Dengue DAY 1 Test

Rapid visual test for the detection of Dengue NS1 Antigen and differential detection of IgM & IgG antibodies in Human Serum/ Plasma

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 mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. 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 5-10 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 DSS 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 upto 15 days. Earlier diagnosis of Dengue reduces risk of complication 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 detection of Dengue NS1 antigen and second 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 complex. This complex migrates along the membrane to the test region and forms the visible pink line at "T" as 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 (IgG or IgM). 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 for use

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 European Standard EN ISO15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instructions for use
LOT	Lot Number Batch Number	4°C 30°C	Temperature Limit
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Contains biological Material of Animal Origin
2	Single use only		Keep away from sunlight
	Keep Dry	IN	Country of Manufacture

WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
6. Do not pipette by mouth.
7. 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.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Assay 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).
11. Follow standard biosafety guidelines for handling & disposal of potentially infective material.

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 at 2-30°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.
4. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
5. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START

The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial. To orifice/puncture the closed nozzle, follow the instruction as illustrated below:

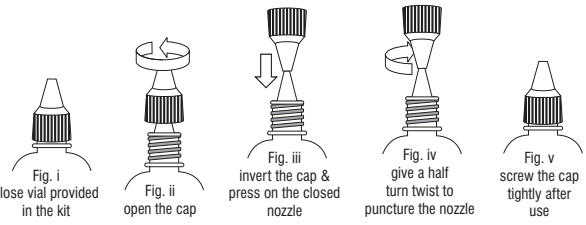

Fig. i close vial provided in the kit | Fig. ii open the cap | Fig. iii invert the cap & press on the closed nozzle | Fig. iv give a half turn twist to puncture the nozzle | Fig. v screw the cap tightly after use

TEST PROCEDURE

1. Bring the required number of Dengue Day 1 Test foil 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.

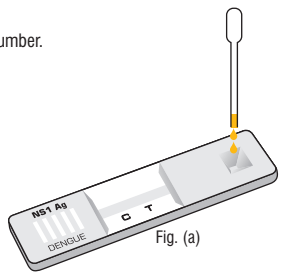

Fig. (a)

B) Dengue IgM/ IgG device:

i) Fill the Dengue Antibody lower circular part of the sample dropper with the specimen upto the mark provided on the dropper as shown in fig. (b-ii). 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 sample using micropipette to the sample well of the antibody device.

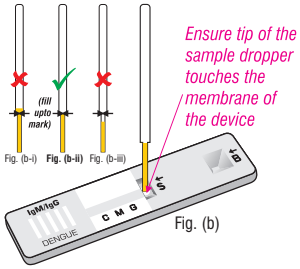

Fig. (b)

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.

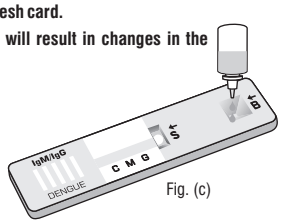

Fig. (c)

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. Do not read result after 30 minutes.

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

Fig. (d)

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

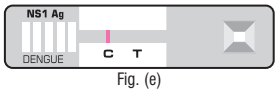

Fig. (e)

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

Fig. (f)

Fig. (g)

The test is invalid, if no control line appears after the completion of test, either with clear background or with complete pinkish/ purplish background fig. (f & g). **The test should be repeated using a new card.**

B) Dengue IgM & IgG Antibodies Device

IgM & IgG REACTIVE

Fig. (h)

As shown in Fig. (h) 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

Fig. (i)

As shown in Fig. (i) 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

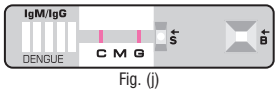

Fig. (j)

As shown in Fig. (j), appearance of red coloured line in the control region 'C' and Test region; IgG region 'G' indicates that the sample is reactive for IgG antibodies. This is indicative of a secondary dengue infection.

Consider the faint test line also a reactive result. Centrifuge the sample and repeat the test using fresh card. If faint test line persist, confirm the test result with ELISA/Clia and co-relate clinically.

NON-REACTIVE

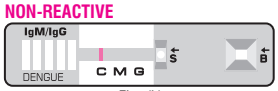

Fig. (k)

As shown in Fig. (k), 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.

INVALID

Fig. (k)

Fig. (l)

Fig. (m)

Fig. (n)

The test is invalid, if no control line appears after the completion of test, either with clear background or with complete pinkish/ purplish background fig. (k, l, m & n). Invalid test may be because of following reasons:

(a) Improper storage at temperature other than the recommended temperature.
(b) Wrong Procedure
(c) Long atmospheric exposure of the test device after opening the pouch.
(d) Use of turbid/ lipemic/ haemolyzed sample.

In case of invalid result, the test sample should be centrifuged at 10,000 rpm for 15 minutes and retest using new Card.

LIMITATIONS OF THE PROCEDURE

1. The test is for in vitro diagnostic use only.
2. This test detects the presence of Dengue NS1 antigen & IgM & IgG antibodies to dengue virus in the specimen and should not be used as the sole criteria for the diagnosis of Dengue virus infection.
3. 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 3-5 days after the first testing date.
4. Serological cross-reactivity across the Flavivirus group (St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.
5. In case of faint test line, repeat the test using fresh card. If faint test line persist, confirm the test result with ELISA/Clia and co-relate clinically.
6. As with all diagnostic tests, all results must be corelated with other clinical findings. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result at any time does not preclude the possibility of an early infection of Dengue virus.
7. This is only a screening test. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and serological test like haemagglutination inhibition test, more specific alternative diagnosis method like ELISA must be used in order to obtain a confirmation of dengue virus infection.

PERFORMANCE CHARACTERISTICS

The Dengue Day 1 Test kit has been evaluated in-house with the known panel of fresh as well as frozen Dengue NS1 antigen positive and Negative samples & dengue IgM & IgG antibody positive and negative samples. The performance of the test was evaluated and compared with ELISA test. The samples included cross-reacting samples; Epstein-Barr virus, Malaria, Rheumatoid factor, Leptospirosis, Japanese encephalitis, yellow fever and West Nile viruses. Following is the in-house evaluation.

Sample Type	No. of Samples tested	Result of ELISA test	Dengue NS1 Ag	Dengue IgM / IgG
Negative for Ag & Ab	4030	4030	3950	3910
Dengue Antigen Positive	175	175	168	-
Dengue IgM Positive	120	120	-	114
Dengue IgG Positive	79	79	-	75

Dengue NS1 Ag :
Sensitivity : 96% **Specificity : 98%**

Dengue IgM / IgG antibody :
Sensitivity : 95% **Specificity : 97%**

External Evaluation:

The performance of Dengue DAY1 Test with reference to Sensitivity & Specificity is also evaluated from NABL lab using clinical patient samples suspected for Dengue. Following are the results:

Sensitivity : 100% **Specificity: 100%**

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of seven samples; one negative, two Dengue NS1 antigen positive, two Dengue IgM positive and two Dengue IgG Positive. The C.V. (%) of all the five samples were within 10% of the time.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

BIBLIOGRAPHY OF SUGGESTED READING

1. Guzman M.G. & Kourig Clinical & Diagnostic Laboratory Immunology (1996) Vol. 3, No. 6, 621-627.
2. Young P.R., Hilditch P.A., etal J. Clinical Microbiology (2000) Vol. 38, No.3, 1053-1057.

in vitro diagnostic reagent, not for medicinal use

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