ONE STEP Dengue NS1 Ag and IgG/IgM Test

SD BIO LINE Dengue Duo

Explanation of the test

Dengue viruses, transmitted by the mosquito, *Aedes aegypti* and *Aedes albopictus* mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world. There are four known distinct serotypes (dengue virus 1, 2, 3 and 4). In children, infection is often subclinical or causes a self-limited febrile disease. However, if the patient is infected second times with a different serotype, a more severe disease, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur. Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes. NS1 is a highly-conserved glycoprotein that is present at high concentrations in the sera of dengue-infected patients during the early clinical phase of the disease. NS1 antigen is found from the first day and up to 9 days after onset of fever in sample of primary or secondary dengue infected patients. Usually IgM does not become detectable until 5 to 10 days after the onset of illness in cases of primary dengue infection and until 4 to 5 days after onset of illness in secondary infections. In primary infections, IgG appear the 14th day and persist for life. Secondary infections show that IgGs rise within 1~2 days after the onset of symptoms and induce IgM response after 20 days of infection.

[Intended Use]

The SD BIOLINE Dengue Duo rapid test is an in-vitro immunochromatographic, one step assay designed to detect both dengue virus NS1 antigen and differential IgG / IgM antibodies to dengue virus in human serum, plasma or whole blood. This SD BIOLINE Dengue Duo rapid test contains two test devices (left side ; Dengue NS1 Ag test, right side ; Dengue IgG/IgM test). The Dengue NS1 Ag rapid test in the left-side is an in-vitro immunochromatographic, one step assay designed for the qualitative determination of dengue virus NS1 antigen in human serum, plasma or whole blood for the diagnosis of early acute dengue infection. This test device contains a membrane strip, which is pre-coated with anti-dengue NS1 Ag capture on test band region. The anti-dengue NS1 Ag -colloid gold conjugate and serum, plasma or whole blood sample move along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms. The Dengue IgG / IgM Rapid Test in the right side is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test is intended for professional use to aid in the presumptive diagnosis between primary and secondary dengu e infection. This SD BIOLINE Dengue Duo provides only a preliminary test result. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and serological test like haemagglutination-inhibition test, more specific alternative diagnosis method must be used in order to obtain a confirmation of dengue virus infection.

[Principle]

The Dengue NS1 Ag test device result window has 2 pre-coated lines, "T" (NS1 Ag Test Line) and "C" (Control Line). Both the Test Line and the Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control and should always appear if the test procedure is performed correctly. The Dengue NS1 Ag can identify dengue virus NS1 antigen in serum, plasma or whole blood specimens with a high degree of sensitivity and specificity. Dengue IgG / IgM rapid test is designed to simultaneously detect and differenciate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test also can detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins. Dengue IgG / IgM test device has 3 pre-coated lines, "G" (Dengue IgG Test Line), "M" (Dengue IgM Test Line) and "C" (Control Line) on the surface of the device. All three lines in result window are not visible before applying any samples. The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple "G" and "M" lines will be visible in the result window if there are enough IgG and/or IgM antibodies to Dengue virus in the sample. If IgG and/or IgM antibodies to Dengue virus are not present in the sample, there is no color appearance in "G" and/or "M". When a specimen is added to the sample well, anti-dengue IgGs and IgMs in the specimen will react with recombinant dengue virus envelope proteins-colloidal gold conjugates and forms a complex of antibodies-antigen. As this complex migrates along the length of the test device by capillary action, it will be captured by the relevant anti-human IgG and/or anti-human IgM immobilized in two test lines across the test device and generate a colored line.

Materials provided/ Active ingredients of main components

1. SD BIOLINE Dengue Duo kit contains the following items to perform the assay.
   - Dengue NS1 Ag and Dengue IgG/IgM Combo device
   - Assay diluent for Dengue IgG/IgM test
   - 10㎕ capillary pipette for Dengue IgG/IgM test
   - Disposable dropper for Dengue NS1 Ag test
   - Instruction for use
2. Active ingredients of main components

   [SD BIOLINE Dengue NS1 Ag]
   - 1 test strip included ; Gold Conjugates (as main component) : Mouse monoclonal anti-dengue NS1 – gold colloid (0.27±0.05㎍), Test Line (as main component) : Mouse monoclonal anti-dengue NS1 (0.72±0.14㎍), Control Line (as main component) : Goat anti-mouse IgG (0.72±0.14㎍)

   [SD BIOLINE Dengue IgG / IgM]
   - 1 test strip included ; Gold Conjugates (as main component) : Recombinant Dengue virus envelope protein – gold colloid (1±0.2㎍), Test Line "G" (as main component) : Mouse monoclonal anti-human IgG (5±1㎍), Test Line "M" (as main component) : Mouse monoclonal anti-human IgM (5±1㎍), Control Line (as main component) : Rabbit anti-Dengue IgG (2.5±0.5㎍),
   - Assay buffer included ; 100 mM Phosphate buffer (5 ml), Sodium azide (0.01% w/w)

Kit Storage and Stability

The SD BIOLINE Dengue Duo test should be stored at room temperature. The test strip is sensitive to humidity and as well as to heat. Perform the test immediately after removing the test strip from the foil pouch. Do not use it beyond the expiration.

Specimen Collection, Storage and Precaution

1. Whole blood
   - Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture.
   - If blood specimens are not immediately tested, they should be refrigerated at 2~8°C.
   - When stored at 2~8°C, the blood specimens should be used within 3 days.
   - For storage period longer than 3 days, freezing is recommended. They should be brought to room temperature (1~30°C) prior to use.
   - Using the blood specimens in the long-term keeping more than 3 days can cause non-specific reaction.
2. Plasma or Serum
   - [Plasma] Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.
     [Serum] Collect the whole blood into the collection tube (NOT containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
   - If plasma or serum specimens are not tested immediately, they should be refrigerated at 2~8°C. For storage period longer than 2 weeks, freezing is recommended. They should be brought to room temperature (1~30°C) prior to use.
   - Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
3. Precaution
   - Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.
   - As known relevant interference, hemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.
   - Use separately disposable capillary pipettes or pipette tips for each sample in order to avoid cross-contamination of either samples which could cause erroneous results.

Warnings

1. For in vitro diagnostic use only. DO NOT RE-USE test device.
2. Do not eat or smoke while handling specimens.
3. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4. Avoid splashing or aerosol formation.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
7. Do not use the test kit if the pouch is damaged or the seal is broken.
8. The instruction must be followed exactly to get accurate results.

Procedure of the test (Refer to figure of Test Procedure)

[SD BIOLINE Dengue NS1 Ag].

1. Remove the test device from the foil pouch, and place it on a flat, dry surface.
2. With a disposable dropper, add 3 drops (about 100㎕) of specimen into the sample well (S).
3. As the test begins to work, you will see purple color move across the result window in the center of the test device.
4. Interpret test results at 15~20 minutes.
5. A positive result will not change once it has been established at 15 ~20 minutes. However, in order to prevent any incorrect results, the test result should not be interpreted after 20 minutes.

[SD BIOLINE Dengue IgG / IgM]

1. Allow all kit components and specimen to room temperature prior to testing.
2. Remove the test device from foil pouch, place it on a flat, dry surface.
3. With 10㎕ capillary pipette, add 10㎕ of specimen drawn to black line into the square sample well marked "S".
4. Add 4 drops (about 90~120㎕) of assay diluent to the assay diluent well round shaped.
5. Interpret test results at 15~20 minutes.

   Caution : Do not read test results after 20 minutes. Reading too late can give false results.

Interpretation of the test (Refer to figure of Interpretation)

[SD BIOLINE Dengue NS1 Ag]

Negative Result : The presence of each one color line within the result window indicates a negative result.

Positive result : The presence of two color lines ("T" band and "C" band) within the result window no matter which line appears first, indicates a positive result.

Invalid Result : If the color line is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

[SD BIOLINE Dengue IgG / IgM]

Negative

The control line is only visible on the test device. No IgG and IgM antibodies were detected. Retest in 3-5 days if dengue infection is suspected.

IgM Positive

The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Dengue virus. This is indicative of a primary dengue infection.

IgG Positive

The control line and IgG line (G) are visible on the test device. This is positive for IgG antibodies. This is indicative of secondary or past dengue infection.

IgG and IgM Positive

The control line, IgM (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies. This is indicative of a late primary or early secondary dengue infection.

Invalid

The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Repeat the test using a new test device.

Limitations of the test

1. A negative result can occur if the quantity of Dengue virus NS1 antigen present in the specimen is below the detection limits of the assay, or the antigens that are detected are not present during the stage of disease in which a sample is collected.
2. A negative test result cannot exclude a recent infection.
3. The presence of detectable Dengue virus NS1 Ag may mean positive for early Dengue Infection. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. In early infections and some secondary infections, detectable levels of IgM antibodies may be low. Some patients may not produce detectable levels of antibody within the first seven to ten days after infection. Where symptoms persist, patients should be re-tested 3-4 days after the first specimen.
5. Serological cross-reactivity across the Flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.

Internal Quality Control

The SD BIOLINE Dengue NS1 Ag test device has "Test Line" and "Control Line" on the surface of the cassette. And the SD BIOLINE Dengue IgG / IgM test device has "G(Dengue IgG Test Line)", "M(Dengue IgM Test Line)" and "C(Control Line)". All the Test Lines and Control Lines in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

Expected value

The NS1 is expected to be detected 1 day after the onset of fever and persist up to 9 days in both primary and secondary dengue infection. But if anti-NS1 antibodies produced, the detection of NS1 is inhibited. Primary dengue is characterized by the presence of detectable IgM 3-5 days after the onset of infection. Secondary dengue is characterized by the elevation of specific IgG 1-2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

Performance Charcteristics

1. Dengue NS1 Ag Sensitivity and Specificity

   The specimens used in this test were confirmed by RT-PCR and/or Viral culture.

		RT-PCR and/or Viral culture		Total
		Positive	Negative	
SD Dengue NS1 Ag	Positive	104	3	107
	Negative	8	186	194
	Total	112	189	301

   * Sensitivity = 104/112 x 100 = 92.8 %
   Specificity = 186/189 x 100 = 98.4 %

2. Dengue IgG/IgM Sensitivity and specificity

   The comparison results of SD BIOLINE Dengue IgG/IgM test with Haemagglutination inhibition (HI) test showed that SD BIOLINE Dengue IgG/IgM test had good correlation with HI test.

		Reference assay : HI test		Total
		Positive	Negative	
SD Dengue IgG/IgM	Positive	159	3	162
	Negative	1	39	40
	Total	160	42	202

   * Sensitivity = 159/160 x 100 = 99.4 %
   Specificity = 39/42 x 100 = 93 %

Technique used	No. of samples teste	IgM positive only	IgG positive only	IgG+IgM Both positive	Total IgM positive	Total IgG positive	Negative
MAC-ELISA	202	39	45	76	115	121	42
Commercial Rapid	202	41	88	32	73	120	41
SD Dengue IgG/IgM	202	33	21	108	141	129	40

3. Sensitivity of SD Dengue Duo related to clinical signs apparition

	Days after symptom onset	No. of samples	No. of positive samples (%)				
			IgG antibody	IgM antibody	Total Antibody(*1)	NS1 Ag	Final Result(*2)
1st	1~7	52	9 (17.3)	21 (40.4)	23 (44.2)	43 (82.7)	49 (94.3)
	8~14	30	26 (86.7)	30 (100)	30 (100)	15 (50)	30 (100)
	15~21	36	35 (97.2)	36 (100)	36 (100)	5 (13.9)	36 (100)
2nd	1~7	36	24 (66.7)	8 (22.2)	24 (66.7)	24 (66.7)	32 (88.9)
	8~14	34	34 (100)	18 (53)	34 (100)	14 (41.2)	34 (100)
	15~21	42	42 (100)	24 (57.2)	42 (100)	3 (7.2)	42 (100)

   *1 : Dengue IgG and/or IgM
   *2 : Dengue NS1 and/or IgG&IgM antibody

4. Cross-reactivity test with other Flavivirus mediated and mosquitoes-borne disease

Disease	Dengue IgM Negative/Total	Dengue IgG Negative/Total	Dengue NS1 Ag Negative/Total
Japanese Encephalitis	25/25	25/25	25/25
Yellow Fever	25/25	25/25	25/25
Malaria P. falciparum	25/25	25/25	25/25
Malaria P. vivax	25/25	25/25	25/25
Total	100/100	100/100	100/100

5. Precision : Within-run and between-run precisions have been determined by the testing of ten specimens three times : 6 of negative, 3 of low positive, 3 of medium positive and 3 of strong positive. All values were collectly identified 100% of the time.
6. To evaluate the interference of Dengue Duo rapid kit with known relevant interfering specimens, the hemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples were investigated. In these studies, those specimens did not interfere with this test kit.
7. Analytical sensitivity : the limit of detection ; the smallest amount of the target marker that can be precisely detected ; have been equal or superior to a leading commercial dengue NS1 Ag and antibody detection rapid test.

Bibliography of suggested reading

1. Pryor MJ. Wright PJ. The effects of site-directed mutagenesis on the dimerization and secretion of the NS1 protein specified by dengue virus. Virology 1993; 194:768-80
2. SHU, P.,HUANG, J. Current advances in dengue diagnosis. Clin. Diagn. Lab. Immunol. 2004 Jul; 11(4):642-50.
3. Alcon S., Talamin A., Debryyne M., Falconar A., Deubel V., Falmand M. 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 non structural protein NS1 reveals circulation of the antigen in the blood during acute phase of disease in patients experiencing primary or secondary infections. J. Clin. Microbiol. 40:376-381.
4. Jan Groen et al. Evaluation of six immunoassays for detection of dengue-virus specific immunoglobulin M and G Antibodies. Clin. Diagn. Lab. Immunol. Vol 7(6) p 867-871, 2000

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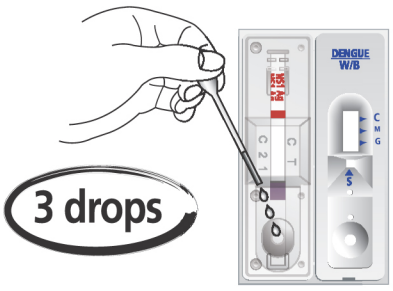
SD BIOLINE Dengue NS1 Ag and IgG/IgM Test

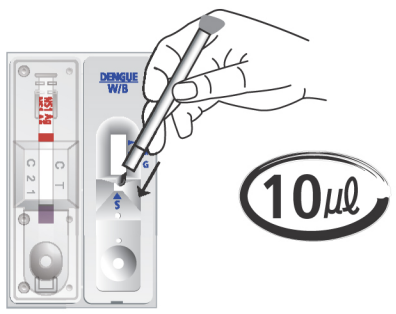
[Dengue NS1 Ag]



Using disposable dropper provided, add 3 drops (about 100μℓ) of serum, plasma or whole blood into the sample well marked "S".

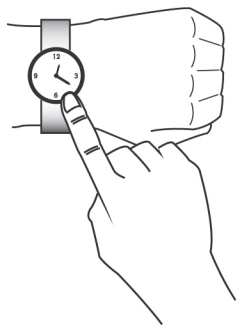
Interpret test results in 15~20 minutes.

[Dengue IgG / IgM]



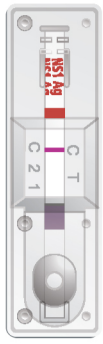
Using capillary pipette provided, add 10μℓ of serum, plasma or whole blood into the sample well marked "S".

Put 4 drops of assay diluent into the assay diluent well round shaped.



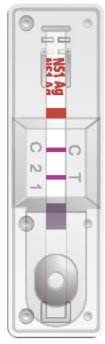
Interpretation

[Dengue NS1 Ag]



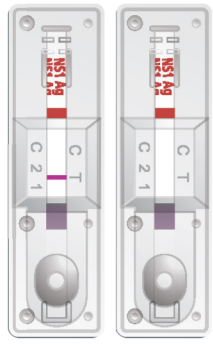
Negative

The presence of only one color line within the result window indicates a negative result.



Positive

The presence of two color lines ("T" band and "C" line) within the result window in left-side, no matter which line appears first, indicates a positive result.

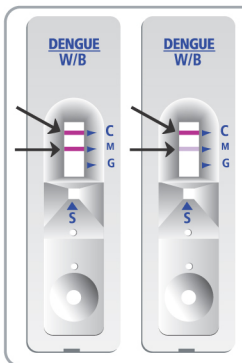


Invalid

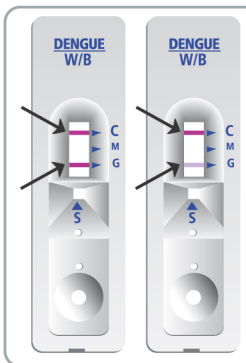
-No control (C) line in result window.
-It is recommended that the specimen be re-tested.

[Dengue IgG / IgM]

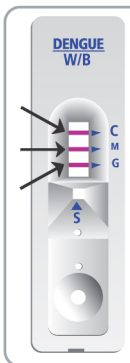
Positive



1. IgM positive
(Primary dengue infection)
-Two lines "C" and "M" in result window.
-It is positive even if "M" line is weak.



2. IgG positive
(Secondary or past dengue infection)
-Two lines "C" and "G" in result window.
-It is positive even if "G" line is weak.



3. IgG and IgM positive
(Late primary or early secondary dengue infection)
-Three lines "C", "M" and "G" in result window.

Negative

(No dengue infection)
-One line "C" in result window at right

Invalid

-No control (C) line in result window.
-It is recommended that the specimen be re-tested.