Dengue NS1/IgG/IgM 3in1 Rapid Test

For detection of Dengue NS1 antigens and IgG/IgM in Human Serum, Plasma or Whole Blood.

IVD *In-vitro diagnostic use only* *Not reuse*

Introduction

Dengue viruses, transmitted by 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 sub-clinical or causes a self-limited febrile disease. However, if the patient is injected a second time 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 associated with it. 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 the onset of fever in samples 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 appears the 14th day and persist for life. Secondary infections show that IgG rise within 1-2 days after the onset of symptoms and induce IgM response after 20 days of infection.

Intended Use

Dengue 3in1 rapid test 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 and for the qualitative and differential detection of IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. Part of 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-colloidal gold conjugate and serum, plasma or whole blood sample move along the membrane chromatographically to the test region and forms a visible line as the antibody-antigen-antibody gold particle complex forms. Other part of this test is intended for professional use as an aid in the presumptive diagnosis between primary and secondary dengue infection. This test provides only a preliminary test result. Therefore, a more specific diagnosis method must be used in order to obtain a confirmation of dengue virus infection

Principle

Dengue NS1 Ag test device (right channel) result window has 2 pre-coated lines, "T" (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 rapid test can identify dengue virus NS1 antigen in serum, plasma or whole blood specimens with a high degree of sensitivity and specificity.

Dengue IgG/IgM test device (left channel) is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test can also 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 membrane. All three lines in result window are not visible before applying any samples. The “Control Line” is used for procedural control. The control line should always appear if the test procedure is performed properly and the test reagents of the control line are working. Pink “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 will be 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 Included and Active Ingredients

1) Dengue 3in1 rapid test kit contains the following items to perform the assay.
   - ❖ Dengue 3in1 rapid test device foil pouched with a desiccant
   - ❖ Disposable pipette (may not provided)
   - ❖ Instruction for use
   - ❖ Test Buffer (one per box)

2) Active ingredients of main components of test stripes

Dengue NS1 Ag test strip (right channel)

- ❖ Gold Conjugates (as main component): Mouse monoclonal anti-dengue NS1 – gold colloid (0.27 ±0.05 µg),
- ❖ Test Line (as main component): Mouse monoclonal anti-dengue NS1 (0.72 ±0.14 µg),
- ❖ Control Line (as main component) Goat anti-mouse IgG (0.72 ±0.14 µg)

Dengue IgG/IgM test strip (left channel)

- ❖ Gold Conjugates (as main component) : Recombinant Dengue virus envelope protein-gold colloid (1 ± 0.2 µg)
- ❖ Test Line “G” (as main component) : Mouse monoclonal anti-human IgG (5 ± 0.2 µg)
- ❖ Test Line “M” (as main component): Mouse monoclonal anti-human IgM (5 ±0.2 ug)
- ❖ Control Line (as main component) Goat anti-dengue IgG (2.5 ±0.14 ug)

Kit Precautions and Storage Instructions

1) For best results, adhere to instructions provided
2) All specimens should be handled as potentially infectious
3) The test device should be stored at room temperature
4) The test device is sensitive to humidity as well as heat
5) Do not use beyond expiration date
6) Do not use test kit if pouch is damaged or seal is broken
7) Use test device immediately after removing from the pouch
8) The components (test device and assay diluents) in this kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

Warnings

1) For in vitro diagnostic use only. DO NOT RE-USE test device
2) The instructions must be followed to obtain accurate results. Anyone performing an assay with this product must be trained in its use and laboratory procedures.
3) Do not eat or smoke while handling specimens
4) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
5) Avoid splashing or aerosol formation
6) Clean up spills thoroughly using an appropriate disinfectant
7) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
8) Do not mix with other specimens.

Specimen Collection, Storage and Precautions

1) Serum (S): Collect the whole blood into a 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
2) Plasma (P): Collect the whole blood into a collection tube (containing anticoagulants such as heparin, EDTA, and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.
3) Whole Blood (WB): Collect WB with a lancing device. WB specimen can be delivered to test card directly. Or if applicable, collect the whole blood into a collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture. Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 24 hours after collection
4) If serum or plasma specimens are not tested immediately, they should be refrigerated at 2-8 ^{o}C. For storage periods longer than 2 weeks, freezing is recommended. They should be brought to room temperature (1-30 ^{o}C) prior to use.
5) Serum or plasma specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
6) Anticoagulants such as heparin, EDTA and sodium citrate do not affect the test results.
7) 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.
8) As known relevant interference, hemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.

Test Procedure (Refer to Figure)

Dengue NS1 test device

1) Allow all test components and specimen to come to room temperature prior to testing.
2) Remove the test device from the foil pouch, and place it on a flat, dry surface
3) With a micropipette (not provided) or a disposable dropper, add 100 µL of serum/plasma/whole blood specimen into the sample well marked “S”
4) As the test begins to work, you will see red color move across the result window in the center of the test device.
5) Interpret test results at 15-20 minutes. Caution: Do not read test results after 20 minutes. Reading too late can give false results.

Dengue IgG/IgM test device

1) Allow all test components and specimen to come to room temperature prior to testing.
2) Remove the test from pouch and place it on a flat, dry surface
3) Using micropipette (not provided), add 10 µL of serum, plasma or whole blood specimen into sample well. Please wait 30 seconds for specimen adsorption.
4) Add 2 drops (approx. 80 µL) of assay diluents to the sample well.
5) Interpret test results in 15-20 minutes. Do not read test results after 20 minutes.

Interpretation of Test Results (Refer to Figure)

Dengue NS1 test device

1) Negative

The control line is the only visible line on the test device. This indicates a negative result.

2) Positive

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

3) Invalid

If the “C” line (control) 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.

Dengue IgG/IgM test device

1) Negative

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

2) IgM Positive

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

3) IgG Positive

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

4) IgG and IgM Positive

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

5) Invalid

The control line fails to appear. Insufficient specimen volume or incorrect procedural tehniques are the likeliest 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 are not present during the stage of disease in which the sample was 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.

Internal Quality Control

Dengue NS1 Ag test device has “Test Line” and “Control Line” on the surface of the cassette. And the 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 antigen 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 are produced, the detection of NS1 is inhibited. Primary dengue infection is characterized by the presence of detectable IgM 3-5 days alter the onset of infection. Secondary dengue is characterized by the elevation of specific IgG 1-2 days alter the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

Evaluation Data

The specimens tests were confirmed by reference ELISA assay.

3in1 rapid test kit		RT-PCR Assay		
		Positive	Negative	Total
Dengue NS1 rapid test strip	Positive	28	2	30
	Negative	2	31	33
	Total	30	33	63

Sensitivity = 24/26 (92.3%)
Specificity = 30/33 (90.9 %)

The specimens tests were confirmed by reference RT-PCR assay.

3in1 rapid test kit		ELISA Assay		
		Positive	Negative	Total
Dengue IgG/IgM test strip	Positive	24	3	27
	Negative	2	30	32
	Total	26	33	59

Sensitivity = 28/30 (93.3%)
Specificity = 31/33 (93.9%)

Suggested Readings

1. Dengue haemorrhagic fever: Diagnosis, treatment, prevention and contril. WHO 2nd Edition,1997
2. Clarke, D. H. and Casals, J. Techniques for Hemagglutination and hemagglutination inhibition with arthropodborne viruses. *Am. J. Trop. Med. Hyg. 1958,* 7, 561-573.
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4. Shu, P., Huang, J. Current advances in dengue diagnosis. *Clin. Diagn. Lab. Immunol.* 2004,11, 642-650.
5. Alcon S., Talamin A., Debryyne M., Falconar A., Deubel V., Falmand M., Enzyme-linked immunosorbent assay specific to dengue virus type 1 non structural protein NS1 reveals circulation of theantigen in the blood during acute phase of disease in patients experiencing primary or secondary infections. *J. Clin. Microbiol.* 2002, 40, 376-381.
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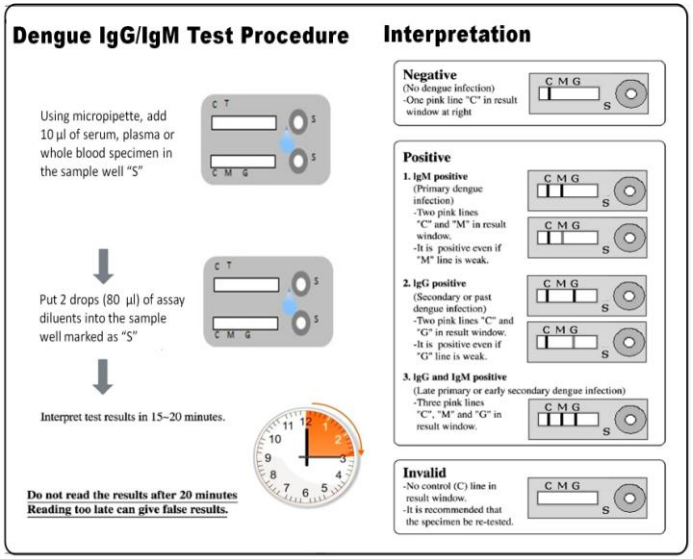
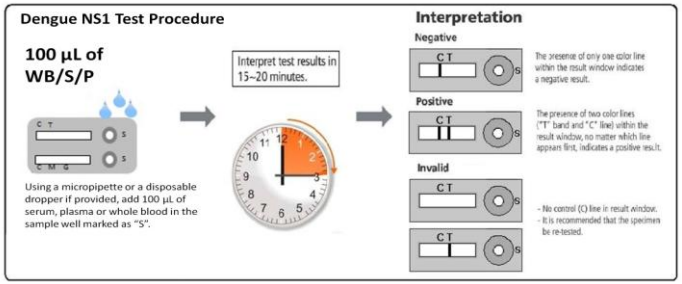
7. Songee L. Ranch and Paul N. Levett. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue viurs. *Clin. Diagn. Lab. Immunol*. 1999, 6, 555-667
8. Sutherst, R. W. (1993). Arthropods as disease Vectors in a changing environment. In environmental change and human health. CIBA foundation symposia 1993, 175, 124-145.





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BIOGATELABS

Manufactured & Quality Controlled by
Biogate Laboratories Ltd.
110-4238 Lozells Avenue, Burnaby, BC
Canada, V5A 0C4
Tel: 1-604-322-2955 Fax 1-604-322-2955
www.biogatelab.com