Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

# One Step Dengue NS1 RapiDip™ InstaTest (Serum)

REF 173110-25-25

IVD 20°C -30°C Σ 25 Tests

| | |
|---|---|
| **Sensitivity** | **76.5 %** |
| **Specificity** | **95.3%** |

## INTENDED USE

The Dengue NS1 Rapid Test is an immunochromatographic strip assay for the qualitative detection of non-structural protein 1 (NS1) in human serum, and serves as an aid in the diagnosis of early Dengue infections. This test will aid in the rapid diagnosis of Dengue virus in human serum even prior to the presence of IgM or IgG antibodies. This test is intended to be used on sera drawn from patients within 1-7 days past the onset of symptoms.

Positive results must be confirmed by CRP when a single sample is collected within the first 7 days (after primary and/or secondary infection) after the onset of symptoms and/or Plaque Reduction Neutralization Test (PRNT) or other acceptable reference standard when paired samples are collected. It is not inteneded to screen blood or blod components and I for professional in vitro diagnostic use only.

## SUMMARY AND EXPLANATION

Dengue is an acute vial disease, 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. Infrequently, more severe disease is seen, manifested by hemorrhage fever which may progress to lethal shock. It isendemic in the tropics and subtropics, worldwide, where an estimated 100,000,000 cases occur annually. 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 epidemic of DHF in 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, 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 bioterrosism agents. Dengue NS1 (non-structural) protein is a multimeric secreted protein that 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 Rapid Test detects NS1 antigen in serum samples following infection.

## PRINCIPLE

The Dengue NS1 Rapid Test is a qualitative, membrane based immunoassay for the detection of NS1 antigen in human serum. The rapid test membrane is pre-coated with a NS1 specific antibody on the test line region and utilizes a separate control to assure assay flow and performance. During testing, the test sample is added directly to the sample region and the test is placed into a well containing 3 drops of buffer. The buffer and serum mix and interact with NS1-specific monoclonal antibodies conjugated to gold nanoparticles. The solution migrates upward on the membrane (via capillary action) to react with the anti-NS1 antibody on the membrane. If NS1 antigen is present, a red line will appear at the test line. The red line at the control region should always appear if the assay is performed correctly. The presence of this red line verifies that proper flow has occurred and catastrophic failure of the conjugate has not occurred. The entire procedure takes approximately 30 minutes.

## MATERIALS PROVIDED

1. Twenty-five (25) Dengue NS1 Rapid Test dipsticks, individually pouched or 25 test strips in a vial with desiccant in the cap. Store at room temperature in vial.
2. One (1) vial of Chase Buffer Type A, 6 ml.

## MATERIALS REQUIRED NOT PROVIDED

1. Pipettor and tips capable of measuring 5-50 µl of solution.
2. Test tube or other sample reservoir well.

## SPECIMEN COLLECTION AND PREPRATION

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

Before beginning, remove the Dengue NS1 Rapid test from the foil pouch or vial and assure that all test serum samples are allowed to reach room temperature. Ensure that no physical damage (e.g., scratched membrane, torn pads, etc.) is apparent on the rapid test. SECURE an individual reservoir well in a microtube holder. Equivalently, a well from a 96-well ELISA plate or small test tube may be used to run the assay. Never re-use reservoir wells. Always run the rapid test with a fresh well.

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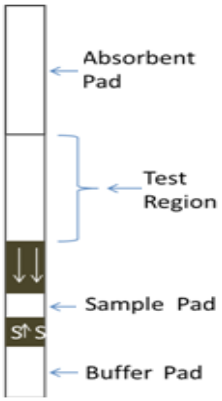


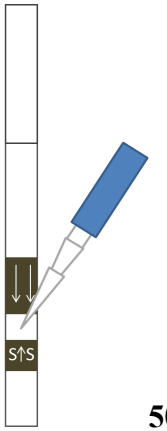

Figure1

1. Add THREE (3) drops (approximately 120 µl) of Chase Buffer Type A to the well.

**3 Drops**

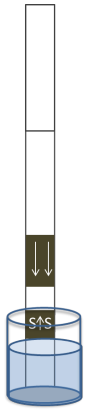
2. Carefully add 50µl of test sample to the Sample Pad. The Sample Pad is located between the arrows, as shown in the diagram.

**DO NOT add the sample directly to the Buffer Pad or to the well. Please see Figure 1.**



**50 µl Sample**

3. Immediately place the rapid test in the well. Ensure that the 'Sample' side of the rapid test is facing downward into the well. If a red color is not seen moving up the membrane within 20 seconds, gently touch the arrows above the sample pad to permit flow of the conjugate and sample up the membrane.



4. Read the rapid test after 30 minutes. Do NOT interpret results after 45 minutes, as this may lead to erroneous results.

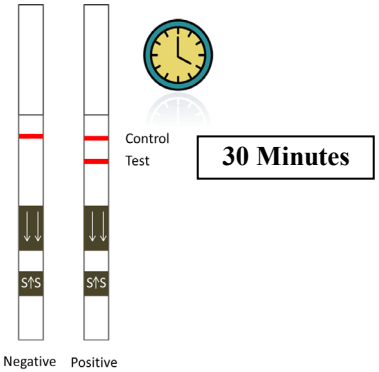
## RESULTS

**Positive:** The test is positive for NS1 antigen when the control line (C) and the test line (T) appear in the test area. A faint line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of the NS1 antigen present. The test line for 'weakly positive' sera samples may show a weak positive but distinctly red line. The presence of a weak red test line should be considered a positive result.

**Negative:** The test is negative when only the control line appears. If no test and control line are present IN 30 MINUTES, it is an Invalid Result.

**Invalid:** No lines appear at the control line areas. The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new Dengue NS1 rapid test and fresh serum.

**Note:** The red color in the test region will vary depending on the concentration of antigens present. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.




## PERFORMANCE CHARACTERISTICS

The analytical sensitivity of the Dengue NS1 Rapid Test was evaluated by peforming serial dilutions of recombinant NS1 diluted

into normal human serum specimens. The approximate limit of detection for each serotype are shown below.

| Dengue-1 | Dengue-2 | Dengue-3 | Dengue-4 |
|---|---|---|---|
| 6.25 ng/ml | 1.56 ng/ml | 3.125 ng/ml | 3.125 ng/ml |

**Sensitivity and Specificity**

Naval Medical Research Center Study: Well characterized serum samples that were identified as Dengue positive or Dengue negative were used to evaluate the performance of the DAI Dengue NS1 Rapid Test. The Dengue positive samples were all collected from patients who reported symptoms consistent with Dengue infection, within the first 6 days post onset of symptoms, from which Dengue virus could be isolated and identified from their sera using an immunoflourescence assay (IFA). The Dengue negative samples were collected from febrile individuals but from whom Dengue virus could not be isolated. Using this panel, the clinical sensitivity of the DAI Rapid test was detertimed to be 76.5% and clinical specificity was determined to be 95.3%.

| | Positive by IFA | Negative by IFA |
|---|---|---|
| Positive by DAI NS1 RapiDip Test | 52 | 2 |
| Negative by DAI NS1 RapiDip Test | 16 | 41 |

Specificity was also evaluated in-house with commercially sourced normal human serum specimens derived from a non-endemic region. 58 out of 60 samples tested negative, for a negative percent agreement of 96.7%.

**Cross-reactivity**

DAI Dengue NS1 Rapid Test was evaluated for cross-reactivity against serum samples positive for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Systemic lupus erythematosus / anti-nuclear antibody (ANA). The kit demonstrated cross-reactivity with one out of ten HCV-positive serum, but did not cross-react with any other disease sera tested.

| | % Cross-Reactive (number positive/total tested) |
|---|---|
| HBV + | 0 % (0/10) |
| HCV + | 10% (1/10) |
| HIV + | 0% (0/10) |
| ANA + | 0% (0/5) |

**Interfering Substances**

Bilirubin, triglycerides, and cholesterol were tested at concentrations well above physiological levels and do not interfere with performance of Dengue NS1 RapiDip Test. No effect from hemoglobin was observed at a concentration of 16 mg/ml. This is well above the normal levels found in human serum samples, but well below that in whole blood samples. Testing with whole blood samples is not recommended.

## LIMITATIONS OF PROCEDURE

1. This test will only indicate the presence of dengue NS1 antigen and should not be used as the sole criterion for the diagnosis of dengue. This test alone must not be used for any clinical treatment decision. As with all diagnostic tests, all results must be considered with other clinical information available to the doctor.
2. This test is intended for serum samples. Testing with whole blood samples is not recommended.
3. If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of dengue.
4. A false positive result may occur. Confirmatory testing (such as by PCR or PRNT) is advised especially in cases where no symptoms exist.
5. Do not use serum samples containing any glycerol or other viscous materials. This will decrease the sensitivity of the assay.

## PRECAUTIONS

1. For professional in vitro diagnostic use only. Do not use after expiration date.
2. 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 sera and used kits.
3. Wear protective clothing, eye protection and disposal gloves while performing the assay. Wash hands thoroughly when finished.
4. Avoid all contact between hands and eyes or mucous membranes during testing.
5. Do not eat, drink or smoke in the area where the sera and kits are handled.
6. Chase buffer contains a preservative; avoid all possible contact with skin and mucous membranes.

## STORAGE

The sealed pouch or vial containing the test strip is designed to be stored at room temperature (20°C-30°C) for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used within 15 minutes after removal from the pouch or vial to prevent exposure to humidity (5 minutes in high humidity areas).

## REFERENCES

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| ISO 13485<br>ISO 9001<br><br>Diagnostic Automation Cortez Diagnostics, Inc.<br>21250 Califa Street, Suite 102 and 116<br>Woodland Hills, California 91367 USA | |
| **Date Adopted** | **2018-06-29** |
| REF 173110-25-25 | **CORTEZ-Dengue NS1**<br>**RapiDip™ InstaTest (Serum)** |
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