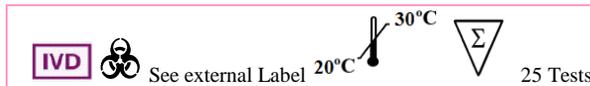


OneStep
Dengue NS1 Antigen & IgG/IgM
Antibody Duo Panel
RapiCard™ InstaTest
(Serum/Plasma/Whole Blood)
REF 173112-25-23



INTENDED USE

Dengue Duo Panel is an *in vitro* qualitative immunochromatographic assay for the rapid detection of Dengue NS1 antigens and anti-dengue IgG/IgM antibodies in human blood, serum and plasma specimens simultaneously. The test results are intended to aid in the diagnosis of dengue infection.

SUMMARY AND EXPLANATION

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. After the anti-NS1 antibody elevate in human body, the detectable NS1 antigens decline quickly. While detectable NS1 antigens declining, the elevated antibodies can be detected for longer time. Usually IgM does not become detectable until 3 to 10 days after the onset of illness in cases of primary dengue infection and until 2 to 3 days after onset of illness in secondary infections. In primary infections, IgG appear the 14th day and may persist for many years. Secondary infections generate an anamnestic IgG antibody response that is characterized by a rapid rise in IgG antibodies detectable at 4-5 days after the onset of the illness.

The detection of both NS1 antigen and anti-Dengue antibodies provides the tool for the diagnosis of dengue infection from the early infection to the stage after the onset of the illness. It can enhance the accuracy of the diagnosis of dengue infection.

TEST PRINCIPLE

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.

Dengue IgG/IgM Antibody Test utilizes the principle of Immunochromatography. Mouse anti-human IgM and human IgG antibodies are immobilized on the nitrocellulose membrane respectively, as two individual test lines (IgM line and IgG line) in the test window of the test device. The IgG line in the test window is closer to the sample well and followed by IgM line. As the test sample flows through the membrane within the test device, the

colored-Dengue specific recombinant antigen-colloidal gold conjugate complexes with specific antibodies (IgM and/or IgG) of Dengue virus, if present in the sample. This complex moves further on the membrane to the test region where it is captured by the anti-human IgM and/or human IgG antibodies coated on the membrane leading to formation of a colored band, which indicates a positive test results. Absence of this colored band in the test window indicates a negative test result. A built-in control line will always appear in the test window when the test has performed properly, regardless of the presence or absence of anti-Dengue virus antibodies in the specimen.

MATERIALS PROVIDED

1. Dengue Duo Panel
 NS1 --- A test strip with NS1 specific antibody on the test region of the membrane and colored anti-NS1 antibody-gold conjugate pad
 IgG/IgM --- A strip with anti-h IgG and anti-h IgM on the test region of the membrane and colored dengue antigen –gold conjugate pad.
2. Instructions for Use
3. Disposable transfer pipette for NS1 test
4. 5 µL Capillary pipette for IgG/IgM test
5. Sample buffer for IgG/IgM test

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.

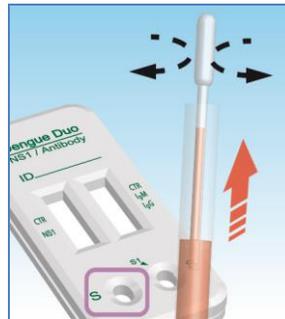
5. Heat inactivation of specimens, which may cause hemolysis and protein denaturation, should be avoided. Avoid to use haemolysed, clotted, contaminated, lipamic and viscous/turbid specimen.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used 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

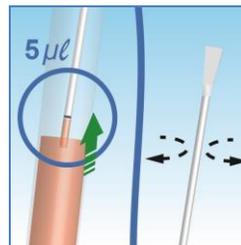
1. Bring the kit components to room temperature before testing.



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. a. Use the provided transfer pipette to transfer the specimen by depressing the bulb of the pipette.



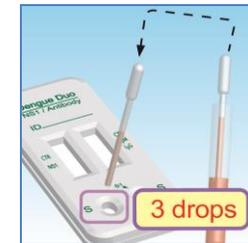
- b. Use provided Capillary pipette to withdraw $5\ \mu\text{L}$ of sample. The black bar near the opening end of pipette indicates the needed $5.0\ \mu\text{l}$ of sample in the stall.



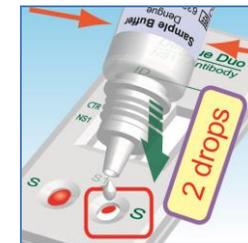
Drop the sample in the corner pointed by "S1 ▼".



5. a. Hold the pipette in a vertical position over the left "S" sample well of the device and deliver 3 drops ($120\text{--}150\ \mu\text{L}$) of sample into the well.

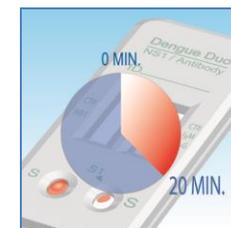


- b. Dispense 2 drops ($80\text{--}100\ \mu\text{L}$) of sample buffer to the right "S" sample well

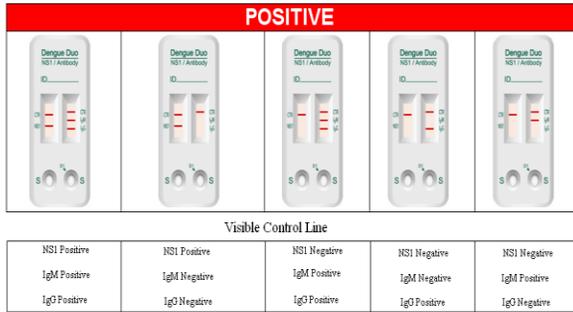


6. At the end of 20 minutes read the results. A strong positive sample may show result earlier.

Note: Result after 20 minutes may not be accurate



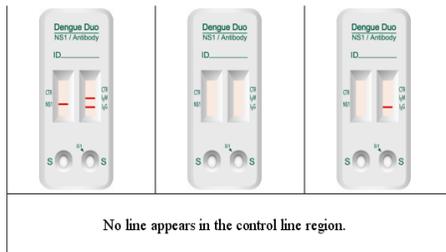
RESULTS



NEGATIVE



INVALID (Samples)



QUALITY CONTROL

- 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.
- 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

- The test is for qualitative detection of Dengue NS1 antigen and/or anti-Dengue antibodies in blood, serum or plasma sample and dose not indicate the quantity of the analytes.
- The test is for *in vitro* diagnostic use only.
- A negative test result cannot exclude a recent infection.
- It is common that viruses belong to 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.
- 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 9th day after the onset of the fever. However, the level of the antigen declines rapidly with the elevated level of antibody.

In primary infection, anti-dengue IgM is detectable 3-5 days after the onset of the illness. Anti-dengue IgG is detectable 14 days after the onset of the illness and may persist for many year.

In secondary infection, anti-IgG antibodies elevate in 1-2 days after the onset of the illness and often accompanied by an elevation of anti-dengue IgM antibodies.

PERFORMANCE CHARACTERISTICS

Dengue IgG/IgM Accuracy

Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test was evaluated on one thousand and forty one (1041) samples. The test results were compared with an approved predicate kit. The results of Dengue IgM accuracy are shown below.

	Predicate Test		Total
	IgM Positive	IgM Negative	
Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel	IgM Positive	249	258
	IgM Negative	771	783
	Negative	261	1041

Out of two hundred and sixty one (261) samples that were tested positive by the predicate kit, two hundred and forty nine (249) were positive on Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test. Out of seven hundred and eighty (780) samples that were tested negative by the predicate kit, seven hundred and seventy one (771) were negative on Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test. Twenty one (21) samples that have disagreed results were verified by IFA. Six (6) samples have the results agreed with Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test while fifteen (15) samples agreed with the predicate kit. The agreement with the predicate kit is summarized as below.

Agreement of positive = $249/261 = 95.40\%$

Agreement of negative = $771/780 = 98.85\%$

Total Agreement = $1020/1041 = 97.98\%$

The results of Dengue IgG accuracy are shown below.

Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel	Predicate Test		Total
	IgG Positive	IgG Negative	
IgG Positive	128	12	140
IgG Negative	12	889	901
Negative	140	901	1041

Out of one hundred and forty (140) samples that were tested positive by the predicate kit, one hundred and twenty eight (128) were positive on Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test. Out of nine hundred and one (901) samples that were tested negative by the predicate kit, eight hundred and eighty nine (889) were negative on Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test. Twenty four (24) samples that have disagreed results were verified by IFA. Seven (7) samples have the results agreed with Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test while seventeen (17) samples agreed with the predicate kit. The agreement with the predicate kit is summarized as below.

Agreement of positive = $128/140 = 91.43\%$
 Agreement of negative = $889/901 = 98.67\%$
 Total Agreement = $1017/1041 = 97.69\%$

Dengue IgG/IgM Specificity

Dengue NS1 Antigen & IgG/IgM Antibody Duo Panel Test has tested sixty (60) samples that were infected by of the following diseases: HAV, HBV, HCV, HEV, Lupus and CMV. All the samples showed no affect on the specificity of the assay. Rheumatoid factor up to 167 IU/mL showed no affect on the specificity of the assay.

Dengue NS1 Antigen 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 & IgG/IgM Antibody Duo Panel 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 & IgG/IgM Antibody Duo Panel Test.

Agreement of positive = $117/125 = 93.60\%$
 Agreement of negative = $114/114 = 100.00\%$

Dengue NS1 Antigen 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 ₅₀)
St. Louis Encephalitis Virus	Parton	$1 * 10^{5.82}$ U/mL
West Nile Virus	NY2001-6263	$1 * 10^{5.66}$ U/mL
West Nile Virus 2	1986	$1 * 10^{8.2}$ U/mL
West Nile Virus Lineage 2	B-956 Uganda	$1 * 10^{5.18}$ U/mL
Yellow Fever Virus	17D	$1 * 10^{5.02}$ U/mL
Zika Virus	N/A	$1 * 10^{4.55}$ 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

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.

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<p>ISO 13485</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa St, Suite 102 and 116, Woodland Hills, California 91367 USA</p>	
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<p>EC REP</p>	<p>CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu</p>
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