

**OneStep**  
**Dengue NS1 Antigen**  
**RapiCard™ InstaTest**  
**(Serum / Plasma / Whole Blood)**  
**REF 173111-25-23**



**INTENDED USE**

Dengue NS1 Antigen Test is an in vitro qualitative immunochromatographic assay for the rapid detection of Dengue NS1 antigens in human blood, serum and plasma specimens. The test results are intended to aid in the diagnosis of acute dengue infection.

**PRINCIPLE**

Dengue fever is one of the most important mosquito-borne diseases in the world in the terms of morbidity, mortality. Dengue fever virus (serotypes 1 – 4) belongs to the group flavivirus, and is transmitted in nature by day-biting Aedes mosquitoes. The most important mosquito vector is highly domesticated and urban species, Aedes aegypti. Primary Dengue infection, also known as Dengue Fever, is the most common type of dengue illness. It is associated with mild to high fever, headache, muscle pain and skin rash. Secondary infection is known as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome, and often results in high fever and in many cases, with hemorrhagic events and circulatory failure. The fatality rate in patients with Dengue Shock Syndrome can be as high as 44%. Dengue presents typically as a fever of sudden onset with headache, retrobulbar pain, pain in the back and limbs (break-bone fever), lymphadenopathy and maculopapular rash. Patients diagnosed with dengue in endemic areas generally have secondary infection, whereas patients in non-endemic areas are usually diagnosed with primary infection. Specific antibody responses to

Dengue virus enable serodiagnosis and differentiation between primary and secondary dengue infections. Dengue NS1 (nonstructural protein 1) is a highly conserved glycoprotein. NS1 antigen was found circulating in samples of infected patient from the first day up to 9 days after the onset of the fever. The detection of NS1 antigen provides the tool for early diagnosis of dengue infection before serological antibodies are detectable. Therefore, it can help the patients to be diagnosed and treated promptly.

Dengue NS1 Antigen Test is a sandwich solid phase immunochromatographic assay. When sample is added to sample pad, it moves through the conjugate pad and mobilizes gold anti-NS1 conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with anti-NS1 antibody that is coated on the test region. If NS1 is present, the result is the formation of a colored band in the test line region. If there is no NS1 in the sample, the area will remain colorless. The sample continues to move to the control area and forms a pink to purple color, indicating the test is working and the result is valid.

**MATERIALS PROVIDED**

1. Dengue NS1 Antigen test card  
Each cassette contains a test strip with NS1 specific antibody on the test region of the membrane and colored anti-NS1 antibody-gold conjugate pad.
2. Instructions for Use
3. Disposable transfer pipet

**MATERIALS REQUIRED NOT PROVIDED**

1. Specimen collection container
2. Timer

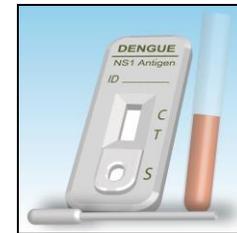
**SPECIMEN COLLECTION AND PREPARATION**

1. The serum, whole blood or plasma specimen should be collected under standard laboratory conditions.
2. No prior special preparation of the patient is required before sample collection by approved techniques.
3. **EDTA may have interference on the test results. Avoid to use EDTA as anti-coagulant for blood tube**

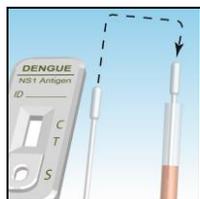
4. The test works best on fresh whole blood / serum / plasma samples. If testing cannot be performed immediately, serum / plasma may be stored at 2-8°C up to 3 days in case of delay in testing. For long-term storage, serum / plasma specimens can be frozen at -20°C for 3 months or -70°C for longer period. Repeated freezing and thawing of the specimen should be avoided. Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
5. Heat inactivation of specimens, which may cause hemolysis and protein denaturation, should be avoided. Avoid to use haemolysed, clotted, contaminated, lipamic and viscous/turbid specimen.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
7. Do not inactivate the sample by heating.
8. Shipment of specimens should comply with local regulations for transportation of etiologic agents.

**ASSAY PROCEDURE**

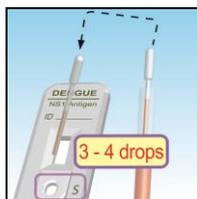
1. Bring the kit components to room temperature before testing.



2. Open the pouch and remove the Card. Once opened, the test card must be used immediately.
3. Label the test card with patient's identity.
4. Use transfer pipet supplied with the device to transfer the specimen by depressing the bulb of the pipet.

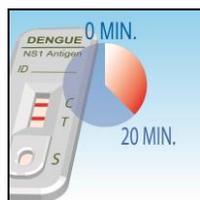


5. Hold the pipette in a vertical position over the sample well of the test card and deliver 3-4 drops (120-160 µL) of sample into the sample well.



6. At the end of 20 minutes read the results. A strong positive sample may show result earlier.

*Note: Result after 20 minutes may not be accurate.*



### QUALITY CONTROL

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.
2. Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which is not provided with this test kit may be commercially available.

### LIMITATIONS

1. The test is for qualitative detection of Dengue NS1 antigen and/or anti-Dengue antibodies in blood, serum or plasma sample and does not indicate the quantity of the analytes.
2. The test is for *in vitro* diagnostic use only.
3. A negative test result cannot exclude a recent infection.
4. It is common that viruses belong to the flavivirus genus, such as dengue virus, Japanese encephalitis, tick-borne encephalitis, yellow fever virus and West Nile virus, have the serological cross reaction on antibody tests.
5. As in case of all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but should rather be made after all the clinical findings have been evaluated.

### EXPECTED VALUES

Dengue NS1 antigen can circulate in human blood from day 1 up to 9<sup>th</sup> day after the onset of the fever. However, the level of the antigen declines rapidly with the elevated level of antibody.

### PERFORMANCE CHARACTERISTICS

#### 1. Accuracy

Out of one hundred twenty five (125 samples) collected from the patients who showed the syndrome of early dengue infection and diagnosed by lab NS1 positive, one hundred and seventeen (117) samples showed positive on Dengue NS1 Antigen Test. Out of one hundred and fourteen (114) blood, plasma and serum samples from healthy blood donor, all of them showed negative on Dengue NS1 Antigen Test.

Agreement of positive =  $117/125 = 93.6\%$

Agreement of negative =  $114/114 = 100\%$

#### 2. Specificity

The following flavivirus were tested at the indicated concentration and showed negative results. The test result is summarized as below.

Flavivirus	Strain	Concentration (TCID <sub>50</sub> )
St. Louis Encephalitis Virus	Parton	1*10 <sup>5.82</sup> U/mL
West Nile Virus	NY2001-6263	1*10 <sup>5.66</sup> U/mL
West Nile Virus 2	1986	1*10 <sup>8.2</sup> U/mL
West Nile Virus Lineage 2	B-956 Uganda	1*10 <sup>5.18</sup> U/mL
Yellow Fever Virus	17D	1*10 <sup>5.02</sup> U/mL
Zika Virus	N/A	1*10 <sup>4.55</sup> U/mL

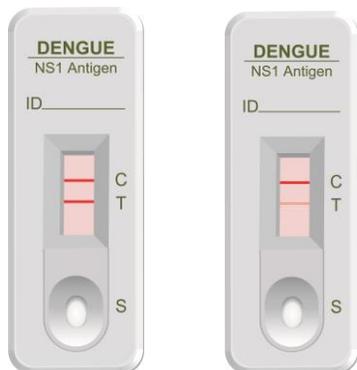
### Interference

The compounds listed below at the mentioned concentration do not interfere the test results.

Acetaminophen	136 µg/mL
Albumin	47 mg/mL
Bilirubin	7.40 mg/mL
Cholesterol	260 mg/mL
Creatinine	6.90 mg/mL
Digoxin	3.00 ng/mL
Ethanol	2.10 mg/mL
Glucose	3.90 mg/mL
Immunoglobulin A	2.20 mg/mL
Immunoglobulin G	10.00 mg/mL
Immunoglobulin M	1.10 mg/mL
Total Protein	70.40 mg/mL
Triglycerides	1.90 mg/mL
Urea nitrogen	700 µg/mL
Uric acid	92 µg/mL

## RESULTS

- **Positive** - A distinct pink colored band appears on test line regions, in addition to a pink line on the control line region.



- **Invalid**- The control line next to the test line does not become visible within 20 minutes after the addition of the sample.



- **Negative** - No line appears in the test line region. A distinct pink line shows on the control line region.



## STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 4-30°C for the duration of the shelf life as indicated on the pouch.

## PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only.
2. This kit is for **PROFESSIONAL** use only.
3. Read the instructions carefully before performing the test.
4. This product does not contain any human source materials.
5. Do not use kit contents after the expiration date.
6. Handle all specimens as potentially infectious.
7. Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. When the assay procedure is completed, dispose specimens after autoclaving them at 121° C for at least 20 min. Alternatively, they can be treated with 0.5% Sodium Hypochlorite for 1-2 hours before disposal.
8. Do not pipette reagent by mouth and no smoking or eating while performing assays.
9. Wear gloves during the whole procedure.

## REFERENCES

1. Alcon S. etc. al, 2002. ELISA specific to dengue virus type1 non-structural protein NS1 reveals circulation of the antigen in the blood during acute phase of disease in patients experiencing primary or secondary infections. J. Clin. Microbiol. 40:376-381
2. Dussart P. etc. al, 2006. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin. Vaccine Immunol. 13:1185-1189.

 <b>ISO 13485</b>  <b>Diagnostic Automation/ Cortez Diagnostics, Inc.</b> 21250 Califa St, Suite 102 and 116, Woodland Hills, California 91367 USA	
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