

DIAGNOS DENGUE CARD



Rapid Visual test for the qualitative & differential detection of IgM & IgG Antibodies to Dengue virus in Human Serum / Plasma

INTENDED USE

DIAGNOS DENGUE CARD is a rapid solid phase immuno-chromatographic assay for the qualitative 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 in the presumptive diagnosis between primary and secondary dengue infection.

PRINCIPLE

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 and IgG test line is coated with anti-human IgG. When a sample is added to the device, IgG and IgM 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 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 positive 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) Diagnos Dengue Card | b) Sample Dropper |
| c) Assay Buffer | d) Instruction Manual |

KIT PRESENTATION

10 Test Pack & 25 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.

| | | | |
|--|-------------------------------------|--|--|
| | Manufactured By | | In vitro diagnostic medical device |
| | No. of tests | | See Instruction for use |
| | Lot Number Batch Number | | Temperature Limitation |
| | Manufacturing Date | | Caution, see instruction for use |
| | Expiry Date | | Catalogue Number |
| | Do not use if package is damaged | | Authorized Representative in the European Community |
| | Single use only | | Keep away from sunlight |

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.

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Tests are for *in vitro* diagnostic use only and should be run by competent person only.
- Do not pipette by mouth.
- 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.
- 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.

- Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
- 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).
- Follow standard biosafety guidelines for handling & disposal of potentially infective material.

PRECAUTION

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

KIT STORAGE & STABILITY

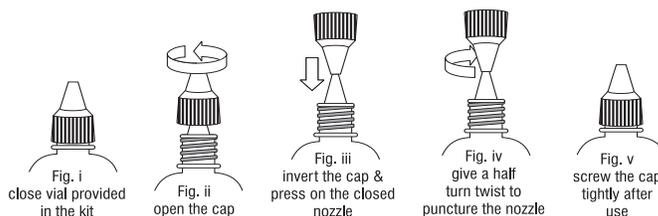
The kit should be stored at 2-30°C in the coolest and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Diagnos Dengue Card should not be frozen and must be protected from exposure to humidity.

SPECIMEN COLLECTION AND PREPARATION

- Serum / plasma samples may be used with this test.
- 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.
- Repeated freezing and thawing of the specimen should be avoided.
- 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.
- The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

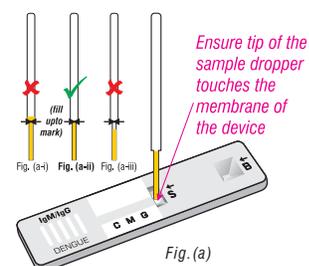
BEFORE YOU START

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

- Bring the required number of Diagnos Dengue Card foil pouches and specimen to room temperature prior to testing.
- Remove the test card from the foil pouch prior to use.
- Label the test card with patient's name or identification number.
- 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. (a). 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 off the dropper considering it to be biohazardous.

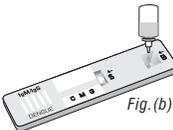


Alternatively, add 10 µl of sample using micropipette to the sample well of the antibody device.

Fig. (a)

Important Note

- (i). 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).
 - (ii) Do not add Assay Buffer until sample starts flow as it may lead to false positive results.
 - (iii) 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.
 - (iv) The devices should not be opened / tampered as it will result in changes in the alignment of the membrane.
5. Add 2 drops (70 µl) of dengue antibody assay buffer to the Buffer well "B" of the device as shown in fig. (b).

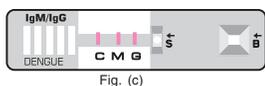


NOTE: Please ensure, no air is entrapped and full drop falls down from the nozzle tip.

6. Allow reaction to occur during the next 20 minutes.
7. Read results at 20 minutes. Positive results may appear as early as 5-10 minutes. However, negative results must be confirmed after 20 minutes only.
8. Discard the Diagnos Dengue Card Test immediately after reading result at 20 minutes, considering it to be potentially infectious.

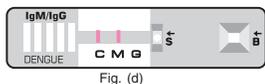
INTERPRETATION OF THE TEST

IgM & IgG REACTIVE



As shown in Fig. (c), 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



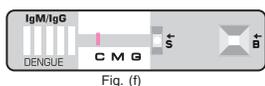
As shown in Fig. (d), appearance of red coloured line in the control region 'C' and Test region; IgM region 'M' indicates that the sample is reactive for IgM antibodies. This is indicative of a primary dengue infection.

IgG REACTIVE



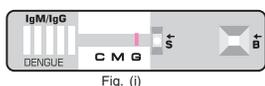
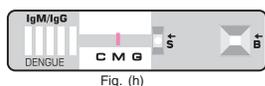
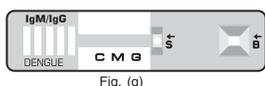
As shown in Fig. (e), appearance of red coloured line in the control region 'C' and Test region; IgG region 'G' indicates that the sample is reactive for IgG antibodies. This is indicative of a secondary dengue infection.

NON-REACTIVE



As shown in Fig. (f), 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



if no control line appears after the completion of test as shown in Fig. (g), (h) & (i), the test should be treated as Invalid which 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.

The test should be repeated using a new device and test sample.

LIMITATIONS OF THE TEST

1. The test is for *in vitro* diagnostic use only.
2. This test detects the presence of 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 (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.
5. As with all diagnostic tests, all results must be correlated 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

An elaborated study has been done on Diagnos Dengue Card to determine its performance as a screening test. The performance of the test was evaluated and compared with a licensed commercially available ELISA test kit in-house by using a known panel of Serum/ Plasma dengue negative & positive samples. The samples included cross-reacting samples; Epstein-Barr virus, Malaria, Rheumatoid factor, Leptospirosis, Japanese encephalitis, yellow fever and West Nile viruses. The results obtained are as follows:

| Sample Type | No. of Samples tested | Result of licensed test | Diagnos Dengue Card results |
|---------------------------|-----------------------|-------------------------|-----------------------------|
| Negative for Ab to Dengue | 4030 | 4030 | 3910 |
| Dengue IgM Positive | 120 | 120 | 114 |
| Dengue IgG Positive | 79 | 79 | 75 |

Sensitivity : 95%

Specificity : 97%

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of 9 samples - 2 negative, 7 Dengue positive (3 weak, 3 medium and 1 strong). 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 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

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

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

J. Mitra & Co. Pvt. Ltd.
A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA
Ph.: +91-11-47130300, 500, 26818971-73
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in