DENGUE AgNS1-IgM/IgG

For in *Vitro* diagnostic use only

Immunochromatographic test for the simultaneous detection and differentiation of IgG anti–dengue virus, IgM anti-dengue virus and dengue antigen (Dengue Ag) in human serum, plasma or whole blood

I. INTENDED USE

The *Dengue Ag NS1-IgM/IgG* rapid test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG anti–dengue virus, IgM anti-dengue virus and dengue antigen (Dengue Ag) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with dengue virus. Any reactive specimen with the *Dengue Ag NS1-IgM/IgG* rapid test must be confirmed with alternative testing method(s).

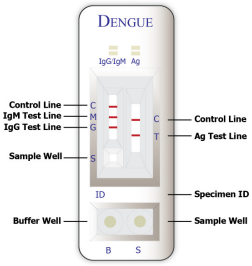
Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (Den 1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting *Stegomyia* family, principally *Aedes aegypti* and *Aedes albopictus*. Today, more than 2.5 billion people living in the areas of tropical Asia, Africa, Australia and the Americas are at risk for dengue infection. An estimated 100 million cases of dengue fever and 250,000 cases of life-threatening dengue hemorrhagic fever occur annually on a worldwide basis.[1-3]

Serological detection is a common method for the diagnosis of infection with dengue virus. IgM anti-dengue virus starts to appear at 3 days after initial exposure and remains in circulation for about 30-60 days. IgG anti-dengue virus is raised at around 7 days, peaks at 2-3 weeks and persists for the duration of life[4-6]. Detection of antigens released during virus replication in the infected patient show very promising results; it enables diagnosis from the first day after the onset of fever up to day 9 once the clinical phase of the disease is over, thus, allowing early detection and prompt treatment[7].

The *Dengue Ag NS1-IgM/IgG* rapid test detects IgG and IgM anti-dengue virus and circulating dengue antigen in one test within 20 minutes. The test is user friendly, does not require cumbersome laboratory equipment and requires minimal staff training.

II. PRINCIPLE

The *Dengue Ag NS1-IgM/IgG* rapid test contains two test strips (left side: Dengue IgG/IgM test; right side: Dengue Ag test).



The Dengue IgG/IgM rapid test on the left-side is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing dengue recombinant envelope antigens conjugated with colloidal gold (dengue Ag conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with the antibody for the detection of IgG anti-dengue virus, the M line is coated with the antibody for the detection of IgM anti-dengue virus, and the C line is pre-coated with a control line antibody.

The Dengue Ag rapid test on the right-side is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing mouse anti-dengue NS1 antigen conjugated with colloidal gold (dengue Ab conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with mouse anti-dengue NS1 antigen, and the C line is pre-coated with a control line antibody. The antibodies to dengue NS1 recognize the antigens from all four dengue virus serotypes.

When an adequate volume of specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. IgG anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated reagent forming a burgundy colored G line, indicating a dengue virus IgG positive test result and suggesting a recent or repeat infection.

IgM anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated reagent forming a burgundy colored M line, indicating a dengue virus IgM positive test result and suggesting a fresh infection.

Dengue NS1 antigen, if present in the specimen, will bind to the dengue Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-dengue NS1 forming a burgundy colored T line, indicating a dengue Ag positive test result.

Absence of any G, M or T lines suggests a negative result. Each test contains an internal control (C lines) which should exhibit a burgundy colored lines of the immunocomplex of the control antibodies in both the left and right panels, regardless of color development of any of the test lines. If the C line does not develop in a panel, the test result is invalid and the specimen must be retested with another device. An invalid result in one panel does not invalidate the test result in the other panel.

III. STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test device unopened, preferably at 2℃-30℃. Do not expose the kit over 40℃. Do not freeze the kit. The positive and negative controls should be kept at 2℃-8℃**.** If stored at 2℃-8℃, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch if it is stored at 2℃-30℃.

IV. PRECAUTIONS

1) For in vitro diagnostic use and professional use only.
2) Read the package insert instruction before use the kit.
3) Do not use beyond the expiration date which appears on the package label.
4) Do not open the sealed pouch, unless ready to conduct the assay.
5) Bring all reagents to room temperature (15℃-30℃) before use.
6) Do not use the components in any other type of test kit as a substitute for the components in this kit.
7) Haemolized blood may be used for the testing, but do not take precipitants.
8) Wear protective clothing and disposable gloves while assaying samples. Wash hands thoroughly after performing the test.
9) Handle all specimens as if they contain infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5 to 1% solution of sodium hypochlorite for one hour before disposal.
10) Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11) The testing results should be read within 30 minutes after a specimen is applied to the sample well or sample pad of the device.

Read result after 30 minutes may give erroneous results.
12) Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.
13) As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
14) Excess sample volume (>5µL) can give false positives.

V. REAGENTS AND MATERIALS SUPPLIED
1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes (for Dengue IgG/IgM test)
3. Plastic droppers (for Dengue Ag test)
4. Sample Diluent (5mL/bottle)
5. Insert (instruction for use)

VI. MATERIAL REQUIRED BUT NOT PROVIDED
Clock or Timer

VII. SPECIMEN COLLECTION AND PREPARATION

Plasma
1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

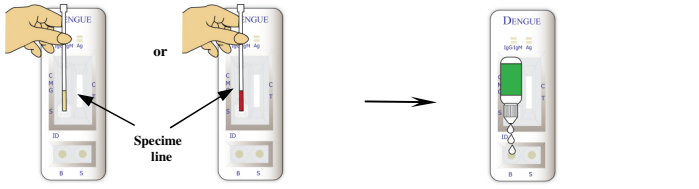
Serum
1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8℃ if not tested immediately. Store specimens at 2-8℃ for up to 5 days. The specimens should be frozen at -20℃ for longer storage.
Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

Blood
Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®). Do not use hemolyzed blood for testing.
Whole blood specimens should be stored in refrigeration (2℃-8℃) if not tested immediately. The specimens must be tested within 24 hours of collection.

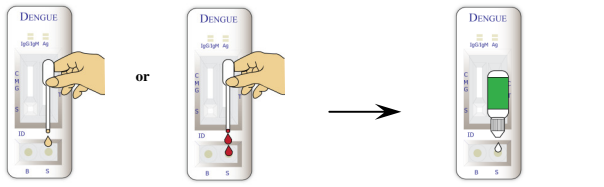
VIII. TEST PROCEDURE
Step 1: Bring the specimen and test components to room temperature, if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
Step 3: Be sure to label the device with specimen's ID number.

For detection of Dengue IgG/IgM
Fill the capillary tube with specimen not to exceed the specimen line as shown in the images below.
Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well **(S well)** making sure that there are no air bubbles.
Immediately add 3 drops (about 90-120 µL) of Sample Diluent into the buffer well **(B well)** with the bottle positioned vertically.



5 µL of specimen to S well — 3 drops of sample diluent to B well

For detection of Dengue Ag
Fill the plastic dropper with specimen.
Holding the dropper vertically, dispense 1 drop (about 30-45 µl) of serum/plasma or 2 drops (about 80-100 µl) of whole blood to the sample well **(S well)** making sure that there are no air bubbles.
Immediately add 1 drop (about 30-40 µL) of Sample Diluent to the sample well **(S well)** with the bottle positioned vertically.



1 drop of serum/plasma to S well — 2 drops of whole blood to S well — 1 drop of sample diluent

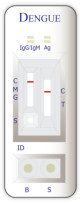
Step 5: Set up timer.
Step 6: Results can be read after 20 minutes.
Do not read result after 25 minutes. To avoid confusion, discard the test device after interpreting the result.

IX. QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30℃.
   e. The temperature of the test area falls outside of 15-30℃.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

X. INTERPRETATION OF RESULTS

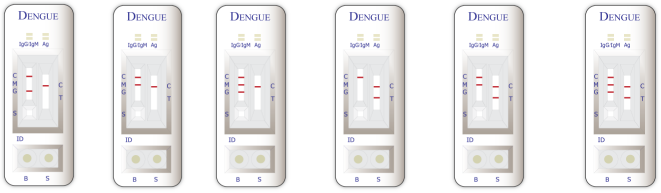


NEGATIVE RESULT:
If only the C line is present, the absence of any burgundy color in the G, M or T lines indicates that neither anti-dengue virus antibodies or dengue virus antigens are detected. The result is negative or non-reactive.

INVALID:
If no C line is developed, the assay is invalid regardless of any burgundy color in the G, M or T lines as indicated below. Repeat the assay with a new device.

POSITIVE RESULT:

IgG Positive	IgM Positive	IgG/IgM Positive	Ag Positive	Ag/IgM Positive	Ag/IgG/IgM Positive



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

XI. PERFORMANCE CHARACTERISTICS

1. Clinical Performance for IgM Test: A total of 387 samples from susceptible subjects were tested with the Dengue IgG/IgM rapid test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue IgG/IgM rapid test		
IgM EIA Test	**Positive**	**Negative**	**Total**
Positive	121	5	126
Negative	7	254	261
Total	128	259	387

Relative Sensitivity: 96.0% , Relative Specificity: 97.3%, Overall Agreement: 96.9%

2. Clinical Performance for IgG Test: A total of 441 samples from susceptible subjects were tested with the Dengue IgG/IgM rapid test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue IgG/IgM rapid test		
IgG EIA Test	**Positive**	**Negative**	**Total**
Positive	153	7	160
Negative	11	270	281
Total	164	277	441

Relative Sensitivity: 95.6% , Relative Specificity: 96.1%, Overall Agreement: 95.9%

3. Clinical Performance For Ag Test: A total of 438 patient samples from susceptible subjects were tested by the Dengue Ag rapid test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue Ag rapid test		
Dengue Ag EIA Test	**Positive**	**Negative**	**Total**
Positive	135	5	140
Negative	12	286	298
Total	147	291	438

Relative Sensitivity: 96.4%, Relative Specificity: 96.0%, Overall Agreement: 96.1%

4. Cross Reactivity: Specimens from other infectious diseases were tested for cross-reactivity with the *Dengue Ag NS1-IgM/IgG* rapid test according to the standard procedure. The results showed that the following specimens did not cross-react with the *Dengue Ag NS1-IgM/IgG* rapid test.

Specimen	Sample size	IgM Test	IgG Test	Ag Test		Specimen	Sample size	IgM Test	IgG Test	Ag Test
HBsAg positive serum	10	-	-	-		Syphilis positive serum	10	-	-	-
HAV positive serum	10	-	-	-		TB positive serum	10	-	-	-
HCV positive serum	10	-	-	-		H. pylori positive serum	10	-	-	-

5. Interference: Common substances (such as pain and fever medication, blood components) may affect the performance of the Dengue Ag NS1-IgM/IgG rapid test. This was studied by spiking these substances into standard controls of Dengue NS1 antigen, Dengue IgG and IgM. The results are presented in the following table and demonstrate that the substances studied do not affect the performance of the Dengue Ag NS1-IgM/IgG rapid test.

Note**:** -: Negative; +: Weak positive;

Interference substances	IgM test		IgG test		Ag Test	
	Negative	Positive	Negative	Positive	Negative	Positive
Control	-	+	-	+	-	+
Bilirubin 20 mg/dL	-	+	-	+	-	+
Glucose 55 mmol/L	-	+	-	+	-	+
Albumin 60 g/L	-	+	-	+	-	+
Salicylic acid 4.34 mmol/L	-	+	-	+	-	+
Heparin 3,000 U/L	-	+	-	+	-	+
EDTA 3.4 □mol/L	-	+	-	+	-	+

XII. LIMITATION OF PROCEDURE

1. The Assay Procedure and Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to dengue virus and dengue Ag in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Dengue Ag NS1-IgM/IgG* rapid test is limited to the qualitative detection of antibodies to dengue virus and dengue Ag in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibody and Ag titers in the specimen.
3. The *Dengue Ag NS1-IgM/IgG* rapid test can not be used to differentiate whether the infection is primary or secondary. No information about dengue serotypes can be provided with this test.
4. Serological cross-reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile, yellow fever, etc.), therefore, it is possible that patients infected with these viruses may show some level of reactivity with this test.
5. A negative or non-reactive result for an individual subject indicates absence of detectable dengue virus antibodies or antigen. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with dengue virus.
6. A negative or non-reactive result can occur if the quantity of the dengue virus antibodies or dengue Ag present in the specimen is below the detection limits of the assay or the antibodies and the Ag that are detected are not present during the stage of disease in which a sample is collected.
7. If the symptoms persist while the result from *Dengue Ag NS1-IgM/IgG* rapid test is negative or non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test device.
8. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
9. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

XIII. BIBLIOGRAPHY

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7. Alcon S, Talarmin A., Debruyne M., et al: Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol, 2002, 40: 376–381.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENTS **Ref. VQ81406**

Test Device	25 items
Sample Diluent	2x 5 mL
5 µL mini plastic droppers/capillary tubes	25 items
Instruction for use	1 item

EDMA Code 15 04 80 11 00 CE IVD