OnSite® Dengue Ag Rapid Test

REF R0063C CE

Instructions for Use

INTENDED USE

The *OnSite* Dengue Ag Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals 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.

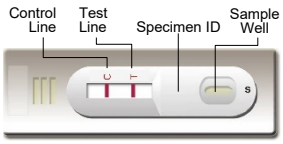
SUMMARY AND EXPLANATION OF THE TEST

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*. 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 of IgM antibody is the most common method for the diagnosis of acute dengue virus infection. Lately, detection of antigens, such as dengue NS1, released during virus replication in the infected patient showed 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[4].

The *OnSite* Dengue Ag Rapid Test detects all four serotype dengue NS1 antigens in human serum, plasma or whole blood. It can be performed within 20 minutes by minimally skilled personnel and without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Dengue Ag Rapid Test is a lateral flow chromatographic immunoassay. The test strip in cassette device consists of: 1) a 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 antigens, 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 antigens forming a colored T line, indicating a dengue Ag positive test result and suggesting an early acute primary or secondary infection.

Absence of the T line suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies, regardless of color development on the test line (T line). 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. Plastic droppers
3. Sample diluent (REF SB-R0063, 5 mL/bottle)
4. Instructions for Use

MATERIALS MAY BE REQUIRED AND AVAILABLE FOR PURCHASE

1. *Positivia* Dengue Ag Rapid Test Assay 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. 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 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. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other bloodborne 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. Read the test results 20-25 minutes after a specimen is applied to the sample well or sample pad 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. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device 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 temperature above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Step 1: **For all specimens:** Collect venous blood by venipuncture into collection tube containing EDTA, citrate or heparin for plasma or whole blood specimens, or collection tube containing no anticoagulants for serum specimens.

Step 2: **For whole blood specimens:** Test immediately or store refrigerated at 2-8°C for up to 24 hours after collection. Do not freeze specimens.

To prepare plasma: Centrifuge collected specimen and carefully withdraw plasma into new pre-labeled tube.

To prepare serum: Allow blood to clot, centrifuge collected specimen and carefully withdraw serum into new pre-labeled tube.

Step 3: **For plasma/serum only:** Test specimens immediately after collection or store refrigerated at 2-8°C for up to 5 days. Specimens can 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.

Note: Do not test specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

ASSAY PROCEDURE

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

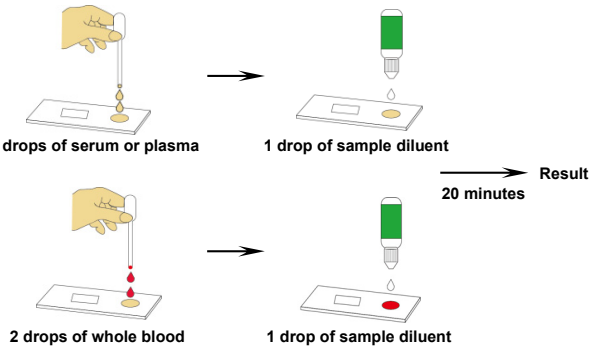
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Label the device with specimen ID number.

Step 4: Fill the plastic dropper with the specimen.

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, making sure that there are no air bubbles.

Immediately add 1 drop (about 30-40 µL) of sample diluent into the center of the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Results should be read at 20-25 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 requirements governing the disposal of devices.***

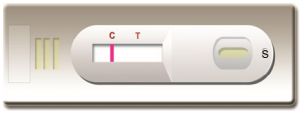
QUALITY CONTROL

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 the C line does not develop, review the whole procedure and repeat 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 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 test indicates that no detectable dengue NS1 antigen is present in the specimen. The result is non-reactive or negative.



2. **POSITIVE RESULT**: If both the C and T lines develop, the test indicates the presence of detectable dengue NS1 antigen in the specimen. The result is reactive or positive.

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

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Limit of Detection**

The *OnSite* Dengue Ag 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**

A total of 350 specimens were collected from susceptible subjects and normal healthy control subjects, and tested by the *OnSite* Dengue Ag Rapid Test and by a commercial Dengue Ag ELISA. Comparison for all subjects is shown in the following table:

	***OnSite* Dengue Ag Rapid Test**		
Reference Test	**Positive**	**Negative**	**Total**
Positive	117	0	117
Negative	1	232	233
Total	118	232	350

Relative Sensitivity: 100% (95% CI: 96.8-100%)
Relative Specificity: 99.6% (95% CI: 97.6-99.9%)
Overall Agreement: 99.7% (95% CI: 98.4-99.9%)

3. **Cross-Reactivity**

No false positive dengue Ag test results were observed on 6-10 positive specimens from the following disease states:

Chikungunya	CMV	HAV	HBV	HCV
HIV	*H. pylori*	HSV-1	HSV-2	Leishmania
Malaria	TB	*T. gondii*	*T. pallidum*	Typhoid
Rubella	Zika	ANA	HAMA	Pregnant women
RF (up to 8,400 IU/mL)				

4. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the *OnSite* Dengue Ag Rapid Test. This was studied by spiking these substances into negative serum and positive serum samples spiked with NS1 antigen from all 4 serotypes (DEN1, 2, 3, 4), respectively. The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Dengue Ag Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Heparin	3,000 U/L
2. Bilirubin	20 mg/dL	7. Salicylic acid	4.34 mmol/L
3. Creatinine	442 µmol/L	8. Sodium citrate	3.8%
4. EDTA	3.4 µmol/L	9. Human IgG	1,000 mg/dL
5. Glucose	55 mmol/L		

5. **Dose Hook Effect**

No hook effect was detected with dengue NS1 antigen concentration up to 200 µg/mL.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of dengue NS1 antigen (DEN1, 2, 3, 4) in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Dengue Ag Rapid Test is limited to the qualitative detection of dengue antigen in human serum, plasma and whole blood. The intensity of the test line does not have a linear correlation with the dengue antigen titer of the specimen.
3. A non-reactive or negative test result does not preclude the possibility of exposure to or infection with dengue viruses.
4. A non-reactive or negative result can occur if the quantity of dengue NS1 antigen present in the specimen is below the detection limits of the assay or the dengue NS1 antigens that are detected are not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptoms persist while the result from *OnSite* Dengue Ag Rapid Test is non-reactive, it is recommended to test with an alternative method such as PCR or ELISA.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. 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; 91:2395–2400.
4. 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. Journal of Clinical Microbiology 2002; 40: 376–381.

Index of Symbols

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