Advantage Dengue NS1 Ag Card

Rapid visual test for the detection of Dengue NS1 Ag 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 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

Advantage Dengue NS1 Ag Card is a rapid solid phase immuno-chromatographic test for the qualitative detection of Dengue NS1 Antigen in human 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)

Advantage Dengue NS1 Ag Card 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 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.

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 NS1 Ag Device
b) Sample Dropper
c) Instruction Manual

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 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
Keep Dry	

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 should not be used. Advantage Dengue NS1 Ag Card should not be frozen and must be protected from exposure to humidity.

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 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.
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. Do not open the foil pouch to remove the product until it attains room temperature and you are ready to perform the test.
10. Take out the cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.
    Optimal test performance requires strict adherence to the test procedure described in the insert.

SPECIMEN COLLECTION AND PREPARATION

1. Advantage Dengue NS1 Ag Card test should be performed in human serum or plasma only immediately after collection.
2. If not tested immediately, specimen should be refrigerated at 2-8°C for upto 24 hours following collection.

3. If testing within 24 hours is not possible, specimen should be frozen at -20ºC for 3 months or -70ºC for longer period.
4. Specimens containing visible precipitate or cloudy specimens may give inconsistent test results. Such specimens should be clarified prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.
5. Heamolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.
6. Repeated freezing & thawing of the specimen should be avoided.

TEST PROCEDURE

1. Bring the required number of Advantage Dengue NS1 Ag Card 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. Add 2 drops (70 μl) of sample using sample dropper to the sample well of device as shown in fig. (a).

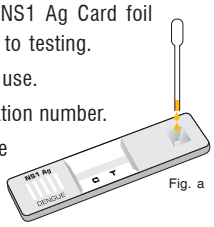

Fig. a

5. Allow reaction to occur for 20 minutes.
6. Read results at 20 minutes. Positive results may appear as early as 2-10 minutes. However, negative results must be confirmed after 20 minutes only.
7. Discard the Advantage Dengue NS1 Ag Card immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF THE TEST

REACTIVE:

Fig. b

As shown in (Fig. b), 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. c

As shown in (Fig. c) 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. d

Fig. e

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. (d) & (e). Invalid test are obtained due to following reasons:

(a) Improper storage at temperature other than the recommended temperature.

(b) Wrong test 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 TEST

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

(i) 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 availabel 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**
Negative for Ag	4030	4030	3950
Dengue Antigen Positive	175	175	168

Sensitivity: 96% **Specificity: 98%**

(ii) Performance of Advantage Dengue NS1 Ag Card with reference to sensitivity and specificity has also been determined by NIV (National Institute of Virology), Pune, India. The evaluation indicate the following sensitivity and specificity:

Sensitivity: 93.33% **Specificity: 100%**

This information is provided for the scientific community enquiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional purpose.

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

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R-03
VER-02

in vitro diagnostic Reagent, 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, 47130500
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

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