TETOX IgG

Enzyme ImmunoAssay (ELISA) for the quantitative determination of IgG antibodies to Tetanus toxoid in human serum and plasma

- for “in vitro” diagnostic use only -

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REF TETG.CE
96 / 480 Tests

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A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative determination of IgG antibodies to Tetanus toxoid in human serum and plasma.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Tetanus is caused by an infection with the bacterium *Clostridium tetani* which is commonly found in soil, dust and manure. Tetanus occurs in all parts of the world but is most frequent in hot and wet climates where the soil contains a lot of organic matter. The bacteria generally enter through a break in the skin such as a cut or puncture wound by a contaminated object. They produce toxins that interfere with muscle contractions, resulting in the typical symptoms. Diagnosis is based on the presenting signs and symptoms. The disease does not spread between people. Infection can be prevented by proper immunization with the inactivated tetanus toxoid vaccine. In those who have a significant wound and less than three doses of the vaccine both immunization and tetanus immune globulin are recommended. In those who are infected tetanus immune globulin or, if it is not available, intravenous immunoglobulin (IVIG) is used.
The determination of IgG to the Tetanus Toxin is quite important for the decision to proceed or not in active or passive vaccination.

C. PRINCIPLE OF THE TEST
Microplates are coated with native inactivated Tetanus toxoid.
The solid phase is first treated with the diluted sample and IgG to Tetanus toxoid are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Tetanus toxoid IgG are detected by the addition of polyclonal specific anti hIgG 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 Tetanus toxoid IgG antibodies present in the sample. A Calibration Curve, calibrated against the WHO 1st International Standard for Tetanus immunoglobulin, Human, NIBSC code TE-3, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
The standard kit contains reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with inactivated native Tetanus toxoid 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° …
5x2ml/vial. Ready to use and color coded standard curve derived from human plasma positive for Tetanus toxoid IgG and titrated on WHO 1st International Standard TE-3 ranging:
CAL1 = 0 WHO IU/ml
CAL2 = 0.1 WHO IU/ml
CAL3 = 0.5 WHO IU/ml
CAL4 = 1.0 WHO IU/ml
CAL5 = 5.0 WHO IU/ml

It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300as preservatives. Standards are blue colored.

3. Positive Control Serum: POS CONTROL
1x2ml. Ready to use. Human serum base reactive for anti Tetanus Toxoid IgG antibodies calibrated at 1.0 ± 20% WHO 1st International Standard TE-3. It contains 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The Control Serum Positive is **green coded**.

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 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and **red coded**. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02 mg/ml gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. **Black vial**. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2) and 4% dimethylsulphoxide.
Note: *To be stored protected from light as sensitive to strong illumination.*

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

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 0.045% ProClin 300 as preservatives. The reagent is **blue coded**.

9. Plate sealing foils n°2

10. Package insert n°1

For the optional method (see proper chapter):

Calibrator 6: CAL 6
1x2.0 ml/vial. **8 WHO IU/ml.** Ready to use (diluted 1:250) **light yellow coded** calibrator derived from human plasma positive for Tetanus toxoid IgG and titrated on WHO 1st International Standard TE-3. ***Note***: *To be used only in the Optional Method.*

Calibrator 7: CAL 7
1x2.0 ml/vial. **20 WHO IU/ml.** Ready to use (diluted 1:250) **dark yellow coded** calibrator derived from human plasma positive for Tetanus toxoid IgG and titrated on WHO 1st International Standard TE-3. ***Note***: *To be used only in the Optional Method.*

Important note: Only upon specific request, Dia.Pro can supply reagents for 480 tests, as reported below**:**

Microplate	n°5
Calibration Curve	5x10ml/vial
Positive Control Serum	1x10ml/vial
Wash buff 20X conc	2x150ml/bottles
Enzyme Conjugate	2x40ml/vial
Chrom/Substrate	2x40ml/bottle
Sulphuric Acid	2x40ml/bottles
SampleDiluent	4x150ml/bottle
Calibrator 6	1x10ml/vial
Calibrator 7	1x10ml/vial
Plate seal foils	n° 10
Pack. insert	n°1
Number of tests	**480**
Code	**TETG.CE.480**

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 microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
6. Calibrated ELISA microplate washer.
7. 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-born 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. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
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 pointed 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 in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 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 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

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 standards. Mix carefully on vortex before use.
***Note**: CAL 6 and CLA 7 have to be used only for the Optional Method.*

Positive Control Serum
Ready to use standard. Mix carefully on vortex before use.

Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
***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.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).
Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
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, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 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. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. 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. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as reported in the specific section.
8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.

12. 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.
Mix carefully all the liquid components on vortex and then proceed as described below.

A. Standard Method:

Intended for the determination of the degree of protection against tetanus infection before or after vaccination.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the calibrators and Controls serum as they are ready to use.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators in duplicate and 100 µl of Positive Control Serum in duplicate.
4. Then dispense 100 µl of diluted samples in each properly identified well. It is advised to dispense them in duplicate for a more precise quantification of IgG.
5. Incubate the microplate for **60 min 37°C**.

Important note: *Strips have to be sealed with the supplied adhesive sealing foil.*
Do not cover strips when using ELISA automatic instruments.

6. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover again with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. Incubate the microplate for **30 min at 37°C**.
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate for **15 minutes** at **18-25°C in the dark**.

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

11. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive standards and positive samples from blue to yellow.
12. 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, mandatory), blanking the instrument on A1 or B1 or both.

B. Optional Method:

Intended for testing high titre samples with an automatic device.

1. Use the ready to use optional standards at 0-8-20 IU/ml.
2. Dilute samples 1:250 with the Sample Diluent. Samples might be dispensed in single.

Important note: *If the dilution is carried out by an automatic device make the instrument take 240 ul of Sample Diluent and then 10 ul of sample dispensing then all into a dilution plate and properly mixing (dilution 1:25). Thereafter make the instrument aspirate 90 ul Sample Diluent and then 10 ul 1:25 diluted sample (dilution 1:10). Dispense into a proper assay well and mix (final dilution 1:250).*

3. Dispense 100 µl of standards 0-8-20 IU/ml possibly in duplicate. Standards are ready to use: do not dilute them.
4. Incubate the assay plate for **30 min at room temperature**.
5. Wash as reported in the standard procedure.
6. Dispense 100 µl ready to use Enzyme Conjugate.
7. Incubate for **30 min at room temperature**.
8. Wash as in step 5.
9. Proceed as reported in the standard method at point 10 and followings

Important note for quality control:

The correct dispensation of samples and reagents is important to avoid in particular false negative results due to lack of sample or of a reagent. The following Procedure of Verification of Dispensation is recommended:
The addition of the diluted Sample (blue), the Conjugate (red) and the Chromogen/Substrate (pale blue) is verified by reading wells at 405nm, according to what defined in the following table:

Steps (addition of)	Volumes dispensed	Verification (reading)
Empty well	/////	OD405nm < 0.070
Diluted sample	100 µl	OD405nm ≥ 0.140
Conjugate	100 µl	OD405nm ≥ 0.180
Chromogen/Substrate	100 µl	OD405nm ≥ 0.050

In case of a non conformity do not proceed further on and check the instrument and the volumes of reagents.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. 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.*
3. *Positive Control Serum does not affect the test results calculation. The Positive Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Standard Method	Operations
Calibrators and Positive Control Serum Samples diluted 1:101	100 µl 100 µl
1st incubation	**60 min**
Temperature	**37°C**
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**30 min**
Temperature	**37°C**
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H_2O_2	100 µl
3rd incubation	**15 min**
Temperature	**18-25°C**
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

Optional Method	Operations
Ready-to-use Standards 0-8-20 IU/ml	100 µl
Samples diluted 1:250	100 µl
1st incubation	**30 min**
Temperature	**18-25°C**
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**30 min**
Temperature	**18-25°C**
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H_2O_2	100 µl
3rd incubation	**15 min**
Temperature	**18-25°C**
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme for the Standard Method is reported below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 2									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 3									
E	CAL2	POSCS	S 4									
F	CAL2	POSCS	S 4									
G	CAL3	S1	S 5									
H	CAL3	S1	S 5									

Legenda: BLK = Blank CAL = Calibrators POSCS= Positive Control Serum
S = Sample

An example of dispensation scheme for the Optional Method is reported below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S1										
B	BLK	S2										
C	CAL1	S3										
D	CAL1	S4										
E	CAL6	S5										
F	CAL6	S6										
G	CAL7	S7										
H	CAL7	S8										

Legenda: BLK = Blank CAL = Calibrators S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Standard Method:

Check	Requirements
Blank well	$_{mean}$OD450nm < 0.050
CAL1 0 IU/ml	$_{mean}$OD450nm < 0.050 after blanking
CAL2 0.1 IU/ml	$_{mean}$OD450nm ≥ 0.150 after blanking
CTRL POS	$_{mean}$OD450nm = $_{mean}$OD450nm of the CAL4 1 IU/ml ± 20%

Optional Method:

Check	Requirements
Blank well	$_{mean}$OD450nm < 0.050
CAL 1 0 IU/ml	$_{mean}$OD450nm < 0.050 after blanking
CAL 7 20 IU/ml	$_{mean}$OD450nm ≥ 1.400 after blanking

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

If they do not, before proceeding any further check out the following:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 0 IU/ml > 0.050 OD450nm after blanking	1. the efficiency of the washer 2. that the proper washing solution has been used 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 occurred by spills of positive samples or the enzyme conjugate; 5. that micropipettes are not contaminated 6. that the washer needles are not blocked or partially obstructed.
Problem	**Check**
CAL 2 0.1 IU/ml OD450nm < 0.150 **CAL7 20 IU/ml** OD450nm < 1.400	1. that the assay 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 settings are correct; 4. that no external contamination of the calibrator has occurred. 5. that the kit is not expired
Positive Control Serum POSCS 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. 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 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.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. RESULTS

Standard Method:
If the test turns out to be valid, use an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm/620-630nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Tetanus toxoid IgG antibody in samples.

Example of a typical Calibration Curve after blanking:
CAL1 0 IU/ml = 0.005 OD450nm/620-630nm
CAL2 0.1 IU/ml = 0.180 OD450nm/620-630nm
CAL3 0.5 IU/ml = 1.000 OD450nm/620-630nm
CAL4 1.0 IU/ml = 1.800 OD450nm/620-630nm
CAL5 5.0 IU/ml = 2.500 OD450nm/620-630nm
Do not use these values to generate the assay standard curve.

Optional Method:
Use a point-to-point fitting program to draw the calibration curve from the values obtained by reading at 450nm/620-630nm.

Then on the calibration curve calculate the concentration of anti Tetanus toxoid IgG antibody in samples.

Example of a typical Calibration Curve after blanking:
CAL 1 0 IU/ml = 0.010
CAL 6 8 IU/ml = 1.500
CAL 7 20 IU/ml = 2.600

Do not use these values to generate the assay standard curve.

Q. INTERPRETATION OF RESULTS
The following table reports how to interpret the results:

IU/ml found	Interpretation
< 0.1	Immunization recommended
0.11-0.5	Vaccination recall required
0.5-1.0	To be controlled after 1-2 years
1.1 – 5.0	To be controlled after 2-4 years
> 5.0	To be controlled after 4-8 years

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. *Anyway the decision to immunize the patient has to be taken by the medical doctor, not by the laboratory.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*

R. PERFORMANCES

Limit of detection
The limit of detection (analytical sensitivity) was calculated as mean OD450nm/620-630nm CAL 0 IU/ml + 5 SD. The assay shows a limit of detection of 0.035 IU/ml when the standard method is used. However for the diagnostic performances reported below a **cut-off of 0.1 IU/ml** was used.

Diagnostic sensitivity:
The diagnostic sensitivity was tested in a study of performance evaluation on a panel of samples composed of about 500 sera, classified positive by a CE marked reference ELISA.
The assay in such study showed a correlation value of 100% with the standard method.

Diagnostic specificity:
The diagnostic specificity was determined in the same evaluation on a panel of negative samples composed of about 200 sera, confirmed negative with a CE-marked reference ELISA.
An overall correlation value of 100% was found in such study.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin) and sera were used to determine the value of specificity.
No interference by other antibodies was observed.

Precision:
It was calculated in the standard method on the CAL2 (0.1 IU/ml) and on the CAL4 (1 IU/ml) examined in 48 replicates in three separate runs for three lots.
A CV% value ranging 3-20 was found.

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 determination of IgG.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
Hemoglobin > 10 mg/ml, bilirubin > 0.5 mg/ml and triglycerides > 5.0 mg/ml could give false results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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