DENGUE IgM QUANTI CARD

Fluorescence immunoassay for qualitative detection of Dengue IgM 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.

INTENDED USE

Dengue IgM Quanti Card is a sensitive immuno- chromatographic test for the qualitative detection of dengue IgM antibodies in human Serum/ Plasma with iQuant Analyzer . This test is for in vitro diagnostic use only and is intended as an aid in the diagnosis of dengue infection.

PRINCIPLE

Dengue IgM Quanti Card is a fluorescence immunoassay. The test area is coated with dengue antigen (1-4). When a sample is added to the device, dengue IgM antibodies in the sample react with anti-human IgM antibodies complex to flourescent dye in the conjugate. 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 iQuant Analyzer.

MATERIALS PROVIDED

Dengue IgM Quanti Card kit contains following components to perform the assay:
a) Dengue IgM Quanti CardDevice 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 IgM 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 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. 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 temper / paste any sticker or write anything on the QR-Code as this will lead to erroneous result.
7. Do not touch the membrane with the pipette tip.

Important Note: Dengue IgM 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. For plasma, collect the whole blood in a clean container (containing EDTA, sodium floride or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If 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.
2. Repeated freezing and thawing of the specimen should be avoided.
3. 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.
4. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erratic 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:

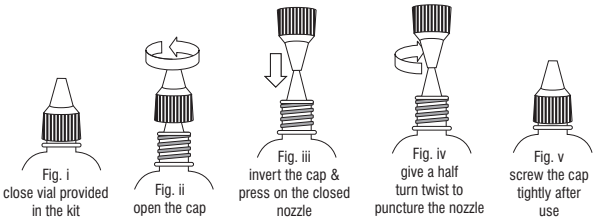


Fig. i close vial provided in the kit

Fig. ii open the cap

Fig. iii invert the cap & press on the closed nozzle

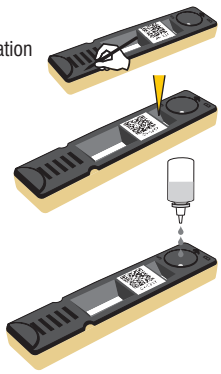
Fig. iv give a half turn twist to puncture the nozzle

Fig. v screw the cap tightly after use

TEST PROCEDURE

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 10μl 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 3 drops of the Assay Buffer in the buffer well 'B'.
6. Allow the reaction to occur for 40 minutes.
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 IgM Quanti Card immediately after reading results at 40 minutes considering it to be potentially infectious.

Important Note: Do not read results after 40 minutes.

INTERPRETATION OF RESULTS

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

IgM Reactive: >1.1 U and above: interpret the result as Dengue IgM Antibody 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 IgM Antibody non-reactive.

LIMITATIONS AND INTERFERENCES

1. The test is for in vitro diagnostic use only.
2. This test detects the presence of Dengue IgM 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. 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.
6. 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 must be used in order to obtain a confirmation of dengue virus infection.

PERFORMANCE CHARACTERISTICS OF DENGUE IgM& IgG QUANTI CARD

The kit has been evaluated in-house with the known panel of fresh as well as frozen Dengue IgM antibody 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 Dengue IgM ELISA test		Dengue IgM Quanti Card	
		Positive	Negative	Positive	Negative
Negative	855	0	855	11	844
Dengue IgM Positive	120	120	0	118	2
Dengue IgG Positive	75	0	75	0	75

Sensitivity: **96.72%** Specificity: **97.45%**

Precision:

Intra assay precision

Within run (Intra assay) precision have been determined by testing 10 replicates of two negative and five dengue IgM antibody positive samples (3 weak, 1 medium & 1 strong). 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 five Dengue IgM antibody positive samples (3 weak, 1 medium & 1 strong) 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

4. Dengue: guidelines for diagnosis, treatment, prevention and control –New Edition WHO/HTM/NTD/DEN/2009.1
2. Rapid Dengue Diagnosis: A prospective study using a Commercial RapidTest* (S.K. Lam+ and L.C.S. Lum++)
3. Dengue disease diagnosis: A puzzle to be solved Jaime Eduardo Castellanos 1.2. Carolina Coronel-Ruiz1 Rev. Fac. Med. 2014 Vol. 62 No. 4:617-629

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

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VER-01 R- 00

MN/DMO/063
Rev. Date: Jul.-18