ZIKV IgM

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

- For “in vitro” diagnostic use only -

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REF ZIKVM.CE
96 Tests

ZIKV IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM antibodies to Zika Virus (ZIKV) in human plasma and sera collected from patients suspected of an ongoing ZIKA Virus infection or at risk of it. 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 non segmented, 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 NS1 synthetic antigen showing minimal crossreactivity with the other Flaviviruses.

In the 1st incubation, the solid phase is treated with diluted samples and anti ZIKV IgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-ZIKV 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-ZIKV IgM antibodies present in the sample.

Neutralization of IgG anti-ZIKV, 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 ZIKV-specific synthetic NS1 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 0.1% Kathon GC as preservatives. The negative control is yellow colour coded.

3. Positive Control CONTROL +

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

The Positive Control is dark green colour coded.

4. 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 0.05% Kathon GC.

5. Enzyme conjugate : CONJ

1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated 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 (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, 0.2% 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 minutes 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 (or 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 also 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 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/calibrators 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/calibrators, 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 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. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u 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 storing.
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 components. Mix carefully on vortex before use. Do not dilute!!

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, possibly sterile disposable container

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

Neutralizing Reagent
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 and correctly optimised using the kit controls/calibrators 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/calibrators 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 and maintenance (decontamination and cleaning of needles) of the washer have to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 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 workstation**, 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 “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/Substrate 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 PROCEDURES

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.

M1. Assay protocol for ZIKV IgM testing in suspected ongoing ZIKA Virus infection

1. Place the required number of strips in the plastic holder and carefully identify the wells for Controls and samples.
2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Controls as they are ready-to-use.
3. Leave the A1 well empty for blanking purposes.
4. Dispense 50 µl Neutralizing Reagent in all the wells, except A1 well, used for blanking operations.
5. In the identified positions pipette 100 µl of the Negative Control in triplicate, 100 µl of the Positive Control in single and then 100 µl of diluted samples.

6. Incubate the microplate **for 60 min at +37°C**.

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

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. In all the wells, except A1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

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.*

9. When the second incubation is finished, wash the microwells as previously described (section I.3)
10. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

11. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples and with positive Control will turn from clear to blue.
12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive Control and the positive samples from blue to yellow.
13. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important Note: *This procedure provides the highest sensitivity. Recommended for IgM detection in acute infection.*

M2. Assay protocol for generic laboratory routine ZIKV IgM testing <u>in absence</u> of clinical symptoms of ongoing infection (short).

Proceed as in the previous protocol but shorten the incubations times to **45min** (sample incubation / point 6) + **45min** (conjugate incubation / point 8) + **15min** (TMB / point 11).

Important Note: *This procedure provides the highest specificity. Recommended for pregnancy monitoring and for general screening purposes.*

General Important notes:

1. *If the second filter is not available ensure that no fingerprints 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 TMB chromogen can occur leading to high background.*

N. ASSAY SCHEMES

Method in suspected acute infection	**Operations**
Neutralizing Reagent	50 µl
Controls	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

Short Method	**Operations**
Neutralizing Reagent	50 µl
Controls	100 µl
Samples diluted 1:101	100 µl
1st incubation	**45 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**45 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4										
B	NEG	S5										
C	NEG	S6										
D	NEG	S7										
E	POS	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Legenda: BLK = Blank // NEG = CTRL- // POS = CTRL+ // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out 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:

Parameter	**Requirements**
Blank well	< 0.100 OD450nm
Negative Control	< 0.200 OD450nm after blanking
Positive Control	OD450nm > 0.500

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 perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control > 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); 4. that no contamination of the Control, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.500 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; 4. that the negative control has been erroneously used instead; 5. that a wrong microplate has been used instead

If any of the above problems has occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

NC + 0.250 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph

***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 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not developed IgM antibodies to 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 an ongoing ZIKV active infection.

Important notes:

1. *Any positive sample should be in principle tested a second time and if positive again submitted to a Confirmatory Assay based on an appropriate IVD product of similar characteristics.*
   RT-PCR is recommended only in the very early stage of acute infection as RNA becomes detectable only for quite a few days from the event. RT-PCR is definitely not a confirmation assay for IgG or anyway in the post-acute stage. In fact, both IgM (for a shorter time) and IgG (for a quite longer one) can be present in post-acute phase, even in absence of RNA.
2. *Any negative sample in presence of clinical evident signs of infection should be submitted to confirmation with an appropriate IVD product of similar characteristics.*
3. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
4. *When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.*
5. *Diagnosis of infection has to be done and released to the patient only by a qualified medical doctor, taking in deep consideration clinical symptoms and anamnesis evidences.*

R. PERFORMANCE CHARACTERISTICS

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

1. Limit of detection

No international standard for ZIKV 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 chronic ZIKV infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Sensitivity and Specificity:

The performances were evaluated in a study conducted in external clinical centers, with excellent experience in the diagnosis of infectious diseases and ZIKV.
The sensitivity was assessed on a panel of 28 samples positive in a CE-marked reference ELISA; a correlation value of 100% was found.
The specificity was determined on panels of 400 negative samples from normal individuals and blood donors.
A panel of potentially interfering samples (RF+, hemolised, lipemic, etc.) was also examined. No interference was observed on the samples examined.
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.

The Performance Evaluation provided the following values:

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

It has been calculated on two samples examined in replicates in different runs. Results are reported below summarized in a table:

Average values N = 48	Negative sample	Positive sample
OD450nm	0.077	1,758
Std.Deviation	0.007	0,071
CV %	9.4	4,0

S. LIMITATIONS

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 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 in presence of symptoms (e.g., clinical signs of infection with 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 is recommended).
Assay results are therefore useful for the presumptive identification of IgM 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. In the south American healthy population presence of false positive reactions has been seen to be slightly higher due to cross-reactions to other endemic microorganism.
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.

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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 EN 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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