ZIKV IgG

Enzyme Immunoassay for the determination of anti Zika Virus IgG antibody in human serum and plasma

- for “in vitro” diagnostic use only -

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REF ZIKVG.CE
96 Tests

ZIKV IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative and semi quantitative determination of IgG antibodies to Zika Virus in human plasma and sera. For "in vitro" diagnostic use only.

B. INTRODUCTION

The Zika virus belongs to Flaviviridae and the genus *Flavivirus*, and is thus related to the Dengue, Yellow Fever, Japanese Encephalitis and West Nile viruses. Like other flaviviruses, Zika virus is enveloped and icosahedral and has a nonsegmented, single-stranded, positive-sense RNA genome.

The RNA genome genes encode for nonstructural proteins and structural proteins, whose most immunogenic ones are NS1 and ENV antigens. The structural proteins encapsulate the virus.

There are two lineages of the Zika virus: the African lineage, and the Asian lineage. Phylogenetic studies indicate that the virus spreading in the Americas is most closely related to the Asian strain, which circulated in French Polynesia during the 2013 outbreak.

The Zika virus is transmitted by daytime-active mosquitoes as its vector. It is primarily transmitted by the female *Aedes aegypti* in order to lay eggs, but has been isolated from a number of arboreal mosquito species in the *Aedes* genus, with an extrinsic incubation period in mosquitoes of about 10 days.

Since 2015, news reports have drawn attention to the spread of Zika in Latin America and the Caribbean.

In 2015, Zika virus RNA was detected in the amniotic fluid of two pregnant women whose fetuses had microcephaly, indicating that the virus had crossed the placenta and could have caused a mother-to-child infection.

According to the WHO on 5 February 2016, a causal link between the Zika virus and microcephaly was "strongly suspected but not yet scientifically proven" and "Although the microcephaly cases in Brazil are spatio-temporally associated with the Zika outbreak, more robust investigations and research is needed to better understand this potential link."

The new recommendations include offering serologic testing to pregnant women without Zika fever symptoms who have returned from areas with ongoing Zika virus transmission in the last 2–12 weeks; and for pregnant women without Zika symptoms living in such areas, they recommend testing at the beginning of prenatal care and follow-up testing in the fifth month of pregnancy.

C. PRINCIPLE OF THE TEST

Microplates are coated with Zika Virus synthetic antigen.

In the 1st incubation, the solid phase is treated with diluted samples and anti-Zika Virus IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti-Zika Virus IgG are detected by the addition of anti hIgG 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-Zika Virus IgG antibodies present in the sample.

The presence of IgG in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples. The test may be quantitative by plotting the results of the controls and determining the content of IgG in arbU/ml.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with ZIKV synthetic antigen. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -

1x2.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is yellow colour coded.

The control contains 0 arbU/ml of anti ZIKV antibodies.

3. High Positive Control CONTROL + HIGH

1x2.0ml/vial. Ready to use control. It contains 1% goat serum proteins, inactivated human anti ZIKV IgG, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.

The High Positive Control is blue colour coded.

It contains 100 arbU/ml of anti ZIKV antibodies

4. Mid Positive Control CONTROL + MID

1x2.0ml/vial. Ready to use control. It contains 1% goat serum proteins, inactivated human anti ZIKV IgG, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.

The Mid Positive Control is pale blue coded.

It contains 20 arbU/ml of anti ZIKV antibodies.

5. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle 20x 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.

6. Enzyme conjugate : CONJ

1x16ml/vial. Ready to use and red colour 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 and 0.02% gentamicine sulphate as preservatives.

7. 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 H2O2).

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

8. Sulphuric Acid: H2SO4 0.3 M

1x15ml/vialIt contains 0.3 M H2SO4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Specimen Diluent: DILSPE

2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.2% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.

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 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/Substrate 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 6 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 RECOMMANDATIONS

1.Blood is drawn aseptically by venipuncture 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. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3.Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. 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.
5. 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 several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. 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

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

Microplates:
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 strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Controls:
Ready to use. Mix well 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. Mix well 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 of 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; (d) 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 section O "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 for blood screening 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 of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
5. 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.
6. Check that the ELISA reader has been turned on at least 20 minutes before reading.
7. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
8. Check that the micropipettes are set to the required volume.
9. Check that all the other equipment is available and ready to use.
10. 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 100 µl of Negative Control in duplicate, 100 µl of Mid Positive Control in triplicate, 100 µl of High Positive Control in duplicate and 100 µl of 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 as reported previously (section I.3).
6. 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.*

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**.

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 *10*. Addition of acid will turn the positive control 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 (mandatory).

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~~. Fingerprints 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. *If the assay has to be semi-quantitative, use the value in ArbU/ml of the controls to draw a point-to-point calibration curve and measure the ArbU/ml of the sample.*

N. ASSAY SCHEME (qualitative / semi-quantitative)

Method	Operations
Controls 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	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630

An example of dispensation scheme for qualitative/semi-quantitative assay is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 1	S 9									
B	NC	S 2	S 10									
C	NC	S 3	S 11									
D	MPC	S 4	S 12									
E	MPC	S 5	S 13									
F	MPC	S 6	S 14									
G	HPC	S 7	S 15									
H	HPC	S 8	S 16									

Legenda: BLK = Blank, NC = Negative Control, MPC =Mid Positive Control HPC =High Positive Control, 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 their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.200 mean OD450nm value after blanking
High Positive Control	OD450nm > 1.000
Mid Positive Control	OD450nm > 0.400

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 (NC) > 0.200 OD450nm after blanking	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 positive control instead of negative control; 4. that no contamination of the negative control 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.

High Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of high positive 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.
Mid Positive Control < 0.400 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of mid positive 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 these problems happen, after checking, report any residual problem to the supervisor for further actions.

P. INTERPRETATION OF RESULTS

In the **qualitative** determination calculate the Cut-Off value (Co) from the MID POSITIVE control (MPC) by the following formulation:

meanOD450nm MPC / 2 = Co

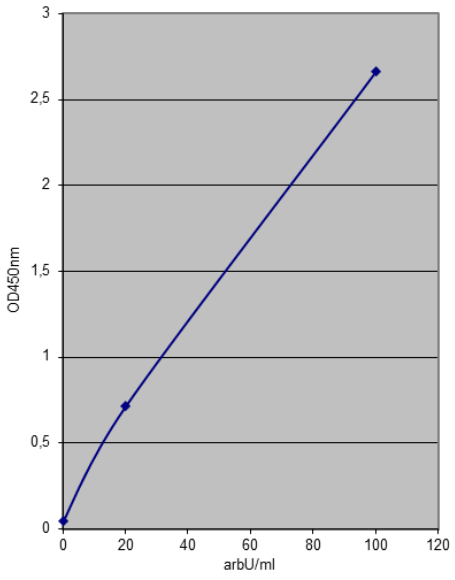
Results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by ZIKV.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined.
A positive result is indicative of a ZIKV infection.

In case of a **semi-quantitative** determination use the ArbU/ml of the controls to draw a point to point calibration curve and then express results in ArbU/ml.

Example of Semi-quantitative assay



Note: Do not use the calibration curve above to make calculations.

Samples showing ArbU/ml < 9 arb/Uml are considered negative for ZIKV IgG antibodies.
Samples showing ArbU/ml > 11 are considered positive.
Samples showing ArbU/ml between 9 and 11 are considered equivocal and patients should be retested.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed by an alternative method capable to detect IgG to ZIKV (example: neutralization assay)*
3. *When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of Zika Virus infection has to be done and released to the patient only by a qualified medical doctor.*
5. *ZIKV infection cannot be diagnosed only on the basis of this ELISA assay.*
6. *See what reported in the chapter LIMITATIONS and WARNINGS*

Q. PERFORMANCES

Evaluation of Performances has been conducted on selected panels carried out in a clinical external center and internally.

Specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. A total of 440 unselected blood donors (including 1st time donors) and non-ZIKV patients were tested. A specificity value of 98% was found.
About 88 healthy pregnant women and potentially interfering samples (hemolized, lipemic, etc.) were examined; no interference was observed.
No false reactivity due to the method of specimen preparation has been observed. 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.
A panel of samples positive for antibodies to other Flavivureses (in particular Dengue Virus and WestNile Virus antibodies) and to Chikungunya Virus was tested; some degree of crossreaction was observed in particular with Chikungunya Virus.

Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
The sensitivity was assessed on a panel of 148 samples positive in a CE-marked reference ELISA; a correlation value of 100% was found.

Precision:

It was calculated on two samples examined in replicates in different runs. CV% ranging 2-16% depending on OD450nm values were seen.

R. LIMITATIONS and WARNINGS

Due to the well-known high genetic homology of ZIKV with the other viruses of the same Flaviviruses family and with Chikungunya Virus, cross-reactions were observed in those populations affected by such infections (mainly South American individuals that could have been infected by Dengue and Chikungunya viruses since years).
The assay should in principle be used only for testing individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or

travel to a geographic region with active Zika virus transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing).
Assay results are therefore useful for the presumptive identification of IgG antibodies to Zika virus. Positive and equivocal results alone are not definitive for diagnosis of Zika virus infection. Confirmation should be done according to CDC indications (neutralization assay in particular).

Repeatable false positive results, not confirmed by other ELISA, were assessed as less than 2% of the normal Flaviviruses and Chikungunya virus free population, due to unidentified interfering substances.
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results. These samples should be tested only after clarification by filtration or centrifugation.

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.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

CE

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