OnSite® Dengue IgG/IgM Combo Rapid Test

REF R0061C CE

Instructions for Use

INTENDED USE

The *OnSite* Dengue IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of anti–dengue virus IgG and IgM (DEN1, 2, 3 and 4) in human serum, plasma or whole blood. It is intended to be used by professionals to aid in the diagnosis of infection with dengue viruses.

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

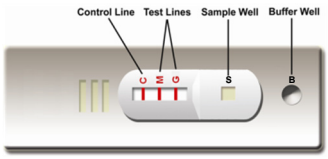
SUMMARY AND EXPLANATION OF THE TEST

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 *Stegemyia* family, principally *Aedes aegypti* and *Aedes albopictus*. Today, more than 2.5 billion people living in 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. Anti-dengue virus IgM starts to appear 3 days after initial exposure and remains in circulation for about 30-60 days. Anti-dengue virus IgG levels rise around 7 days, peak at 2-3 weeks and persist for the duration of life[4-6].

The *OnSite* Dengue IgG/IgM Combo Rapid Test detects anti-dengue virus IgG and IgM 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* Dengue IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a 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 antibodies for the detection of anti-dengue virus IgG, the M line is pre-coated with antibodies for the detection of anti-dengue virus IgM, and the C line is pre-coated with a control line antibody.

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

Anti-dengue virus IgM, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated anti-human IgM, forming a colored M line, indicating an anti-dengue virus IgM positive test result and suggesting either an acute primary or secondary dengue infection. An IgM and IgG positive result indicates a 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 line) which should exhibit a colored line of the control antibodies regardless of color development on any of the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (REF SB-R0061, 5 mL/bottle)
4. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Sterile lancets, sterile gauze and wipes for fingerstick whole blood specimens

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions may lead to 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 of any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen 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. 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 testing results should be read 20-25 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 20-25-minute window 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 until 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/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C 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 with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (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.

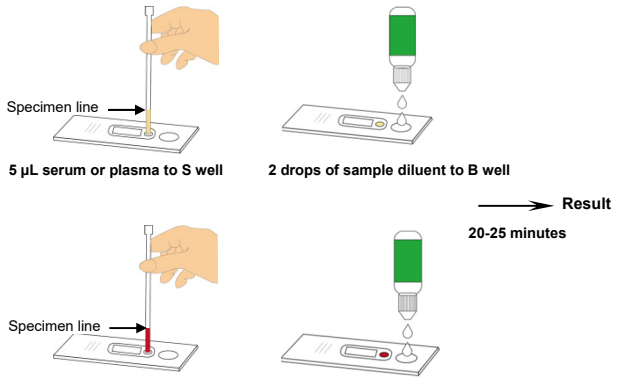
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the capillary tube with the serum, plasma or whole blood specimen not exceeding the specimen line as shown in the image below. The volume of the specimen is around 5µL. ***For better precision, transfer the specimen by a pipette capable of delivering 5µL of volume.***

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 2 drops (about 90-120 µL) of sample diluent into the buffer well **(B well)** with the bottle positioned vertically.



5 µL serum or plasma to S well **2 drops of sample diluent to B well**

5 µL whole blood to S well **2 drops of sample diluent to B well**

Step 5: Set up a 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. ***Any results interpreted outside of the 20-25 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local laws governing the disposal of devices.***

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 there is no visible C line, review the whole procedure and repeat the test using a new device.
2. **External Control:** Good Laboratory Practice recommends using the 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 kit is used.
   c. A new shipment of 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 color in both test lines (G and M) indicates that no anti-dengue virus antibodies are detected. The result is negative or non-reactive.

2. **POSITIVE RESULT:**

2.1 In addition to the presence of C line, if only the G line develops, the test result indicates that anti-dengue virus IgG is detected. The result is anti-dengue virus IgG positive or reactive.

2.2 In addition to the presence of C line, if only the M line develops, the test result indicates that anti-dengue virus IgM is detected. The result is anti-dengue virus IgM positive or reactive.

2.3 In addition to the presence of C line, if both G and M lines develop, the test result indicates that anti-dengue virus IgG and IgM are detected. The result is anti-dengue virus IgG and IgM positive or reactive.

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

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color in the test lines (G and M) as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance for IgG Test**

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

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

Relative Sensitivity: 97.3% (95% CI: 85.8-99.9%),
Relative Specificity: 99.3% (95% CI: 97.5-99.9),
Overall Agreement: 99.1% (95% CI: 97.3-99.8%).

2. **Clinical Performance for IgM Test**

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

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

Relative Sensitivity: 96.9% (95% CI: 83.8-99.9%),
Relative Specificity: 98.9% (95% CI: 96.9-99.8%),
Overall Agreement: 98.7% (95% CI: 96.8-99.7%).

3. **Cross Reactivity**

No false positive anti-dengue virus IgG and IgM test results were observed on 1-13 specimens from the following disease states or specific conditions, respectively:

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

4. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* Dengue IgG/IgM Combo Rapid Test. This was studied by spiking these substances into IgG anti-dengue negative and positive, and IgM anti-dengue negative and positive specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Dengue IgG/IgM Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

Albumin	60 g/L	Caffeine	20 mg/dL
Acetominophen	20 mg/dL	EDTA	3.4 µmol/L
Atropine	20 mg/dL	Hemoglobin	2 g/L
Aspirin	20 mg/dL	Heparin	3,000 U/L
Ascorbic acid	20 mg/dL	IgG	1,000 mg/dL
Bilirubin	20 mg/dL	Glucose	55 mmol/L
Creatinine	442 µmol/L	Salicylic acid	4.34 mmol/L
Sodium citrate	3.80%		

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to dengue virus in serum, plasma and whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Dengue IgG/IgM Combo Rapid Test is limited to the qualitative detection of anti–dengue virus IgG and IgM in human serum, plasma and whole blood. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. Information about the dengue virus serotype(s) present in a specimen cannot be provided from this test.
4. The *OnSite* Dengue IgG/IgM Combo Rapid Test cannot differentiate primary or secondary infection.
5. 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.
6. A negative or non-reactive result for an individual subject indicates absence of detectable dengue virus antibodies. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with dengue virus.
7. A negative or non-reactive result can occur if the quantity of antibodies to dengue virus present in the specimen is below the limits of detection, or if the antibodies that are detected are not present during the stage of disease in which a sample is collected.
8. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
9. Infection may progress rapidly. If the symptoms persist, while the result from *OnSite* Dengue IgG/IgM Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
10. The results obtained with this test should be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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3. Monath TP. Dengue: The risk to developed and developing countries. Proc Natl Acad Sci U S A 1994;91:2395–2400.
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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. Trap. Med. Hygiene. 1989: 40: 418-427.
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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number		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.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

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

PI-R0061C Rev K
Date released: 2021-08-02
English version

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