DENGUE NS1 ANTIGEN FP

Finger Prick rapid visual test for the detection of Dengue NS1 Antigen in Human Whole Blood/ 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, prinicipally 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 haemorrhagic 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. Secondry 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 symtoms 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, haemorrhagic evets, 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 NS1 Antigen FP is a rapid solid phase immuno- chromatographic test for the qualitative detection of Dengue NS1 Antigen in human Whole Blood/ Serum/ Plasma. This test is for in vitro diagnostic use only and is intended as an aid in the earlier diagnosis of dengue infection.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

Dengue NS1 Antigen FP is an immunoassay based on the “sandwich” principle. Dengue NS1 antigen device contains two lines; “C” (Control Line) & “T” (Dengue NS1 Antigen detection Test Line). Test line is coated with anti-dengue NS1 antibodies. 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. On addition of assay buffer, 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.

The intensity of the test band in the device will vary depending upon the amount of antigen present in the sample. The appearance of any pink/red colour in test region should be considered as reactive for Dengue NS1 antigen. A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

MATERIALS PROVIDED

Dengue NS1 Antigen FP kit contains following components to perform the assay:

a) Dengue NS1 Antigen Device	b) Sample Dropper	c) Assay Buffer
d) Swab	e) Lancet	f) Instruction Manual

KIT PRESENTATION

10 Test Pack 50 Test Pack

STORAGE AND STABILITY

The kit should be stored at 2-30ºC in the coolest and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Dengue NS1 Antigen FP should not be frozen and must be protected from exposure to humidity.

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 BS EN 15223-1:2012.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 30°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Single use only		

CLASSIFICATION: These are modified immunological products/ Antisera & other blood fractions, Classified under chapter heading 30.02 of Central Excise Tariff. As held by Hon. Supreme Court Judgement in our own case, Citation 2007(211) ELT 521(SC) / Civil appeal 1076 of 2002 SC. (Available at website of Hon. Supreme Court of India: “supremecourtofindia.nic.in”)

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. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC 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.
7. 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.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. 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).
10. Follow standard biosafety guidelines for handling & disposal of potentially infective material.

PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. Use disposable gloves while handling potentially infectious samples and performing the assay. Wash hands thoroughly afterwards.
2. Do not use the kit beyond the expiry date.
3. Do not mix reagents from different batches.
4. Do not open the foil pouch until it attains room temperature.
5. Do not re-use the test device.
6. Use separate sample dropper or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
7. For best results, follow the given test procedure and storage instructions strictly.

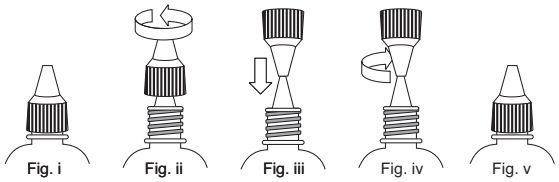
SAMPLE / SPECIMEN COLLECTION AND STORAGE

1. Collect the whole blood in a clean container (containing EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8ºC for not more than 3 days, otherwise false / erroneous results may be obtained.
2. Fresh blood from finger prick may also be used as a test sample.
3. Heamolysed or clotted sample or sample with microbial contamination should not be used.
4. If serum/ plasma specimens cannot be tested immediately, they should be refrigerated at 2-8ºC. For storage for more than 3 days, freeze the specimen at -20ºC or below.
5. Repeated freezing and thawing of the specimen should be avoided.
6. 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.
7. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START

1. 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.

2. To orifice the closed nozzle, press the inverted cap on the respective closed nozzle and give a half turn twist to ensure nozzle is properly orificed/ punctured as illustrated below in Fig. iii & iv, before use:



TEST PROCEDURE

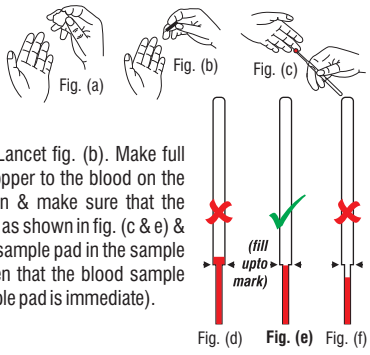
RT 25-30°C

1. Bring the complete kit and specimen to be tested to room temperature prior to testing.

2. Remove the test card from the foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test card from the foil pouch.

3. Label the test card with patient's name or identification number.

4. When finger prick blood is being used, clean fig. (a) the skin surface of the finger tip with the Alcohol swab. Let it dry and then prick the lateral part of the finger tip with the Lancet fig. (b). Make full blood drop and touch the sample dropper to the blood on the finger prick while hands facing down & make sure that the dropper is full of sample upto the mark as shown in fig. (c & e) & immediately blot the sample onto the sample pad in the sample well 'S' fig. (g). (Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate).



OR

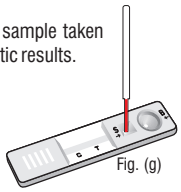
Alternatively, using a micropipette take 4μl of the anti-coagulated or finger prick specimen.

5. Take 4μl whole blood/ serum/ plasma using the sample dropper upto the mark as shown in fig. (e).

Note: Sample taken above the mark as shown in fig. (d) and sample taken below the mark as shown in fig. (f) are wrong and will lead to erratic results.

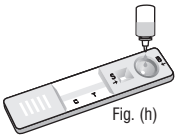
OR

6. Add whole blood/ serum/ plasma sample using the sample dropper/ micropipette onto the sample pad in the sample well 'S'. fig. (g)



NOTE : Make sure that the test sample from the sample dropper has been completely transferred to the sample pad.

7. Add 3 drops of the Assay Buffer in the buffer well 'B'. fig. (h)



8. Allow the reaction to occur for 20 minutes.

8. Read the results at 20 minutes.

9. Discard the Dengue NS1 Antigen FP card immediately after reading results at 20 minutes as it is potentially infectious.

INTERPRETATION OF THE RESULTS

REACTIVE

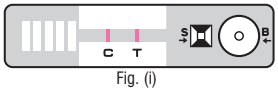


Fig. (i)

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

NON-REACTIVE

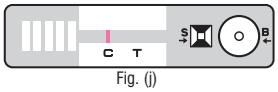


Fig. (j)

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

INVALID

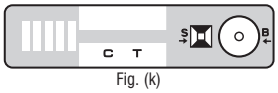


Fig. (k)

When neither control line nor the test line appears on the membrane as shown in (Fig. k) the test should be treated as invalid. Repeat the test with new card.

LIMITATIONS AND INTERFERENCES

1. The test is for in vitro diagnostic use only.
2. This test detects the presence of Dengue NS1 antigen in the specimen and should not be used as the sole criteria for the diagnosis of Dengue virus infection.
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 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.
5. This is only a screening test. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and more specific alternative diagnosis method must be used in order to obtain a confirmation of dengue virus infection.

PERFORMANCE CHARACTERISTICS OF DENGUE NS1 ANTIGEN FP CARD TEST

The kit has been evaluated in-house with the known panel of fresh as well as frozen Dengue NS1 antigen positive and Negative samples. The performance of the test kit was evaluated and compared with the a license commercially available ELISA test kit. 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 licensed test	Dengue NS1 Antigen FP
Dengue NS1 Antigen Negative	2530	2530	2529
Dengue NS1 Antigen Positive	255	255	255

Sensitivity : **100%** Specificity : **99.96%**

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

NOTICE: Every effort is made to supply ordered consignment as per the sample submitted but due to continuous development, the company reserves the right to improve/change any specifications/ components without prior information/notice to the buyer.

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-manual, 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. Dengue haemorrhagic fever: Diagnosis, Treatment, Prevention and Control. WHO 2009 edition.
2. Evaluation of diagnostic test: Dengue, Rosanna W. Peeling, Harrey Artsob etal. (2010). Nature reviews.
3. Use of dengue Ns1 antigen for early diagnosis of dengue virus infection. Kassim FM; Izate MN, etal. (2011) Southeast Asian J. Trop. Med. Public Health. May; 42(3); 562-9.
4. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. S. Datta, C Wattal (2010). Indian Journal of Medical Microbiology, vol 28, No. 2; 107-110.

For *in-vitro* diagnostic use only ,not for medicinal use

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MN/DAF/048