OnSite™ Duo Dengue Ag-IgG/IgM Rapid Test

REF R0062C CE

INTENDED USE

The *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG anti-dengue virus, IgM anti-dengue virus and dengue NS1 antigen (DEN1, 2, 3, 4) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with dengue virus.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

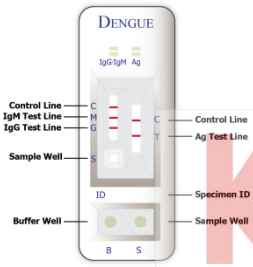
Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (DEN1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting *Stegomyia* family, principally *Aedes aegypti* and *Aedes albopictus*. Today, more than 2.5 billion 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 3 days after initial exposure and remains in circulation for about 30-60 days. IgG anti-dengue virus rises around 7 days, peaks at 2-3 weeks and persists for the duration of life[4-6]. Detection of antigens, such as dengue NS1, 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 *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test detects IgG and IgM anti-dengue virus and circulating dengue NS1 antigen (DEN1, 2, 3, 4) in human serum, plasma or whole blood. It can be performed within 20-25 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The *OnSite* Duo Dengue Ag-IgG/IgM 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 strip consists of: 1) a burgundy colored conjugate pad containing recombinant dengue 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 antibodies for the detection of IgG anti-dengue virus, the M line is pre-coated with antibodies 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 strip consists of: 1) a burgundy colored conjugate pad containing antibodies to 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 antibodies to 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. 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 antibodies to dengue NS1 antigen forming a burgundy colored T line. IgG and/or 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 G and/or M line, respectively.

Suggested result interpretation: Ag positive: Early acute primary or secondary infection. IgM positive: acute primary or secondary infection. IgG positive: secondary or past infection. IgM and IgG positive: Late primary or early secondary acute infection.

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 line of the immunocomplex of the control antibodies in both the left and right panels, regardless of color development on 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.

REAGENTS AND MATERIALS PROVIDED

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 (REF SB-R0062, 5mL/bottle)
5. One Package Insert (instruction for use)

MATERIALS MAY BE REQUIRED AND AVAILABLE FOR PURCHASE

1. *Positivia* Dengue Ag Rapid Test Control Kit (Cat # C0063) contains positive control and negative control.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may give inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read 20-25 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside 20-25 minutes should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, 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. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
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 venipuncture.
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. If not tested immediately, store specimens at 2-8°C for up to 5 days. For longer storage, specimens should be kept frozen at -20°C.

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 specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. 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 at 2-8°C if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature, if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

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.

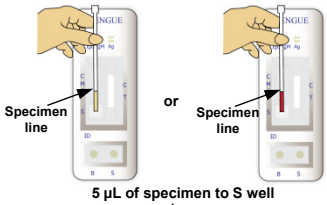
Step 4:

For detection of Dengue IgG/IgM

4.1 Fill the capillary tube with serum/plasma/whole blood specimen not to exceed the specimen line as shown in the images below.

4.2 Holding the capillary tube vertically, dispense the entire specimen (5 µL) 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

20 minutes

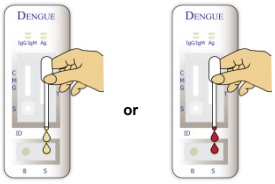
Result

For detection of Dengue Ag

4.1 Fill the plastic dropper with specimen.

4.2 Holding the dropper vertically, dispense 2 drops (about 60 µL) of serum/plasma or 2 drops of whole blood (about 70 µL) into the center of 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.



2 drops of specimen to S well

1 drop of sample diluent to S well

20 minutes

Result

Step 5: Set up timer.

Step 6: Read results at 20 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 25 minutes only. ***However, any results interpreted outside of the 20-25 minute window should be considered invalid and must be repeated. Discard used device after interpreting the result following local laws governing the disposal of device.***

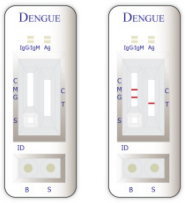
QUALITY CONTROL

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. 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 during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

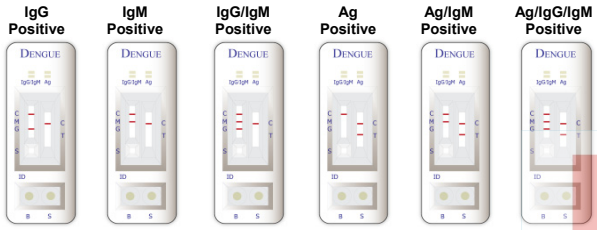
INTERPRETATION OF ASSAY RESULT

1. **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 nor dengue virus NS1 antigen are detected. The result is negative or non-reactive.

2. **INVALID:**
If no C line develops, 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.



3. **POSITIVE RESULT:**



Specimens with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

PERFORMANCE CHARACTERISTICS

1. **Limit of Detection**
The *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test was found to detect NS1 protein in all 4 types of dengue virus lysate I, II, III, and IV. The limit of detection is 0.25 ng/mL as determined on recombinant dengue NS1 antigen from serotype 2 (DEN2).

2. **Clinical Performance for Ag Test**
A total of 100 specimens were collected from susceptible subjects and normal healthy control subjects, and tested by the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial Dengue Ag ELISA. Comparison for all subjects is shown in the following table:

	OnSite Duo Dengue Ag-IgG/IgM Rapid Test		
IgM EIA Test	**Positive**	**Negative**	**Total**
Positive	20	0	20
Negative	1	79	80
Total	21	79	100

Relative Sensitivity: 100%, Relative Specificity: 98.75%, Overall Agreement: 99.0%

3. **Clinical Performance for IgG Test**
A total of 326 specimens were collected from susceptible subjects, and tested with the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	OnSite Duo Dengue Ag-IgG/IgM Rapid Test		
IgM EIA Test	**Positive**	**Negative**	**Total**
Positive	36	1	37
Negative	2	287	289
Total	38	288	326

Relative Sensitivity: 97.3%, Relative Specificity: 99.3%, Overall Agreement: 99.1%

4. **Clinical Performance for IgM Test**
A total of 314 specimens were collected from susceptible subjects and tested with the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	OnSite Duo Dengue Ag-IgG/IgM Rapid Test		
IgG EIA Test	**Positive**	**Negative**	**Total**
Positive	31	1	32
Negative	3	279	282
Total	34	280	314

Relative Sensitivity: 96.9%, Relative Specificity: 98.9%, Overall Agreement: 98.7%

4. **Cross Reactivity**
Specimens from other infectious diseases were tested for cross-reactivity with the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test according to the standard procedure. The results showed that the following specimens (n=1-10) did not cross-react with the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test.

Chikungunya	CMV	HAV	HBV	HCV
HIV	hCG	*H.pylori*	TB	*T. gondii*
Typhoid	Rubella	ANA	HAMA	RF (up to 8,400 IU/mL)

5. **Interference**
Common substances (such as pain and fever medication, blood components) may affect the performance of the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test. This was studied by spiking these substances into negative and positive standard controls for dengue NS1 antigen, dengue IgG and IgM. The results are presented in the following table and demonstrate, at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test.

List of potentially interfering substances and concentrations tested:

1.	Albumin	60 g/L	5.	Glucose	5.5 mmol/L
2.	Bilirubin	20 mg/dL	6.	Heparin	3,000 U/L
3.	Creatinine	442 µmol/L	7.	Sodium citrate	3.8%
4.	EDTA	3.4 µmol/L	8.	Salicylic acid	4.34 mmol/L

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to dengue virus and dengue NS1 antigen in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to dengue virus and dengue NS1 antigen in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibodies and NS1 antigen titers in the specimen.
3. Information about the dengue virus serotype(s) present in a specimen cannot be provided from this test.
4. Serological cross-reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile virus, yellow fever, etc.). Therefore, it is possible that patients who were exposed to 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 NS1 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 antibodies to dengue virus or dengue NS1 antigen present in the specimen is below the detection limits of the assay or the antibodies and antigen that are detected are not present during the stage of disease in which a sample is collected. For example, some patients may not produce detectable levels of IgM antibodies in early infection or repeat infection.
7. Infection may progress rapidly. If the symptoms persist while the result from the *OnSite* Duo Dengue Ag-IgG/IgM Rapid Test is negative or non-reactive, it is recommended to test with an alternative method, such as PCR or ELISA.
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.

REFERENCES

1. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever. The emergence of a global health problem. Emerg Infect Dis.1995;1(2):55–57.
2. Gubler DJ, Trent DW. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. Infect Agents Dis 1993;2:383–393.
3. Monath TP. Dengue: the risk to developed and developing countries. Proc Natl Acad Sci U S A 1994;91:2395-400.
4. Price DD, Wilson SR, "Severe Dengue Infection." Medscape Reference Drugs, Diseases & Procedures, May 2011. Web. http://www.emedicine.com/EMERG/topic124.htm.
5. Innis BL, and Nisalak A, et al: An enzyme-linked immunosorbent assay to characterize dengue infections where denude and Japanese encephalitis co-circulate. Am J Trop Med Hygiene 1989: 40: 418-27.
6. Anonymous. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: World Health Organization, 1997.
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: 376–81.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
San Diego, CA 92121, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R0062C Rev. J
Date released: 2017-05-22
English version

For Export Only, Not For Re-sale In the USA