

Dengue IgG & IgM Card Test (Whole Blood)

NOT APPROVED FOR USE IN THE UNITED
STATES

INTENDED USE

The Dengue IgM and IgG Combo Rapid Test is a qualitative test for the detection of IgM and IgG antibodies to dengue virus in human serum, plasma or whole blood. The test provides a differential detection of antidengue IgM and anti-dengue-IgG antibodies and can be used for the presumptive distinction between a primary and secondary dengue infection. This test is for *in-vitro* diagnostic use only.

INTRODUCTION

Dengue virus, a virus belonging to the Flavivirus group of viruses, is one of the most significant mosquito borne diseases in the world in terms of morbidity and mortality. Transmitted by principally by the mosquito types, *Aedes aegypti* and *Aedes albopictus*, the virus is found commonly throughout the tropics and subtropic regions of the world. There are four known serotypes of Dengue. Symptoms of Dengue fever range from high fever, headache, muscle pain and skin rash. The complications often associated with this infection are Dengue hemorrhagic fever or dengue shock syndrome. The immune response to this virus includes the production of IgM antibodies by 5th day of symptoms which remain in the circulatory system for 30-60 days. IgG antibodies appear by the 14th day of infection and persist for life. A secondary infection often results in high fever and, in many cases, initiates hemorrhagic events and circulatory failure. A secondary infection also induces an IgM antibody response after 20 days of infection and IgG antibodies rise within 1-2 days after the onset of symptoms. Therefore, patients with secondary infections will have a positive IgG result usually with a positive IgM result. Thus, the use of a reliable and sensitive rapid serological test that can simultaneously detect the presence of anti-dengue IgG and IgM antibodies is of great clinical utility. This rapid test provides an excellent methodology for specifically detecting anti-dengue IgG and IgM antibodies. The presence of high titers of IgG antibodies does not interfere with the detection of IgM antibodies in the sample. By using a mixture of highly purified proteins, the test is able to detect all 4 Dengue serotypes.

PRINCIPLE OF THE TEST

Serum, plasma or whole blood samples may be used with this test. When a specimen is added to the test, IgG and IgM antibodies in the specimen sample react with blue particles coated with recombinant Dengue envelope proteins. As this specimen/particle mixture migrates along the length of the test strip, the anti-Dengue IgG or IgM antibody particle complex is captured by the relevant IgG and or IgM test bands located on the test strip causing a pale to dark blue band to form at the IgG or IgM region of the test strip. The intensity of the bands will vary depending upon the amount

of antibody present in the sample. The appearance of any color in a specific test region (IgG or IgM) should be considered as positive for that particular antibody type (IgG or IgM). A red procedural control line should always develop on the test strip to indicate that the test has been performed properly.

MATERIALS AND REAGENTS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label:

- 25 test devices packaged in foil pouches. Each device contains two (2) test lines: one that captures human IgG antibodies and another that captures IgM antibodies. The device also contains a third procedural control line.
- 1 Product Insert
- 1 Dropper bottle of buffer
- 25 – 1 microliter plastic sample transfer loops.
- 25 Pippette/Stir Sticks

MATERIALS REQUIRED, BUT NOT PROVIDED

- Glass, borosilicate or plastic tubes, 12 x 75 mm preferred
- Timer capable of timing from 0 to 60 minutes

STORAGE

Store kit between 2°C and 30°C. Do not remove only the required number of them immediately. The test kit may be found on the package label.

WARNINGS AND PRECAUTIONS

1. All Specimens should be handled as being potentially infectious. The U.S. Centers for Disease Control (CDC) and the National Institutes of Health (NIH) recommend that all potentially infectious agents be handled at a Biosafety Level 2.
2. Biological decontamination procedures should be followed for all equipment, containers, surfaces, etc. that come in contact with potentially infectious specimens. All disposables that come in contact with these samples should be disposed of as infectious waste.
3. For best results, strict adherence to these instructions is required. Be careful not to touch the tip of the buffer bottle to the sample tube when adding buffer to the tube. This will greatly minimize the likelihood of contaminating the buffer reagent.
4. The buffer contains a low concentration of sodium azide as a preservative (less than 0.1 %). Sodium azide is toxic. Do not drink this buffer. High concentrations of sodium azide may also react with lead and copper in plumbing to form explosive compounds. If you dispose of this buffer down a drain, flush the drain with excess amounts of water

to minimize the accumulation of potentially explosive metal-azide compounds.

5. Do not use the test device(s) or reagents beyond the stated expiration date marked on the package label.
6. Store the test kits and reagents according to the temperature range stated on the package label.
7. All test devices, buffers and specimens must be at room temperature (15-30°C) before running the assay.
8. Do not re-use the test devices or buffer.

QUALITY CONTROL

1. For the assay to be considered valid, the control line must appear. If it does not appear, the test results are not valid and the test must be repeated.
2. In addition to your laboratory's standard quality control procedures, the NCCLS recommends that a positive and negative external control be tested at least once within each 25-test kit and by each operator performing testing within a kit. This will verify that the reagents and test strips are working properly and the operator is able to correctly perform the test procedure. Please refer to this NCCLS publication C24-A for recommendations on appropriate Quality Control practices. Region (T). A negative result indicates there is no presence of PfHRP-II.

SPECIMEN COLLECTION AND PREPARATION

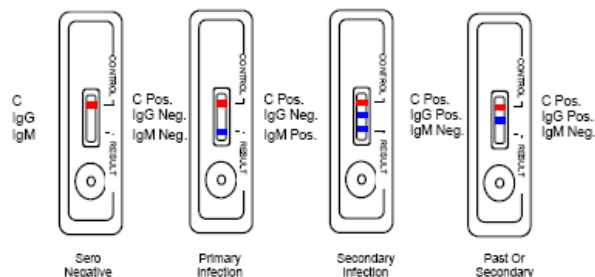
1. Handle all specimens as capable of transmitting infectious diseases. Dispose of all materials that come in contact with the specimen as infectious waste.
2. Specimens should be collected aseptically by venipuncture according to the standardized methods such as those recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The use of grossly lipemic or turbid samples should be avoided.
3. Whole blood samples should be used immediately, if possible. NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H1SA. 1990).
4. If serum or plasma specimens cannot be tested immediately, they should be refrigerated at 2 to 8°C. For storage periods greater than three (3) days, freeze the specimen at -20°C or below.

PROCEDURE

1. Remove the appropriate number of Dengue Combo Test devices from their pouch(es).
2. Add 4 drops (120 ul) of Dengue Wash Buffer into glass or plastic test tube.
3. Using a clean, unused 1 uL plastic loop (provided), dip the circular end of the plastic loop into the specimen, then carefully place the circular end of the plastic loop into the test tube and stir the buffer with the loop. This will add 1 ul of specimen to the buffer. Remove the loop and dispose of it as a biohazard. Do not reuse the loop.

4. If using a pipetter instead of the loop, add 1 uL of specimen directly into the buffer in the test tube and mix. Dispose of the pipette tip as a biohazard.
5. Use pipette/stir stick to transfer the specimen/buffer solution to the sample well of the test device.
6. Read the test results after 15 to 30 minutes. **Negative results must be confirmed at 30 minutes.**

INTERPRETATION OF RESULTS



1. The test is not valid if the red control line does not appear, regardless of the presence of a blue IgM or IgG line. Repeat the test with a new strip.
2. Specimens with positive IgM antibodies will generate a red line in the control region and a blue line in the IgM region.
3. Specimens with positive IgG antibodies will generate a red line in the control region and a blue line in the IgG region.
4. Specimens with positive IgG and IgM antibodies will generate a red line in the control region and blue lines in the IgG and IgM regions.
5. Positive results may appear as early as 2-5 minutes. Negative results must be confirmed after 30 minutes.

IgM Positive.

The control line and IgM lines are visible on the test strip. The test is positive for IgM antibodies. This is indicative of a primary dengue infection (see limitations).

IgM and IgG Positive.

The control line, IgM and IgG lines are visible on the test strip. The test is positive for IgM and IgG antibodies. This is indicative of a secondary dengue infection (see limitations).

IgG Positive

The control line and IgG lines are visible on the test strip. The test is positive for IgG antibodies. This is indicative of a past dengue infection.

Control Positive

The control line is the only line visible on the test strip. No IgG or IgM antibodies were detected. The result does not exclude dengue infection. If symptoms persist, a new

sample should be drawn from the patient in 3-5 days and then should be retested.

Control Negative

The test results are INVALID, regardless of the presence or absence of lines in the IgG or IgM region of the strip. Repeat the test using a new strip.

LIMITATIONS

1. This test detects the presence of antibodies to Dengue in the specimen and should not be used as the sole criterion for the diagnosis of a Dengue viral 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 seven to ten days of infection. If symptoms persist, a fresh sample should be drawn from the patient 3-4 days after the first testing date and the new specimen should be retested.
3. As with all diagnostic tests, the result must be correlated with clinical findings. If the test result is negative and a dengue infection suspicion still exists, additional follow-up testing using other clinical methods is recommended.
4. A negative serological result at any time does not preclude the possibility of an early infection of Dengue virus.
5. The use of Icteric or Lipemic samples should be avoided.
6. Strict adherence to the test procedure is required. Do not re-use negative strips. Do not adulterate the wash solution reagent.
7. This test cannot be used to monitor therapy or to estimate the relative antibody titer.
8. This test should not be used on specimens from immuno-suppressed individuals.
9. A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

EXPECTED VALUES

Primary dengue is characterized by the presence of detectable IgM antibodies 5 days after the onset of infection. Secondary dengue is characterized by the elevation of specific IgM antibodies and the elevation of specific IgG antibodies.

BIBLIOGRAPHY

1. Sabin, AB and Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice: *Science* (1945), 101:640.
2. Lam, SK. Dengue haemorrhagic fever. *Rev. Med. Micro.* (1995), 6:39-48.
3. Innis, BL, Nisalak, A., et.al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am. J. Trop. Med. Hygiene* (1989), 40:418-427.

4. CDC/NIH Guidelines. Biosafety in Microbiological and Biomedical Laboratories. 2nd Edition, 1988
5. Siti-Strong. Diagnosis, prevention, and treatment of tropical disease, 7th ed., Philadelphia, The Ablakiston Company

E102: 07/07

**NOT APPROVED FOR USE IN THE
UNITED STATES**