DENGUE NS1 Ag
QUANTI CARD

Fluorescence immunoassay (antigen-antibody) for qualitative detection of Dengue NS1 Antigen 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, 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 fever, 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 Ag Quanti Card is a sensitive immuno- chromatographic test for the qualitative detection of Dengue NS1 Antigen in human Serum/ Plasma with iQuant Analyzer . 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 Ag Quanti Card is a fluorescence immunoassay based on sandwich principle. The test area is coated with specific anti-dengue NS1 Ag antibodies. When a sample is added to the cartridge, Dengue NS1 antigen if present will form a complex with another specific NS1 antibodies conjugate to fluorochorme. On addition of Assay Buffer, this complex migrates along the nitrocellulose membrane to the test region and forms an antibody-antigen-antibody fluorescence immunocomplex. The result will be displayed by i-Quant Analyzer.

MATERIALS PROVIDED
Dengue NS1 Ag Quanti Card kit contains following components to perform the assay:
a) Dengue NS1 Ag Quanti Card Device b) Assay Buffer
c) Instruction Manual

KIT PRESENTATION
24 Test Pack 48 Test Pack 96 Test Pack

STORAGE AND STABILITY
The kit should be stored at 2-8ºC 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 Ag Quanti Card 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 EN ISO 15223-1:2016.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2ºC 8ºC	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
	Single use only		

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 before disposal or by autoclaving at 121ºC at 15psi for 60 min. They should be disposed off in accordance with established safety procedures and standard biosafety guidelines for handling & disposal of potentially infective material.
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).

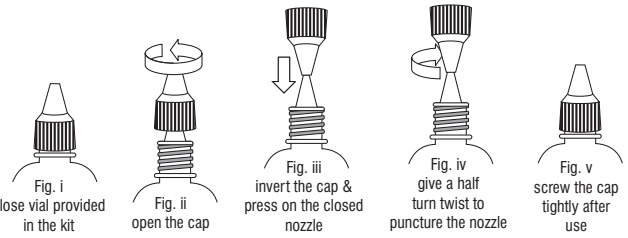
PRECAUTIONS
In order to obtain reproducible results, the following instructions must be followed:

1. Do not use the kit beyond the expiry date.
2. Do not mix reagents from different batches.
3. Do not open the foil pouch until it attains room temperature.
4. Do not re-use the test cartridge.
5. Follow the given test procedure and storage instructions strictly.
6. **Do not paste any sticker or write anything on the QR-Code as this will lead to erroneous result.**
7. Do not temper the QR-Code as this will lead to erroneous result.
8. Do not touch the membrane with the pipette tip.

Important Note: Dengue NS1 Ag Quanti Card is only operational in conjection with iQuant Analyzer.

SAMPLE / SPECIMEN COLLECTION AND STORAGE
1. Serum / plasma samples may be used with this test.
2. If serum / plasma specimens cannot be tested immediately, they should be refrigerated at 2-8ºC. For storage for more than 3 days, freeze the specimen at -20ºC 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 at 10,000 rpm for 15 minutes 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.

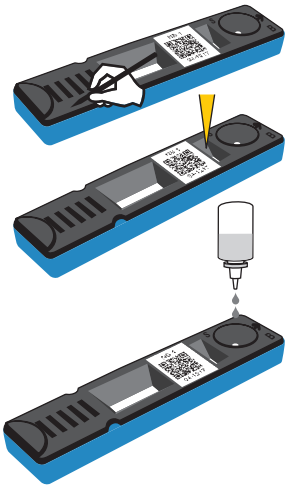
BEFORE YOU START
The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial. To orifice/puncture the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



TEST PROCEDURE

RT 20-30°C

1. Bring the complete kit and specimen to be tested to room temperature prior to testing.
2. Remove the test cartridge 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 cartridge with patient's name or identification number. **Do not write on QR code.**
4. Add 50μl Serum/ Plasma sample using micropipette onto the sample pad in the sample well 'S'. **Care should be taken to avoid any spillage on the QR Code.**



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

5. Add 2 drops of the Assay Buffer in the buffer well 'B'.
6. Allow the reaction to occur for 30 minutes. In the meantime enter the patient's details in the iQuant analyzer testing window and select the NS1 test from the pop down menu in the testing window of the iQuant analyzer.
7. Insert the test cartridge into the i-Quant Analyzer with arrow (←) marked side on the top of cartridge facing towards the analyzer and press RUN icon. Note down the value displayed on the screen of i-Quant Analyzer & interpret the result as mentioned below.
8. Discard the Dengue NS1 Ag Quanti Card immediately after reading results at 30 minutes considering it to be potentially infectious.

Important Note: Do not read results after 30 minutes.

INTERPRETATION OF RESULTS

The iQuant Analyzer will display results as Reactive, Equivocal or Non-Reactive as follows:

NS1 Antigen Reactive: >1.1 U and above: interpret the result as Dengue NS1 Antigen reactive.

Equivocal: >0.9 to ≤1.1 U: Interpret the result as equivocal. Repeat the test after centrifuging the sample at 5000 rpm for 20 minutes. Even after repeating the test, if result comes equivocal, further test the sample with alternative method or collect another sample.

Non-Reactive: Below ≤0.9 U: Interpret the result as Dengue NS1 Antigen non-reactive.

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 Ag QUANTI 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 Ag Quanti Card
Dengue NS1 Antigen Negative	2070	2070	2069
Dengue Antigen Positive	255	255	255

Sensitivity : **100%** Specificity : **99.95%**

Precision:

Intra assay precision (Reproducibility)

Within run (Intra assay) precision have been determined by testing 10 replicates of two negative and four dengue NS1 antigen positive samples; 3 weak and 1 medium. The C.V. (%) of all the samples were within 10% of the unit.

Inter assay precision (Reproducibility)

Between run (Inter assay) precision have been determined by testing 10 replicates of two negative and four dengue NS1 antigen positive samples; 3 weak and 1 medium in 10 sequential days. The C.V. (%) of all the samples were within 10% of the unit.

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.

TROUBLE SHOOTING

	PROBLEM	POSSIBLE CAUSE	SOLUTION
1.	False negative	a) Low sample volume along with less volume of Assay Buffer used.	Repeat the test with proper volume of samples and/or Assay Buffer.
2.	False positive	a) Less volume of Assay Buffer added/used.	Repeat the test with proper volume of Assay Buffer

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.
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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.
5. Dengue: guidelines for diagnosis, treatment, prevention and control –New Edition WHO/HTM/NTD/DEN/2009.1

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

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