA.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. In the follow-up of pregnancy for Toxoplasma Gondii infection a positive result (presence of IgG antibody > 50 IU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what indicated in the standard prEN 13612.

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 3rd international standard produced by the World Health Organization (WHO).

The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.

The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay.

OD450nm values

WHO IU/ml	TOXOG.CE Lot # 0503	TOXOG.CE Lot # 0403	TOXOG.CE Lot # 0303
250	0.816	0.853	0.974
100	0.365	0.398	0.445
50	0.209	0.244	0.246
10	0.094	0.125	0.108
Std 0	0.033	0.031	0.056

The assay shows a limit of detection better than 10 IU/ml.

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested in a Performance Evaluation trial on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of Toxoplasma gondii Virus infection were tested.

The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

No interference was observed.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested. No crossreaction was observed.

An overall value > 98% of specificity was found when examined on more than 100 specimens.

4. Precision:

It has been calculated on three Calibrators, examined in 16 replicates in three separate runs with three lots.

Results are reported as follows

TOXOG.CE: lot 0503

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.067	0.066	0.070	0.067
Std.Deviation	0.006	0.005	0.006	0.006
CV %	9.3	7.7	9.0	8.7

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.276	0.259	0.268	0.267
Std.Deviation	0.025	0.006	0.010	0.014
CV %	9.1	2.4	3.6	5.0

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.768	2.657	2.707	2.711
Std.Deviation	0.118	0.098	0.101	0.106
CV %	4.3	3.7	3.7	3.9

TOXOG.CE: lot # 0403

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.067	0.065	0.068	0.066
Std.Deviation	0.003	0.004	0.006	0.004
CV %	5.2	6.3	8.3	6.6

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.270	0.262	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.5	3.4	3.1	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.765	2.652	2.718	2.712
Std.Deviation	0.115	0.101	0.092	0.103
CV %	4.2	3.8	3.4	3.8

TOXOG.CE: lot # 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.068	0.067	0.069	0.068
Std.Deviation	0.004	0.004	0.006	0.004
CV %	5.1	6.1	8.0	6.4

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.268	0.261	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.6	3.3	3.2	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.766	2.651	2.719	2.712
Std.Deviation	0.115	0.100	0.091	0.102
CV %	4.2	3.8	3.3	3.8

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy

The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 4.000 IU/ml.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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