Dengue IgG/IgM Rapid Test

For detection of Dengue IgG/IgM antibodies 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.

Rapid and reliable tests for primary and secondary infections of dengue are essential for patient management. Primary dengue infection is associated with mild to high fever, headache, muscle pain, and skin rash. Immune response includes IgM antibodies produced by the 3rd to 5th day of symptoms and persist for 30-60 days. IgG appear the 14th day and persist for life. Secondary infections often result in high fever and in many cases with haemorrhagic events and circulatory failure. 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

Dengue IgG/IgM Rapid Test is a solid phase immunochromatographic assay for the 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 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 IgG/IgM Rapid Test 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 form 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 IgG/IgM kit contains the following items to perform the assay.
- ❖ Dengue IgG/IgM test device foil pouched with a desiccant
- ❖ Instructions for use
- ❖ Buffer (one per box)

2) Active ingredients of main components of one Dengue IgG/IgM test strip
- ❖ Gold Conjugates (as main component) : Recombinant Dengue viru 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 and is sensitive to humidity as well as heat.
4) Do not use beyond expiration date.
5) Do not use test kit if pouch is damaged or seal is broken.
6) Use test device immediately after removing from the pouch.
7) 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.
8) The assay diluents contain a low concentration of sodium azide as a preservative.
9) Sodium azide is toxic and should be handled carefully to avoid ingestion and skin contact.

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. 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 mix with other specimens.

Specimen Collection, Storage and Precautions

1) Serum: 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: 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 by pipette 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) Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 24 hours alter collection.
5) If serum or plasma specimens are not tested immediately, they should be refrigerated at 2-8℃. For storage periods longer than 2 weeks, freezing is recommended. They should be brought to room temperature (1-30℃) prior to use.
6) Serum or plasma specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
7) Anticoagulants such as heparin, EDTA and sodium citrate do not affect the test results.
8) Use separately disposable dropper or pipette tips for each sample in order to avoid cross-contamination of either samples which could cause erroneous results.

Test Procedure (Refer to Figure)

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 marked “S”
4) Add 2drops (approx. 80 μL) of assay diluents (provided) to the assay diluents well.
5) Interpret test results in 15-20 minutes. Do not read test results after 20 minutes.

Interpretation of Test Results (Refer to Figure)

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 techniques are the likeliest reasons for control line failure. Repeat the test using a new test device.

Limitations of the Test

1) This test detects the presence of antibodies to dengue in the specimen and should not be used as the sole criterion of Dengue virus infection.

2) 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 7-10 days after infection. If clinical symptoms persist, patients should be retested in 3-4 days.

3) Serological cross-reactivity across the Flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.

4) As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

5) If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of an early infection of dengue virus.

6) The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.

Internal Quality Control

The control line is used for procedural control. Control lines should always appear if the test procedure is performed properly and the test reagents of the control line are working. It confirms sufficient specimen volume and correct procedural technique. A clear background
is also required.

Expected value

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.

Evaluation Data

The specimens tests were confirmed by reference ELISA assay.

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

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

Suggested Readings

1. Dengue haemorrhagic fever: Diagnosis, treatment, prevention and contril. WHO 2nd Edition,1997

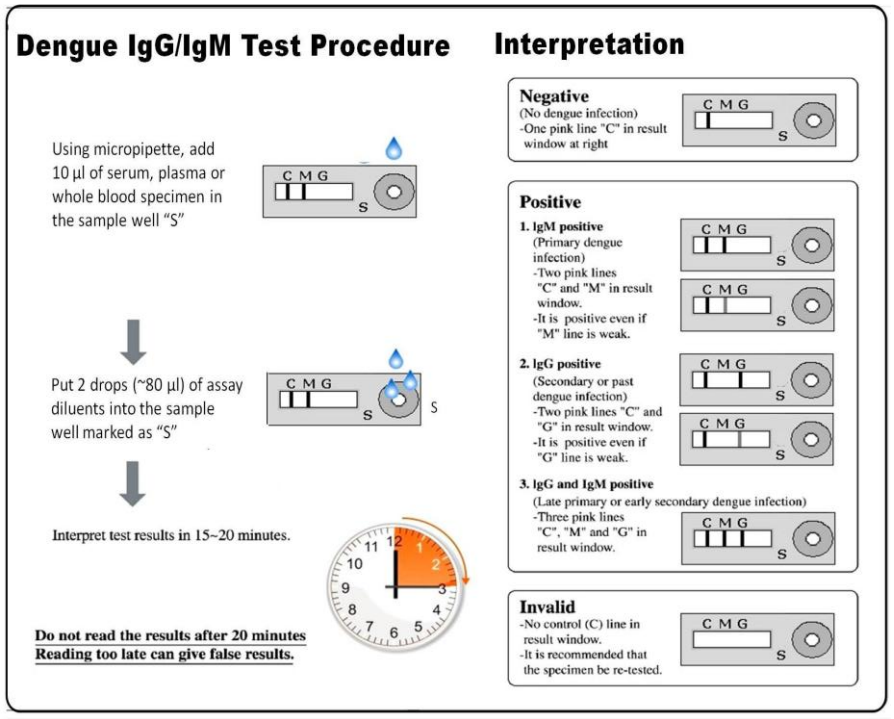
2. Clarke, D. H. and Casals, J. Techniques for Hemagglutination and hemagglutination inhibition with arthropodborne viruses. *Am. J. Trop. Med. Hyg. 1958,* 7, 561-573.

3. Innis, B. L., Nisilak, A., Nimmannitya S. et al. An enzyme-linked immunoabsorbent assay to characterise dengue infections where dengue and Japanese encephalitis cocirculate. *Am. J. Trop. Med.1989,* 40, 418-427

4. Mikano Y et al. Studies on Serological cross-reaction in sequential Flavivirus infections. *Microbiol. Immunol*. 1994, 38, 951-955.

5. 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

6. 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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