



READ THE PACK INSERT CAREFULLY BEFORE PERFORMING THE TEST

Catalogue No.: DC

Introduction : The Dengue Virus belongs to the Flavivirus group of viruses transmitted by the mosquito, *Aedes aegypti* and *Aedes albopictus* mosquitoes, widely distributed throughout the tropical and subtropical areas of the world. The dengue fever is very common in rainy season. The symptoms of Dengue fever are sudden onset of fever, headaches, pain in the back and limbs, lymphadenopathy, maculopapular rash and retrobulbar pain. There are four known distinct serotypes (dengue virus 1, 2, 3 and 4). In children infection is often subclinical or causes a self-limited febrile disease. However, if the patient is infected a second time with a different serotype a more severe disease, dengue hemorrhagic fever or dengue shock syndrome is more likely to occur. Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes. Traditionally haemagglutination-inhibition (HAI) test has been the most commonly used serological assay for dengue diagnosis.

Primary Dengue Infection is associated with mild to high fever, headache, muscle pain and skin rash. Immune response includes IgM antibodies produced by 5th day of symptoms and persist for 30.60 Days. IgGs appear in the 14th day and persist for life. Secondary infections often result in high fever and in many cases with hemorrhagic events and circulatory failure. Secondary infections show that IgGs rise within 1.2 days after the onset of symptoms and induce IgM after 20 days of infection. Traditionally, HAI titers have been used to classify infection. The current definition as primary or secondary depends on an assay of paired serum specimens separated in time by at least 7 days, although any acute specimen with a HAI titer > 1:2560 is defined as coming from a patient with secondary Flavivirus infection.

Intended Use : Dengue IgG/IgM rapid card test is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in human serum or plasma or whole blood. This test is intended for Professional use as aid in the presumptive diagnosis between primary and secondary dengue infection. This test provides only a preliminary test result. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and serological test like haemagglutination-inhibition test, more specific alternative diagnostic method must be used in order to obtain a confirmation of dengue virus infection. This kit can detect all 4 Dengue serotypes by using a mixture of recombinant Dengue envelope proteins.

Principles : The Dengue IgG/IgM test kit is a sandwich immunochromatographic membrane based screening test to detect the antibodies for dengue virus. The test can be used either with serum, plasma or whole blood. The test employs the use of colloidal gold particle conjugated with IgG & IgM binding proteins and a unique combination of dengue antigen immobilized on the membrane at T. After the addition of sample and running buffer in the sample well of test device, the mixture passes through the conjugate gold and makes an immune complex. The immune complex will bind with the antigen immobilized at test zone (IgM/IgG) of the test device, if antibodies to dengue are present in the sample. The remaining complex continues to migrate to a control area (C) in the test device and produces a blue colored band in the control (C) zone. The control band indicates that the test has been performed properly and is in working condition. Appearance of pink/purple band at test (IgM & IgG) in addition to band at control (C) indicates that sample is positive for dengue antibodies.

Note : A Red Color Control line will be present in Control region before testing. This should not be misunderstood as tested cassette. The Red Line will disappear while testing and it will turn into blue color.

Specimen: Fresh Serum or Plasma or whole blood can be used.

Storage: Store at 2-30°C. Do not freeze. The kit is stable till the expiry date mentioned on the pouch when stored under the above condition.

Pack Size: Available in packs of 5's, 10's, 20's & 50's.

Contents of the Kit

Pack Size	5 Test	10 Test	20 Test	50 Test
1. Test Device	5	10	20	50
2. Assay Buffer	0.75 ml	1.5 ml	3.0 ml	7.5 ml
3. Silicagel	5 Nos.	10 Nos.	20 Nos.	50 Nos.

Materials Required but not Provided: Micropipette (1-10 µl)

Assay Procedure :

1. Remove as many test units from the pouches as needed and put on flat surface.
2. Label them with sample/patient identity.
3. Add 5 µl of serum/plasma or 10 µl of whole blood to the sample well of the device using pipette.
4. Add 2 drops of the running buffer provided in the dropper bottle by holding the bottle vertically over the sample well.
5. Read results within 5-20 minutes after sample & buffer addition.

DO NOT INTERPRET THE RESULT AFTER 20 MINUTES.

NOTE : Additional drops of running buffer may be added in the sample well if background of result window is not clear.

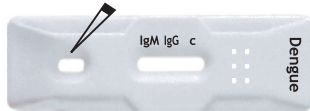
Precautions :

1. Read the pack insert carefully.
2. Remove the test device from the foil pouch prior to use.
3. Assay should be conducted at room temperature (15^o to 30^o C).
4. The Dengue test pack, if refrigerated, should be brought down to room temperature before testing.
5. Refrigerated Specimen must be brought to room temperature before testing.
6. Specimen should not be repeatedly frozen and thawed.
7. Handle all specimens carefully as potentially infectious material. On completion of assay, dispose off specimen after autoclaving at 15 lb & 121° C for 20 minutes.
8. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
9. For in-vitro diagnostic use only.

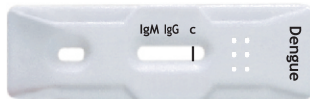
Interpretation of Test Result :

NEGATIVE: Only blue band appears in the control zone (C) of the result window. No band at test zone (T).

SAMPLE ADDITION & RUNNING BUFFER ADDITION



Negative



POSITIVE : Appearance of two colored bands, one in the test zone (IgM and or IgG) and another in the control zone (C), indicates that sample is positive for antibodies of dengue virus.

Positive for IgG & IgM



Positive for IgG

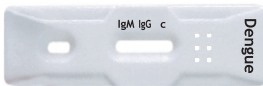


Positive for IgM



INVALID : If a colored band does not appears in the control zone (C). or Only Test Line appears, then the result is Considered Invalid.

Invalid (Retest)



Invalid (Retest)



Expected Value : Primary dengue is characterized by the presence of detectable IgM 3-5 days after the onset of infection. Secondary dengue is characterized by the elevation of specific IgG 1- 2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

Sensitivity: 98.4%

Specificity: 99%.

Limitations of the Test

1. This test detects the presence of antibodies to dengue in the Specimen and should not be used as the sole criteria for the diagnosis of Dengue virus infection.
2. 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 7-10 days after infection. Where symptoms persist, patients should be re-tested 3-4 days after the first specimen.
3. If the test result is negative and clinical symptoms persists, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of an early infection of Dengue virus.

References :

1. Dengue haemorrhagic fever : Diagnosis, treatment, prevention and control. WHO 2nd Edition 1997.
2. Songee L. ranch and Paul N. Levett. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. Clin. Diagn. Lab. Immunol. Vol6 (4) p 555-557, 1999.
3. Lamm S.K. (1995), dengue haemorrhagic fever. Rev. Med. Micro, 6-39-48.
4. Seth, J. (1991. standardization & quality assurance. In principle and practice of immunoassay, Ed. C.P. Price & D.J. Newman. Macmillan Publishers, pp.154-189.



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