Chlamydia Trachomatis IgM

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM antibodies to Chlamydia Trachomatis in human serum and plasma

- for “in vitro” diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni (Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF CTM.CE
96 Tests

Chlamydia Trachomatis IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Chlamydia Trachomatisin human plasma and sera. For "in vitro" diagnostic use only.

B. INTRODUCTION

Chlamydia trachomatis is a bacterium-like obligate intracellular organism that counts at least 15 recognized serotypes. C.trachomatis is one of the three distinct species within the genus Chlamydia (trachomatis, psittaci and pnemoniae).
C.trachomatis infection in adults is responsible of most of sexually acquired urethritis in men, mucopurulent cervicitis in women, pelvic inflammatory disease, lymphogranuloma venereum, most of acute urethral syndromes, ocular infections, proctocolitis and epididymitis. In infants, the organism is responsible of pneumonia and conjunctivitis.
Infections due to C.trachomatis stimulates the patient to generate a strong immunological response both in IgG, lasting a long time, and IgA and IgM, whose presence is more correlated with an ongoing infection or a recent event.
The determination of species-specific IgG, IgM and IgA is a helpful tool for the clinician to identify the infective agent and to decide the right therapy.

C. PRINCIPLE OF THE TEST

Microplates are coated with a species-specific polypeptide derived from C.trachomatis major outer membrane antigen.
In the 1st incubation, the solid phase is treated with diluted samples and anti-CT IgM are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-CT IgM are detected by the addition of anti hIgM antibody, labeled 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-CT IgM antibodies present in the sample.
The presence of IgM in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples.
Neutralization of IgG anti-CT, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with CT specific immunodominant native antigens *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 4°C.

2. Negative Control: CONTROL -
1x2.0 ml/vial. Ready to use. It contains, human IgM antibodies negative to Ch. Trach., 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.
The Negative Control is pale yellow color coded.

3. Positive Control: CONTROL +
1x2.0 ml/vial. Ready to use. It contains high titer human IgM antibodies positive to Ch. Trach., 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.
The Positive Control is green yellow color 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 *0.1% Kathon GC*.

5. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated *goat* polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

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 (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
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 H_2SO_4 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. Neutralizing Reagent: SOLN NEUT
1x8ml/vial. It contains goat anti hIgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

10. Plate sealing foils n°2

11. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled 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 possibly 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-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 tem perature 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 pointed out any relevant loss of activity up to six 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 u p to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any fr ozen samples should not be freezed/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 is not turned to dark green, indicating a defect of manufacturing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C. When opened the first time, residual str ips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Negative Control
Ready to use components. Mix carefully on vortex before use.

Positive Control
Ready to use components. Mix carefully on vortex before use.

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.

Neutraling Reagent
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 (tole rance 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 section "Internal quality Control". 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 ideally with a second filter (620-630nm) 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 "Internal Quality Control". 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. 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 manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
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.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Controls as they 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 A1 well empty for the operation of blanking.

3. Dispense 50 µl Neutralizing Reagent in all the wells, except A1 used for blanking operations and in the wells used for the Positive and Negative Controls.

4. Dispense 100 µl of Negative Control in triplicate, 100 µl of Positive Control in duplicate and 100 ul of diluted samples in each properly identified well.
5. 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.*

6. Wash the microplate with an automatic as reported previously (section I.3).

7. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

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 **60 min at +37°C** .

9. Wash microwells as in step 6.

10. 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** .

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

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step *10.* Addition of acid will turn the positive calibrators, the control serum and the 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 possibly at 620-630nm (background subtraction), blanking the instrument on A1.

General 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.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control	100 µl
Neutralizing Reagent	50 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/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	NC	S6										
E	PC	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
PC = Positive Control 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 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
Negative Control	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Positive Control	OD450nm > 0.750

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/Sustrate solution has not got contaminated during the assay
Negative Control > 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 control instead of the negative one; 4. that no contamination of the negative control or of their wells has occurred due spills of positive samples or 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.
Positive Control < 0.750 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong control) ; 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.

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

P. RESULTS

If the test turns out to be valid, results are calculated from the mean OD450nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

Cut-Off = NC + 0.250

Important note*: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Test results are interpreted as a ratio of the sample OD450nm value (S) and the cut-off value (Co), or S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9≤S/Co<1.0	*Equivocal*
≥ 1.0	Positive

A negative result indicates that the patient has not developed IgM antibodies to C. Trachomatis.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of an ongoing C. Trachomatis infection and therefore the patient should be treated accordingly.

Important notes:
1. *C. Trachomatis IgM results alone are not enough to provide a clear diagnosis of Chlamydia Trachomatis infection. Other tests for Chl. Trachomatis (supplied by Dia.Pro Diagnostic BioProbes s.r.l. at code CTA.CE and CTG.CE), should be carried out.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

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

Negative Control: 0.100 – 0.120 – 0.080 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.150 – Accepted

Positive Control: 1.000 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 0.100+0.250 = 0.350

Sample 1: 0.080 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co ≥ 1.0 = positive

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted on panels of positive and negative samples with reference to a CE marked reference kit.
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1. Limit of detection
No international standard for Ch. Trachomatis IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past Ch. Trachomatis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:
The diagnostic performances were evaluated on samples supplied by two external centers, with excellent experience in the diagnosis of infectious diseases.
The diagnostic **sensitivity** was studied on more than *60* samples, positive with the reference kit. Positive samples were collected from patients with a clinical history of Chlamydia trachomatis infection.
The diagnostic **specificity** was determined on panels of more than 100 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
Potentially interfering samples (pregnancy, emolyzed, lipemic, RF+) were tested.
No crossreaction was observed.
The Performance Evaluation provided the following values :

Sensitivity	≥ 98 %
Specificity	≥ 98 %

3. Precision:
It has been calculated on three samples, a negative, a low positive and a high positive, examined in 16 replicates in three separate runs for three lots.
Results are reported as follows:

CTM.CE: lot P1

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.051	0.051	0.053	0.052
Std.Deviation	0.005	0.006	0.006	0.006
CV %	10.1	10.9	10.8	10.6

Low Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.616	0.609	0.607	0.610
Std.Deviation	0.051	0.048	0.046	0.048
CV %	8.2	7.8	7.6	7.9

High Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.255	1.270	1.262	1.262
Std.Deviation	0.050	0.049	0.058	0.052
CV %	4.0	3.9	4.6	4.1

CTM.CE: lot P2

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.050	0.048	0.049	0.049
Std.Deviation	0.005	0.005	0.005	0.005
CV %	10.4	10.0	10.2	10.2

Low Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.603	0.591	0.596	0.596
Std.Deviation	0.048	0.046	0.045	0.046
CV %	8.0	7.8	7.5	7.7

High Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.212	1.231	1.245	1.229
Std.Deviation	0.049	0.043	0.051	0.048
CV %	4.0	3.5	4.1	3.9

CTM.CE: lot P3

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.051	0.050	0.050	0.050
Std.Deviation	0.005	0.005	0.005	0.005
CV %	10.0	9.9	9.4	9.8

Low Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.618	0.615	0.616	0.616
Std.Deviation	0.049	0.046	0.045	0.047
CV %	7.9	7.5	7.4	7.6

High Positive Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.216	1.239	1.233	1.229
Std.Deviation	0.048	0.046	0.050	0.048
CV %	3.9	3.7	4.1	3.9

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

4. Accuracy

The assay accuracy has been checked by the dilution test. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out.

S. LIMITATIONS

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.

False positivity has been assessed as less than 2% of the normal population.

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Dia.Pro. Diagnostic Bioprobes Srl.
Via G. Carducci n°27 – Sesto San Giovanni (MI) - Italy

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