Dengue virus NS1 Ag

Enzyme Immunoassay (ELISA) for the determination of Dengue Virus NS1 Antigen in human serum and plasma

- for "in vitro" diagnostic use only -

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REF DENS1AG.CE
96 Tests

Dengue virus NS1 Antigen

A. INTENDED USE

Qualitative or semi-quantitative Enzyme Immunoassay (ELISA) for the determination of Dengue virus NS1 Antigen in human plasma and sera.
The kit is intended for the identification of Dengue Virus (DNGV) infected patients, in particular in the first acute infection when antibodies are not present.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Dengue virus is a member of the virus family *Flaviviridae* and is transmitted to people through the bite of the mosquitos *Aedes aegypti* and *Aedes albopictus*.
Each year, 100 million people become infected with dengue virus. However, the majority of deaths that result from dengue infection result from Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).
The incubation period of dengue fever is approximately four days. It is difficult to distinguish dengue fever from other viral diseases, in particular due to other viruses of the same family, and the person usually recovers in 5 days.
The Dengue virus has been shown to have 4 subtypes. These 4 subtypes are different strains of dengue virus that have 60-80% homology between each other. After a person is infected with dengue, they develop an immune response to that dengue subtype. The immune response produced specific antibodies to that subtype's surface proteins that prevents the virus from binding to macrophage cells (the target cell that dengue viruses infect) and gaining entry. However, if another subtype of dengue virus infects the individual, the virus will activate the immune system to attack it as if it was the first subtype. The immune system is tricked because the 4 subtypes have very similar surface antigens. The antibodies bind to the surface proteins but do not inactivate the virus.
The DNGV’s genome is about 11000 bases of positive-sense single stranded RNA (ssRNA) that codes for three structural proteins (capsid protein C, membrane protein M, envelope protein E) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5).
NS1 antigen is present in the blood in the very early stage of infection usually together with the RNA of the virus and before the development of antibodies. Therefore its detection is particularly useful in the identification of the virus affecting the patient in the acute infection.

C. PRINCIPLE OF THE TEST

A couple of mouse monoclonal antibodies specific to DNGV NS1, able to capture such antigen from all the subtypes, is fixed to the surface of microwells. In the first incubation the patient’s serum/plasma is added to the microwell to capture NS1 antigen if present.
After washing, a second mouse monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific to all the subtypes, binds the captured NS1 antigen, if present.
At the end of the second incubation, microwells are washed to remove unbound HRP conjugate.
The chromogen/substrate is then added and, in the presence of captured DNGV NS1, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The color intensity is proportional to the amount of DNGV NS1 present in the sample.

D. COMPONENTS

The standard configuration contains reagents to perform 96 tests and is made of the following components:

Microplate MICROPLATE
n°1. 12 strips of 8 breakable wells coated with anti DNGV NS1, affinity purified mouse monoclonal antibody and sealed into a bag with desiccant.

Negative Control CONTROL -
1x2.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

Positive Control CONTROL +
1x2.0ml/vial. Ready to use control. It contains goat serum, noninfectious recombinant NS1, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The positive control is color pale blue coded.

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 0.1% Kathon GC.

Enzyme Conjugate CONJ
1x32ml/vial. Ready to use. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibody to DNGV NS1, 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

Sample Diluent DILSPE
1x16ml/bottle. It contains 0.1 M Tris-citrate buffer pH 7.4 +/-0.1, 0.1% Tween 20 and 0.1% Kathon GC as preservatives. To be used as additive to the sample.

Chromogen/Substrate SUBS TMB
1x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

Sulphuric Acid H_2SO_4 O.3 M
1x15ml/bottle. It contains 0.3 M H2SO4 solution.
Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

Plate sealing foils n° 2

Package insert

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
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. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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.
6. Upon receipt, store the kit at +2…+8°C into a temperature controlled refrigerator or cold room.
7. 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.
8. 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.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. 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 has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. 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.
13. 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.
14. 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.
15. 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.
16. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
17. 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. 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. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and lipemic ("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 well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive results.
5. Sera and plasma can be stored at +2°…+8°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months, if the plate is correctly stored sealed at +2…+4°C in presence of the desiccant.

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

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use. The positive control does not contain any infective material as it is composed of recombinant synthetic DNGV NS1.

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.

Chromogen/Substrate:
Ready to use. Mix well by end-over-end mixing.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Sulphuric Acid:
Ready to use. Mix well by end-over-end mixing.
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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm ±150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator 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/calibrator 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 has to be carried out according to the instructions of the manufacturer.
5. **Incubation times** have a tolerance of ±5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 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.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) 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 by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
8. 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.
9. **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 full compliance with the essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination 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. 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 manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
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.

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 100 ul Sample Diluent and then 100 ul controls and samples. Then follow the instructions reported below.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay:

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Dispense 100µl Sample Diluent in all the wells except A1.
4. Pipette 100µl of the Negative Control in duplicate, 100ul in single of the Positive Control and then 100ul of samples.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells).
6. Incubate the microplate for **60 min at +37°C**.

Important notes: *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 over wash the microwells as described in section I.4.
8. Dispense 200ul of the ready-to-use conjugate in all the wells except A1, check that the color of the wells is red and then incubate the microplate for **60 min at +37°C**.
9. When the second incubation is over, wash the microwells as previously described (section I.4)
10. Pipette 200 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 **18-24°C for 20 min.** Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
12. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
13. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important general notes:

1. *If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Operations	Procedure
Sample Diluent	100 ul
Controls	100 ul
Sample	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing steps	n° 4-5
Enzyme Conjugate	200ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200 ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported in the following section:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S5										
B	NC	S6										
C	NC	S7										
D	PC	S8										
E	S1	S9										
F	S2	S10										
G	S3	S11										
H	S4	S12										

Legenda: BLK = Blank NC = Negative Control PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.
Ensure that the following results are met:

Parameter	Requirements
Blank well	OD450nm < 0.050
Negative Control (NC)	OD450nm < 0.100 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 OD450nm > 0.050	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) OD450nm > 0.100 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 the negative one); 4. that micropipettes have not become contaminated with positive samples 5. that the washer needles are not blocked or partially obstructed.

Positive Control OD450nm < 0.500	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.050). 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.

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

P. CALCULATION OF THE CUT-OFF

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

$$_{mean}\text{OD450nm NC} + 0.200 = \text{Cut-Off (Co)}$$

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 calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm (S) and the Cut-Off value (Co), mathematically 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 DNGV NS1 is not detectable.
Any patient showing an equivocal result should be retested on a second sample taken 2-5 days after the initial time of collection of the sample.
A positive result is indicative of the presence of NS1 antigen and therefore of an ongoing DNGV infection.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to further analysis of confirmation. A confirmed positive result cannot in principle be used as the only parameter for the diagnosis of DNGV infection. Other diagnostic tests (example: DNGV RNA) and clinical evidences have to be considered.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of DNGV infection has to be taken 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: 0.040 OD450nm
Lower than 0.100 – Accepted
Positive Control: 1.057 OD450nm
Higher than 0.500 – Accepted
Calibrator Mean value:0.325 OD450nm
Higher than 0.200 - Accepted
CUT-OFF = 0.325
Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS

1.Analytical and Diagnostic Sensitivity

The **Analytical Sensitivity** was studied by examining a preparation of recombinant glycosylated NS1derived from the four subtypes of DNGV diluted in NS1 Ag&Ab negative serum by a limiting dilution assay.
All the four subtypes are detected by the system down to a limiting concentration of 5 pg/ml.
The **diagnostic sensitivity** was studied on a panel of 80 samples positive for NS 1 Antigen with reference kit collected in the early phase of acute infection.
A sensitivity of 100 % was observed in this study.
To verify that the assay is able to detect NS1 Antigen deriving from all the four serotypes, serotype specific samples were tested. NS1 was detected in all of the four ones.

2.Diagnostic Specificity

About 1500 samples collected in Italy, region not yet endemic for DNGV infection, were tested for NS1 Antigen.
A specificity of 99% was observed in this study.
Samples collected in Brazil, country where DNGV has been present since years, from healthy individuals, without any sign of an ongoing DNGV infection, were tested as well and a specificity > 95% was observed. The few positive samples turned out to be positive also in a CE-marked reference ELISA.
No cross-reactions were observed in ZIKA and Chikungunya IgM positive samples.
In pregnant women just a few false positive samples were detected starting from 8th -9th month of pregnancy. In the study we carried out an about 80 pregnant women we found a specificity more than 91.5%.

3.Precision

CV% values ranging 5-20% were observed depending to the OD450nm values of the negative and positive samples used for testing.

S. LIMITATIONS

Repeatable false positive results were found in far less than 5% of the normal Brazilian population, mostly due to unknown interferences.
Interferences in fresh samples were observed when they were not particles-free, heavily hemolized or hyperlipemic or anyway when they were badly collected (see chapter G).
Old or frozen samples, presenting fibrin clots, crioglobulins, lipid-containing micelles or microparticles after storage or thawing, generated false positive results.

REFERENCES

1. Hapugoda MD, Batra G, Abeyewickreme W, Swaminathan S, Khanna N.Single antigen detects both immunoglobulin M (IgM) and IgG antibodies elicited by all four dengue virus serotypes.Clin Vaccine Immunol. 2007 Nov;14(11):1505-14. Epub 2007 Sep 26.
2. Kumarasamy V, Chua SK, Hassan Z, Wahab AH, Chem YK, Mohamad M, Chua KB.Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection.Singapore Med J. 2007 Jul;48(7):669-73.
3. Chen YC, Huang HN, Lin CT, Chen YF, King CC, Wu HC.Generation and characterization of monoclonal antibodies against dengue virus type 1 for epitope mapping and serological detection by epitope-based peptide antigens.Clin Vaccine Immunol. 2007 Apr;14(4):404-11. Epub 2007 Feb 7.
4. Ageep AK, Malik AA, Elkarsani MS.Clinical presentations and laboratory findings in suspected cases of dengue virus.Saudi Med J. 2006 Nov;27(11):1711-3.
5. Falconar AK, de Plata E, Romero-Vivas CM.Altered enzyme-linked immunosorbent assay immunoglobulin M (IgM)/IgG optical density ratios can correctly classify all primary or secondary dengue virus infections 1 day after the onset of symptoms, when all of the viruses can be isolated.Clin Vaccine Immunol. 2006 Sep;13(9):1044-51.
6. Tran TN, de Vries PJ, Hoang LP, Phan GT, Le HQ, Tran BQ, Vo CM, Nguyen NV, Kager PA, Nagelkerke N, Groen J.Enzyme-linked immunoassay for dengue virus IgM and IgG antibodies in serum and filter paper blood.BMC Infect Dis. 2006 Jan 25;6:13.
7. Anandarao R, Swaminathan S, Fernando S, Jana AM, Khanna N.Recombinant multiepitope protein for early detection of dengue infections.Clin Vaccine Immunol. 2006 Jan;13(1):59-67.
8. Vázquez S, Pérez AB, Ruiz D, Rodríguez R, Pupo M, Calzada N, González L, González D, Castro O, Serrano T, Guzmán MG.Serological markers during dengue 3 primary and secondary infections.J Clin Virol. 2005 Jun;33(2):132-7. Epub 2004 Dec 18.
9. De Paula SO, Fonseca BA.Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis.Braz J Infect Dis. 2004 Dec;8(6):390-8. Epub 2005 May 9. Review.
10. Kao CL, King CC, Chao DY, Wu HL, Chang GJ.Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health.J Microbiol Immunol Infect. 2005 Feb;38(1):5-16. Review.

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

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