

AccuDiag™
Dengue NS1 Antigen
ELISA Kit

REF 8404-25



Test	Dengue NS1 Antigen ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Two-step sandwich-type immunoassay
Detection Range	Qualitative: Positive; Negative Control
Sample	50 µL
Total Time	~ 110 min
Shelf Life	12 Months from the manufacturing date
Specificity	95%
Sensitivity	95%

INTENDED USE

The Diagnostic Automation Inc. Dengue NS1 ELISA for early detection of Dengue virus (DENV), is an ELISA assay system for the detection of NS1 antigen in human serum. This test will aid in the early diagnosis of Dengue virus in human serum even prior to the presence of IgM or IgG antibodies. It is not intended to screen blood or blood components, and is for investigational use only. Not for use in diagnostic procedures. Not for sale or distribution in the United States of America.

SUMMARY AND EXPLANATION

Dengue is an acute viral disease of man, which is transmitted by *Aedes aegypti* mosquitoes. Dengue is characterized clinically by biphasic fever, rash and hematopoietic depression, and by constitutional symptoms such as malaise, arthralgia, myalgia and headache (1). Infrequently, more severe disease is seen, manifested by hemorrhage fever which may progress to lethal shock (2, 3). It is endemic in the tropics and subtropics, worldwide, where an estimated 100,000,000 cases occur annually (4). It has been estimated that about 50 to 100 million cases of Dengue Fever (DF) occur every year with about 250,000 to 500,000 cases of Dengue Hemorrhagic Fever (DHF). During 2002, more than 30 Latin American countries reported over 10,000,000 (DF) cases with large number of DHF cases. This has been followed by extensive epidemics of DHF in several parts of India during 2003 through 2005. In the Americas, the reported incidence has more than tripled from 1996 to 2002. The incidence of Dengue outbreak has been reported in Hawaii (5), and in Laredo, Texas. The potential for the virus to cause a severe disease has also resulted in the inclusion of this pathogen on the CDC "category A" list for potential biological warfare and bioterrorism agents. Dengue NS1 (non-structural) protein is a hexameric secreted protein. It is believed to play a role in viral RNA replication. It is strongly immunogenic eliciting antibodies with complement fixing activity. NS1 antigen can be detected in circulating blood during acute Dengue infection. The Dengue NS1 ELISA can detect NS1 antigen in serum samples almost immediately following infection.

TEST PRINCIPLE

The Dengue NS1 ELISA is a highly sensitive, rapid and reliable assay. It uses one enzymatically amplified "two-step" sandwich-type immunoassay to detect low levels of NS1 in serum.

In this assay, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples are then, "sandwiched" between the capture and secondary antibodies. The presence of NS1 antigen is confirmed by the colorimetric response obtained using an enzyme-conjugate-HRP and liquid TMB substrate. Once the reaction is stopped, using an acidic solution, the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. The values obtained for the negative and positive sera serve as guidelines as to determining if a sample contains NS1 antigen.

Note: A set of negative, positive and cut-off controls are provided as internal controls in order to monitor the integrity of the kit components.

MATERIALS AND COMPONENTS

Materials provided with the test kits

The DengueNS1 ELISA - kit contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each.

Warning: Do not use any reagents where damage to the packing has occurred.

The kit contains the following reagents:

- Coated Microtiter Strips for Dengue NS1:** ELISA Strip holder in ziplock foil, containing 96 polystyrene microtiter wells. Store at 2-8°C until expiry.
- Dengue NS1 Negative Control (1X300µL):** The negative control will aid in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.
- Dengue NS1 Positive Control (1X300µL):** The positive control will aid in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.
- Dengue NS1 Cut-Off Control (1X300µL):** The cut-off control will aid in determining the cut-off value for the ELISA. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.
- Sample Diluent for Dengue NS1 (1X15ml):** This solution contains the secondary antibody. Proclin (0.02-0.03%) is added as a preservative. Store at 2-8°C until expiry.
- 100x Conjugate for Dengue NS1 (1x150µl):** This contains horse radish peroxidase-labeled polyclonal antibody. Mix well prior to use. Store at 2-8°C until expiry. Undiluted 100X Conjugate for Dengue NS1 is stable for the kit's shelf life.
- Conjugate Diluent for Dengue NS1 (1x12ml):** This contains the diluent solution for the 100x Conjugate. The 100x conjugate is diluted directly into this solution. After diluting 100x Conjugate into this solution, the now ready-to-use conjugate may be stored for 2 weeks at 2-8°C before it should be discarded. Store at 2-8°C until expiry.
- 10 X Wash Buffer: (1X 120 mL)** To be used as directed in Test Procedure. Store at 2-8°C until expiry.
- Liquid TMB Substrate (1X12ml):** To be used as directed in Test Procedure. Store at 2-8°C until expiry.
Note: This substrate is light-sensitive and should be stored in the original bottle.
- Stop Solution (1X6ml):** To be used to terminate the reaction as directed in Test Procedure. Store at room temperature until expiry.
Caution: This is a strong acid, wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.



Materials required but not provided

1. ELISA Spectrophotometer capable of absorbance measurement at 450 nm
2. Biological or High-Grade Water
3. Vacuum Pump
4. Automatic Plate Washer
5. 37°C Incubator
6. 1-10 µL Single-Channel Pipettors, 50-200 µL Single- and Multi-Channel Pipettors
7. Polypropylene tubes or 96 well dilution plates
8. Parafilm or similar plate cover
9. Timer
10. Vortex

SPECIMEN COLLECTION AND PREPARATION

1. Human serum must be used with this assay. Reagents have not been optimized, or tested with whole blood or plasma so they cannot be tested directly.
2. Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
3. Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
4. Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days, or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
5. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
6. If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
7. Do not use sera if any indication of growth is observed.

ASSAY PROCEDURE

Caution: This kit has not been optimized by Diagnostic Automation, Inc. for use with any automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the acceptance tolerances described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagent may be required.

Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion.

Preparation of Reagents:

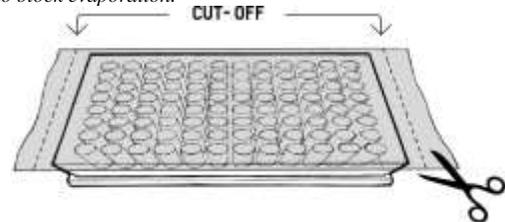
- **Preparation of 1X Wash Buffer**
Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized water). Mix thoroughly to ensure that any precipitate is dissolved and that the solution is uniform. Once diluted to 1X, the solution can be stored at room temperature for up to 6 months. Check for contamination prior to use. Discard if contamination is suspected.
- **Microtitration Wells**
Select the number of coated wells required for the assay. The remaining unused wells should be repackaged immediately with the supplied desiccant and stored at 2-8°C until ready to use or expiration.
- **Preparation of Conjugate Solution**
Add 120 µl of 100x Conjugate for Dengue NS1 ELISA directly to the 12 ml bottle of Conjugate Diluent for Dengue NS1 (1 part : 100 parts). Mix by inverting solution several times. This solution may be stored for up to 2 weeks if stored at 2-8°C. After 2 weeks, this conjugate solution should be discarded and no longer used in this assay. Please note that smaller volumes of the 100X Conjugate may be diluted into the corresponding volume of Conjugate Diluent (1 part: 100 parts), particularly if the storage time is expected to exceed 2 weeks or if the number of samples to be tested is low. Undiluted 100X Conjugate for

Dengue NS1 ELISA that is stored at 2-8°C is stable for the duration of the kit shelf life.

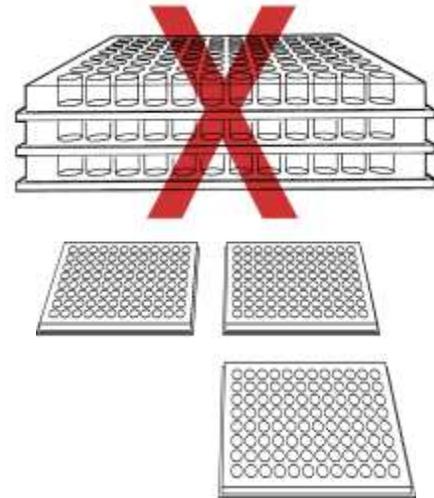
ASSAY PROCEDURE

1. Positive, negative, and cut-off controls should be assayed in duplicate (and run each time assay is performed on every plate). Unknown serum samples may be tested in singlet. (However, it is recommended to run samples in duplicate until the operator is familiar with the assay.) Ninety test specimens can be tested in singlet on each plate.
2. Using a single channel or multichannel pipettor, aliquot 50 µl of Sample Diluent for Dengue NS1 ELISA into each of the required wells.
3. Add 50 µl of each undiluted sera (test samples and control samples) directly to the center of the wells containing the Sample Diluent. Rock the plate gently from side to side 5 times.
4. Cover the top of the plate with parafilm and remove excess.

Note: This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.



Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO₂ or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.



CORRECT METHOD

5. Incubate the plate at 37°C for 1 hour in an incubator.
6. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µl per well in each wash cycle.
7. Prepare the Conjugate Solution (120 µl of 100x Conjugate: 12 ml of Conjugate Diluent) and add 100 µl per well of this Conjugate Solution into all wells using a multi-channel pipettor. Discard the remaining Conjugate Solution or store for up to 2 weeks at 2-8°C.
8. Cover the plate with parafilm, as shown above, and incubate at 37°C for 30 minutes in an incubator.



9. After the incubation, wash the plate 6 times with the automatic plate washer using 1x Wash buffer.
10. Add 100 µl per well of Liquid TMB substrate into all wells using a multi-channel pipettor.
11. Incubate the plate in the dark, at room temperature for 20 minutes
12. Add 50 µl per well of Stop Solution into all wells using a multi-channel pipettor and let the plate stand, uncovered at room temperature for 1 minute.
13. Read the optical density at 450nm (OD₄₅₀) value with a Microplate reader. DO NOT SUBTRACT OR NORMALIZE ANY BLANK VALUES OR WELLS.
14. Record the raw OD₄₅₀ and evaluate the sample status as indicated in the Quality Control section.

Example

Calculate the mean Cut-Off Control:

Example: Cut-Off Control OD

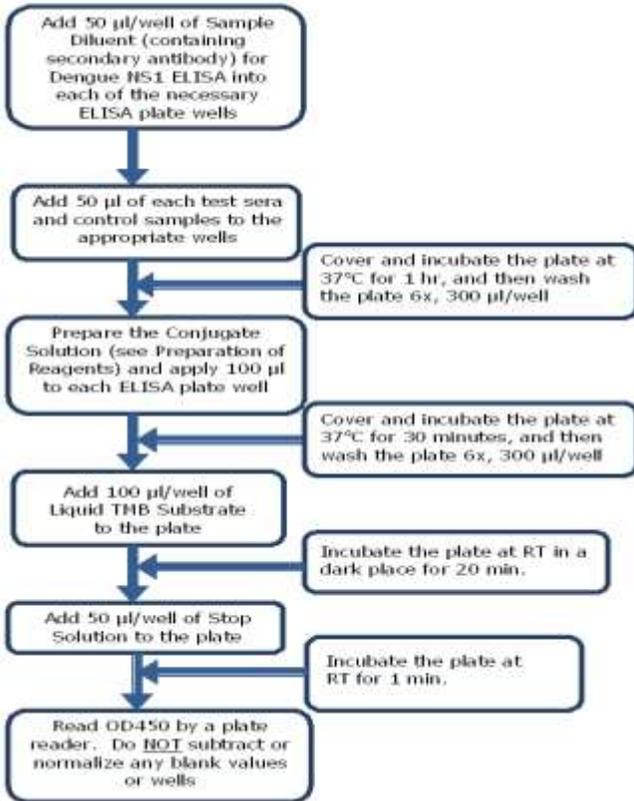
No 1 0.152
 No 2 0.189
Total 0.341

Mean of Cut-Off Control = 0.341 ÷ 2 = 0.171

Example Cut-Off Value: 0.171

Note: It is recommended to verify cut-off using sera from geographically relevant population.

Dengue NS1 ELISA Flow Chart



Calculate Immune Status Ratio (ISR): The immune status ratio (ISR) is calculated from the ratio of the optical density (OD) obtained with the test sample divided by the calculated Cut-Off Value. Calculate the ISR for each test sample.

Example

Calculate the ISR for each sample:

Example: Test Sample OD

Test Sample OD 0.431

Test Sample ISR = OD of Test Sample ÷ Cut-Off Value
 Test Sample ISR = 0.431 / 0.171 = 2.52

Sample Interpretation Chart

Sample Status	ISR
Positive Sample	≥ 1
Negative Sample	< 1

Calculation of Cut-off: Endemic control sera were not used for the cut-off calculation. It is recommended to verify cut-off using sera from geographically relevant population.

Interpretation of results: OD values ≥ cut-off (ISR values ≥ 1) will be considered positive for the presence of circulating NS1 antigen. Those sera with OD values close to cut-off (1.1 > ISR > 0.9) should be repeated in duplicate to verify sample status.

RESULTS

The status of the unknown sample is determined by first calculating the cut-off of the assay, followed by calculating the ratio of the optical density (OD₄₅₀) divided by the cut-off.

Calculation of Cut-off: The cut-off is calculated based on the average OD values obtained with the cut-off control sample.

PERFORMANCE CHARACTERISTICS

Sensitivity Studies:

Site 1 – A retrospective study utilized serially collected archived samples from individuals displaying signs and symptoms of Dengue infection. Archived serum samples from 334 patients (median age = 9 yrs. old), collected within 7 days of the onset of clinical symptoms, were evaluated with the DAI DENGUE NS1 ELISA at a reference laboratory located in Southeast Asia. All patients were evaluated for dengue virus infection by RT-PCR, Dengue IgM ELISA and Dengue IgG ELISA. All four Dengue serotypes were represented amongst these clinical samples and the DAI DENGUE NS1 ELISA kit was able to detect positive samples amongst all four serotypes.

Positive and negative percent agreement of DAI DENGUE NS1 ELISA with final diagnosis of archive samples at Site 1



		Final Diagnosis (RT-PCR <i>and</i> ELISA)		
		Positive	Negative	Total
DAI DENGUE NS1 ELISA	Positive	270	0	270
	Negative	44	20	64
	Total	314	20	334
Positive % Agreement		86.0% (95% C.I.: 81.7% - 89.4%) [†]		
Negative % Agreement		100.0% (95% C.I.: 83.9% - 100%) [†]		

[†] Wilson score method for calculating 95% confidence intervals

Site 2 - Prospectively collected serum samples were collected from patients in Barbados displaying symptoms characteristic of dengue fever. A total of 488 patients (with only one time point per individual, median age = 23 yrs) were collected within 7 days (inclusive) after the onset of clinical symptoms. Samples were sent for confirmatory RT-PCR testing to the Centers for Disease Control and Prevention (CDC) in Fort Collins, CO. Samples were serotyped and the vast majority of the RT-PCR positive samples were found to be Dengue-1 (65/68, 95.6%).

Positive and Negative Percent Agreement of DAI DENGUE NS1 ELISA with final diagnosis of prospective samples at Site 2

		Final Diagnosis (RT-PCR)		
		Positive	Negative	Total
DAI DENGUE NS1 ELISA	Positive	52	52	104
	Negative	16	368	384
	Total	68	420	488
Positive % Agreement		76.5% (95% C.I.: 65.1% - 85.0%) [†]		
Negative % Agreement		87.6% (95% C.I.: 84.1% - 90.4%) [†]		

[†] Wilson score method for calculating 95% confidence intervals

Specificity Study:

Archived serum samples from 193 patients, who presented with clinical symptoms associated with dengue virus, were evaluated with the DAI DENGUE NS1 ELISA at a reference laboratory in Florida. Samples were sent for confirmatory RT-PCR testing to the Centers for Disease Control and Prevention (CDC) in Fort Collins, CO. Samples were collected within 7 days (inclusive) of the onset of clinical symptoms of dengue virus.

Negative Percent Agreement of DAI DENGUE NS1 ELISA with final diagnosis of archive samples at Site 3

		Final Diagnosis (RT-PCR)		
		Positive	Negative	Total
DAI DENGUE NS1 ELISA	Positive	0	1	1
	Negative	1	191	192
	Total	1	192	193
Negative % Agreement		99.5% (95% C.I.: 97.1% - 99.9%) [†]		

[†] Wilson score method for calculating 95% confidence intervals

Reproducibility Study:

The reproducibility study of the DAI DENGUE NS1 ELISA kit was performed at 3 different sites by 2 different individuals (6 operators total) on 5 different days. The study was conducted at Diagnostic Automation, Inc. All assays were performed according to the kit insert. Identical panels of samples plus kit controls were tested in triplicate using the same lot of DAI DENGUE NS1 ELISA. The panels consisted of four clinical serum specimens diluted in an analyte-negative matrix, and included a positive specimen, a specimen within the equivocal range, a specimen just below the equivocal range and a negative specimen. The serum dilutions selected also ensured that the analyte concentration in the specimens represented a clinically relevant range. The total precision %CV (from "total" standard deviation from triplicate results) for the raw OD and ISR values is shown in the table below.

The DAI DENGUE NS1 ELISA's total precision %CV for the raw OD values varied from 9 - 16.2%, depending on the sample. The ISR total precision %CV varied from 3.7 - 11.9%, depending on the sample. This variability measurement inherently included noise due to operator-to-operator variation and lab-to-lab variation.

	%CV _{Total} - OD450	%CV _{Total} - ISR
panel #1	9.81%	10.73%
panel #2	9.75%	8.42%
panel #3	12.29%	7.61%
panel #4	15.17%	8.44%
Positive Control	12.88%	8.25%
Negative Control	16.18%	11.85%
Cut-off control	13.53%	3.71%

%CV = % Coefficient of Variation.
ISR = Immune status ratio

Cross-reactivity Study:

Eighty-four sera (including 48 antigen or viral load positive samples) that tested positive for other potentially cross-reactive pathogens were evaluated with the DAI DENGUE NS1 ELISA in order to determine cross-reactivity. Samples that were antibody positive (IgM and IgG) were also selected and evaluated with the DENGUE NS1 ELISA. However, this does not remove the possibility of cross-reactivity of this ELISA with other diseased specimens that were not evaluated in this panel. The data is presented as % specificity with 95% confidence intervals (Wilson Score method).

Disease	Number of Samples	Number of Positives	Number of Negatives	% Specificity (95% Confidence Interval)*
West Nile Virus (PCR +)	18	0	18	100% (82.4 - 100%)
HBV (Ag +)	10	0	10	100% (72.2 - 100%)
HCV (Viral Load +)	10	0	10	100% (72.2 - 100%)
HIV (Viral Load +)	10	0	10	100% (72.2 - 100%)
Rubella (IgG +)	5	0	5	100% (56.6 - 100%)
Rubella (IgM +)	5	0	5	100% (56.6 - 100%)
Lupus (ANA +)	5	0	5	100% (56.6 - 100%)
EBV (IgM +)	5	0	5	100% (56.6 - 100%)



CMV (IgM +)	6	0	6	100% (61.0 - 100%)
Syphilis (IgG +)	5	0	5	100% (56.6 - 100%)
HIV (EIA +)	5	0	5	100% (56.6 - 100%)
Total	84	0	84	100% (95.6 - 100%)

*95% confidence interval based on Wilson Score method.

Interference Study:

Five potentially interfering substances commonly occurring in serum were tested for their effect on the DAI DENGUE NS1 ELISA test. In addition to the kit's positive, negative and cut-off controls, a panel of four simulated clinical specimens were tested, ranging in strength from negative to weakly positive to strongly positive. The five potentially interfering substances were Bilirubin (0.01 & 0.02 mg/mL), Cholesterol (3 & 5 mg/mL), human anti-mouse antibody (HAMA) (12, 44, 46 ng/mL), Triglycerides (5 & 30 mg/mL), Hemoglobin (16 & 160 mg/mL). There were no noticeable effects of bilirubin or cholesterol at the concentrations tested. HAMA does not appear to increase the incidence of false positives in the assay. HAMA may reduce ISR values for positive samples, although the limited samples available did not show a consistent effect proportional to the levels of HAMA in the samples. The results of this study indicated that high levels of triglycerides (30 mg/mL) may reduce the ISR values of positive samples. Also, high levels of hemoglobin (>16mg/mL), well above the normal levels found in serum samples, caused a significant deleterious effect by lowering the ISR values for the positive samples and causing negative samples to test as false positives. Thus, samples containing high levels of triglycerides or samples that are hemolyzed should be avoided for analysis with this assay.

Freeze Thaw Study:

Ten NS1-positive serum samples were exposed to five freeze-thaw cycles and assayed for NS1 using the DAI NEGUE NS1 ELISA kit after each cycle. All ten samples still tested positive after all five freeze-thaw cycles. It is recommended that serum samples for use in the DENGUE NS1 ELISA test not be frozen and thawed for more than five times. However, as we did not have access to never frozen dengue NS1-positive samples, it is not possible to comment whether positive samples may have lost some positive signal upon the first freeze-thaw relative to a sample that has never been frozen.

QUALITY CONTROL

Each kit contains positive, negative and cut-off control samples. An acceptable Discrimination Capacity ($R_{PC/NC}$) must be obtained to ensure assay validity. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff. The test is invalid and must be repeated if the ($R_{PC/NC}$) value is too low or if the control samples do not

meet the specifications. If the test is invalid, the results cannot be used. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and applicable for spectrophotometric readings only.

First, calculate the $R_{PC/NC}$ as shown in the example.

Example

Calculate the mean Negative Control(NC):

Example: Negative Control OD

No 1 0.108
No 2 0.084
Total 0.192

Average of Negative Control = $0.192 \div 2 = 0.096$

Calculate the mean Positive Control(PC):

Example: Positive Control OD

No 1 1.112
No 2 1.089
Total 2.201

Average of Positive Control = $2.201 \div 2 = 1.101$

Calculate the ratio ($R_{PC/NC}$) between Positive and Negative Control:

Example: ($R_{PC/NC}$) = $1.101 \div 0.096 = 11.47$

Next, ensure that the quality control requirements, listed in the table below, are fulfilled.

Quality Control Requirements

Control	Requirement
Positive Sample	OD \geq 0.5
Negative Sample	OD < 0.2
Cut-Off Sample	OD > Negative Sample
$R_{PC/NC}$	\geq 8

Summary:

The results on the table above must be obtained for the assay to be considered valid. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

LIMITATION OF PROCEDURE

1. Since this is an indirect screening method, the presence of false positive and negative results must be considered.
2. All reactive samples must be evaluated by a confirmatory test.
3. The reagents supplied in this kit are optimized to measure Dengue NS1 levels in serum specimens.
4. Serological cross-reactivity across the flavivirus group is common. Certain sera from patients infected with Japanese Encephalitis, West Nile, and/or Saint Louis viruses may give false positive results. Therefore any Dengue positive sera must be confirmed with other tests.
5. The assay performance characteristics have not been established for visual result determination.
6. Results from immunosuppressed patients must be interpreted with caution.
7. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

8. High triglyceride levels (> 3000 mg/dL) appeared to lower the observed ISR values.
9. Hemoglobin levels (> 1600mg/dL) appear to affect some serum samples by lowering the ISR values.

5. Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ; Hawaii Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis. 2005; 11(5):742-9
6. Henchal EA, Putnak JR. The dengue viruses. Clin Microbiol Rev. 1990;3(4):376-96.
7. Alcon-LePoder S, Sivard P, Drouet MT, Talarmin A, Rice C, Flamand M. Secretion of flaviviral non- structural protein NS1: from diagnosis to pathogenesis.

PRECAUTIONS

1. For research or export use only.
2. All human source materials used in the preparation of controls have tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
3. A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
4. Do not mix various lots of any kit component within an individual assay.
5. Do not use any component beyond the expiration date shown on its label.
6. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
7. Some reagents may form a slight precipitate, mix gently before use.
8. Incomplete washing will adversely affect the outcome and assay precision.
9. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
10. Avoid microbial contamination of reagents, especially of the conjugate concentrate and the conjugate diluent. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
11. Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
13. Cover working area with disposable absorbent paper.

ISO 13485 ISO 9001 	
 Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116 Woodland Hills, California 91367 USA	
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EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
Revision Date: 2015-06-08	

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD

Material Safety Data Sheets (MSDS) are available for all components of this kit. MSDS sheets are available through our website or it can be sent upon request. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

REFERENCES

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3. Halstead SB. Neutralisation and antibody-dependent enhancement of dengue viruses. Advances in Virus Research 2003;60:421-67.
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