INTRODUCTION

Dengue virus is a flavivirus found largely in areas of the tropic and sub-tropics. There are four distinct but antigenically related serotypes of dengue viruses, and transmission is by mosquito, principally Aedes aegypti and Aedes albopictus. The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in third world tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by fever, rash, and joint pain, lasting 5 to 10 days. Secondary infection is the more common form of the disease in many parts of Southeast Asia and South America. IgM antibodies are not detectable until 2-5 days in case of primary dengue infection and until 4-5 days in secondary infection after the onset of illness. IgG appear after 14 days and persist for life in case of primary infection and rise within 1-2 days after the onset of symptoms in secondary infection. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, hemorrhagic events, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DHF is particularly important, as patients may die within 12 to 24 hours if appropriate treatment is not administered. Primary dengue virus infection is characterized by elevations in specific NS1 antigen levels 0 to 9 days after the onset of symptoms; this generally persists up to 15 days. Earlier diagnosis of Dengue reduces risk of complications such as DHF or DSS, especially in countries where dengue is endemic.

INTENDED USE

Dengue Day 1 Test is a rapid solid phase immuno-chromatographic test for the qualitative detection of Dengue NS1 Antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum / plasma. This test is for in vitro diagnostic use only and is intended as an aid to an early diagnosis of dengue infection & presumptive diagnosis between primary and secondary dengue infection.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

Dengue Day 1 Test kit consists two devices; one device for the differential detection of Dengue IgM/ IgG antibodies in human serum/plasma. Dengue NS1 antigen device contains two lines; “C” (Control Line) & “T” (Dengue NS1 Antigen detection Test Line). Test line is coated with antibodies, anti-dengue NS1 Ag. When a sample is added to the device, Dengue NS1 antigen if present in the sample will bind to the anti-dengue NS1 gold colloid conjugate making antigen antibodies complexes. This complex migrates along the membrane to the test region and forms the visible pink line “T” at antibody-antigen-antibody gold conjugate complex.

Dengue IgM/IgG test device contains three lines; “C” (Control line), “M” (IgM test line) & “G” (IgG test line). IgM test line is coated with anti-human IgM monoclonal antibodies and IgG test line is coated with anti-human IgG monoclonal antibodies. When a sample is added to the device, IgM and IgG antibodies in the sample react with anti-human IgM or IgG antibodies coated on the membrane respectively. Colloidal gold complexes containing dengue 1-4 antigens prepared from dengue virus culture is captured by the bound anti-dengue IgM or IgG on respective test bands located in the test window causing a pale to dark red band to form at the IgG or IgM region of the test device window. The intensity of the test bands in the respective device will vary depending upon the amount of antigen/ antibody present in the sample. The appearance of any pink/red colour in a specific test region should be considered as reactive for that particular antigen and/or antibody type (IgM or IgG). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

KIT CONTENTS

a) Dengue Day 1 Test Card  
b) Dengue Antibody Assay Buffer  
c) Antigen Test Sample Dropper  
d) Antibody Test Sample Dropper  
e) Instruction Manual

KIT PRESENTATION

10 Test Pack  
30 Test Pack  
50 Test Pack

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

1. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.  
2. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.  
3. Tests are for in vitro diagnostic use only and should be run by competent person only.  
4. Do not pipette by mouth.  
5. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures and guidelines.  
6. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.  
7. If serum / plasma specimens cannot be tested immediately, they should be refrigerated at 2-8°C. For storage for more than 3 days, freeze the specimen at -20°C or below.  
8. The use of hemolytic, lipemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.  
9. Dengue Buffer contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline “Safety Management No. CDC-22”. Decontamination of Laboratory Sink Drains to remove Azide salts” (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).

PRECAUTION

1. Do not open or remove test card from their individually sealed pouches until immediately before their use.  
2. Do not reuse test cards.  
3. All test device, buffer and specimens must be at room temperature before running the test.  
4. Do not use kit beyond the stated expiry date mentioned on the kit.  
5. Do not mix components from different lot numbers.  
6. Interpret the results at the end of 20 minutes only.

KIT STORAGE & STABILITY

The kit should be stored under 0-30°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Dengue Day 1 Test should not be frozen and must be protected from exposure to humidity.

SPECIMEN COLLECTION AND PREPARATION

1. Serum / plasma samples may be used with this test.  
2. If serum / plasma specimens cannot be tested immediately, they should be refrigerated at 2-8°C. For storage for more than 3 days, freeze the specimen at -20°C or below.  
3. Repeated freezing and thawing of the specimen should be avoided.

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TEST PROCEDURE

1. Bring the required number of Dengue Day 1 Test foils pouches and specimen to room temperature prior to testing.  
2. Remove the test card from the foil pouch prior to use.  
3. Label the test card with patient’s name or identification number.  
4. Perform the test on both the devices as follows:

A) Dengue NS1 antigen device:

i) Add 2 drops (70 µl) of sample (serum/ Plasma) using Dengue Antigen Test sample dropper to the sample well of antigen device as shown in fig. (a).  
ii) Allow reaction to occur for 20 minutes.  
iii) Read results at 20 minutes. Positive results may appear as early as 2-10 minutes. However, negative results must be confirmed at 20 minutes only.

B) Dengue IgM & IgG antibodies device:

i) Close vial provided in the kit.  
ii) Open the cap & press on the closed nozzle.  
iii) Give a half turn twist to puncture the nozzle.  
iv) Screw the cap lightly after use.

Fig. (a)
B) Dengue IgM/IgG device:

i) Fill the Dengue Antibody lower circular part of the sample dropper with the specimen up to the mark provided on the dropper as shown in fig. (b-i). Then add the specimen to the sample well “S” of antibody device as shown in fig. (b). This will add 10 µl of specimen to the device. Dispose of the dropper considering it to be biohazardous. Alternatively, add 10 µl of specimen using micropipette to the sample well of the antibody device.

Important Note:
1. During addition of sample to sample window “S”, gently press the sample dropper onto the membrane of the device for 1 to 2 seconds. Ensure that the sample has been dispensed on the membrane and sample starts to flow on the membrane. This can be seen by observing the flow of the sample in device window. If the sample does not flow, again press the dropper tip gently and touch the membrane pad to remove entrapped air so that the sample starts to move and flow. Even if, still the sample does not flow, it could be that it contains particulate matter or is turbid. If so, rerun the test, after centrifuging at 10,000 rpm. For 10 minutes or more (in case clear sample is not obtained after centrifugation).
2. Do not add Assay Buffer until sample starts flow as it may lead to false positive results.
3. Never add assay buffer in the sample well “S” as it will stop the flow of conjugate. If in case the device does not show conjugate flow, it could be that the assay buffer has been added accidentally in the sample well. Repeat the test with fresh card.
4. The devices should not be opened / tampered as it will result in changes in the alignment of the membrane.

ii) Hold the Assay Buffer vial vertically and add 2 drops (70 µl) of dengue antibody assay buffer to the Buffer well “B” of the device as shown in fig. (c) and screw cap the vial after use.

NOTE: Please ensure, no air is entrapped and full drop falls down from the nozzle tip.

iii) Allow reaction to occur during the next 20 minutes.

iv) Read results at 20 minutes. Positive results may appear as early as 2-10 minutes. However, negative results must be confirmed at 20 minutes only.

5. Discard the Dengue Day 1 Test immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF THE TEST

A) Dengue NS1 Ag Device

REACTIVE

As shown in Fig. (d), appearance of pink coloured line, one each in test region “T” and control region “C” indicates that the sample is REACTIVE for Dengue NS1 Ag.

NON-REACTIVE

As shown in Fig. (e) appearance of one distinct pink line in the control region “C” only, indicates that the sample is “NON REACTIVE” for Dengue NS1 Ag.

INVALID

When neither control line nor the test line appears on the membrane as shown in Fig. (f) the test should be treated as invalid.

B) Dengue IgM & IgG Antibodies Device

IgM & IgG REACTIVE

As shown in Fig. (g), appearance of red coloured line in the control region “C” and Test region; IgM region “M” and IgG region “G” indicates that the sample is reactive for both IgM & IgG antibodies. This is indicative of a secondary dengue infection.

IgM REACTIVE

As shown in Fig. (h), appearance of red coloured line in the control region “C” and Test region; IgM region “M” indicates that the sample is reactive for IgM antibodies. This is indicative of a primary dengue infection.

IgG REACTIVE

As shown in Fig. (i), appearance of red coloured line in the control region “C” only, indicates that the sample is reactive for IgG antibodies. This is indicative of a secondary dengue infection.

NON-REACTIVE

As shown in Fig. (j), appearance of one distinct red coloured line in the control region “C” only (with no line in the IgM region “M” & IgG region “G”) indicates that the sample is non-reactive for dengue antibodies.