Dengue Combi Pak
(NS1 Ag+IgG/IgM Ab Card)

IMMUNOPAK
Last update 02-2017

INTENDED USE

Dengue Combi rapid test is an *in-vitro* immunochromatographic one step assay designed to detect both dengue NS1 antigen and differential IgG/IgM antibodies to dengue virus in human serum/plasma.

INTRODUCTION

Dengue viruses transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, are widely distributed throughout the tropical and sub topical areas of the world. There are four known distinct serotypes (dengue virus 1,2,3 and 4). Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality associated with it. NS1 is a highly-conserved glycoprotein that is present at high concentrations in the sera of dengue-infected patients during the early clinical phase of the disease. NS1 antigen is found from the first day and up to 9 days after onset of fever in sample of primary or secondary dengue infected patients. Usually IgM does not become detectable until 5 to 10 days after the onset of illness in cases of primary dengue infection and until 4 to 5 days after onset of illness in secondary infections. In primary infections, IgG appears on the 14th day and persist for life. Secondary infections shows that IgG rise within 1~2 days after the onset of symptoms and induces IgM response after 20 days of infection.

PRINCIPLE

Dengue NS1 antigen test utilizes the human serum / plasma followed by solid-phase Immuno-chromatographic technology for the qualitative detection of dengue virus NS1 antigen. The membrane strip of the device is pre-coated anti-dengue NS1 monoclonal antibody on the test region (T), and goat anti-mouse IgG is pre-coated on the control region (C). During testing, if the sample is containing dengue NS1 Ag, the complex of the antibody-dengue NS1 Ag-gold conjugate moves laterally on the membrane by capillary action. In this case, the pink-purple line will appear on the membrane in test line (T). Control line (C) should always appear if the test procedure is performed properly.

Dengue IgG / IgM test device has 3 pre-coated lines, "G" (Dengue IgG Test Line), "M" (Dengue IgM Test Line) and "C" (Control Line) on the surface of the device. Dengue-specific antigen complexed with gold conjugate is placed in the conjugate pad and anti-human IgG and anti-human IgM are immobilized on the membrane.
When dengue antibody-positive specimen is loaded into sample well, the antibodies are captured by the immobilized anti-human antibodies. And then, the antibodies are reacted with dengue-specific antigen-gold complex to make visible pink-purple line in the test region. To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, this will result in the formation of pink-purple line upon contact with the conjugate as a control line

PRESENTATION

	10 Tests
Dengue NS1 + IgG/IgM combo Test Cards	10 Cards
Dengue IgG/IgM Assay Buffer	1 Bottle
Antibody Test dropper (10µl)	10 Droppers
Antigen Test dropper	10 Droppers

PRECAUTION

1. For *in-vitro* diagnostic use only. Do not re-use the test device.
2. The instruction must be followed exactly to get accurate results.
3. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
4. Do not eat or smoke while handling specimens.
5. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
6. Avoid splashing or aerosol formation.
7. Clean up spills thoroughly using an appropriate disinfectant.
8. Decontaminate and dispose the specimens, reaction waste in a biohazard container.
9. Do not mix and interchange different specimen.
10. The presence of humidity may decrease the stability of the reagents. Thus, carry out the test immediately after removing the device from the foil pouch.
11. Do not use it beyond the expiration date.

STORAGE AND STABILITY

Dengue NS1 + IgG/IgM combo test card should be stored at 4-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on Pouch as well as carton label. The test kit should be kept away from direct sunlight, moisture and heat.

SPECIMEN COLLECTION AND STORAGE

1. **Specimen collection**
   The test is performed on human Serum/Plasma.
   Collect the whole blood into the collection tube (Not containing Anticoagulants such as heparin, EDTA, and sodium citrate) by Venipuncture, leave to settle for 30 minutes for blood coagulation and centrifuge blood to get serum specimen or supernatant.
2. **Specimen storage**
   All the specimens should be tested as soon as they are prepared. If the specimens are not immediately tested, they should be stored at 2~8 °C for 3 days, -20 °C for longer period than 3 days.

TEST PROCEDURE

Dengue NS1 Ag test

1. Place all the specimens, test device and solution on a flat surface. Allow them to attain room temperature prior to testing (15~30 min.)
2. Please perform the test immediately after removing the device from the foil pouch.
3. With a disposable dropper, add 2-3 drops (approx. 50-75µl) of specimen into the sample well (S) in the test device.
4. Interpret the test results between 15~20 minutes. Do not read the results after 20 minutes.

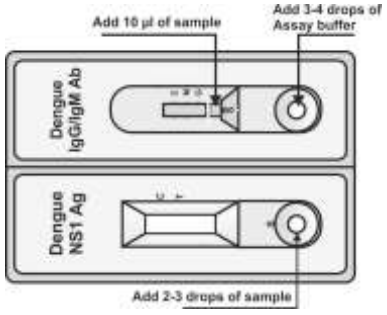
Dengue IgG/IgM test

1. Take a device from the pouch and place it on a flat surface.
2. Add 10µl of serum / plasma blood into the square shape of sample well (S) directly.
   Apply serum / plasma to the "S" area as mentioned in the figure.
3. Add 3-4 drops (approx. 90-120µl) of assay buffer (Diluent) into the assay buffer well.
4. Interpret the test results in 15-20 minutes after dropping buffer. **[Caution: Do not read the test result after 20 minutes, the reading too late can give false results]**

Important Note

It is essential during addition of sample into sample window "s" that the tip of the sample dropper touches onto the membrane of the device for 1 to 2 seconds to ensure that the complete sample is transferred on the membrane. This is to avoid sticking of very small volume (10 µl) sample on the side of sample well. 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 onto the membrane so that flow can happen. Even if, still the sample does not flow, it might contain particulate matter or is turbid, if so, re-run the test, after centrifuging at 10,000 rpm, for 10 minutes or more (in case clear sample is not obtained after centrifugation).

It is essential, that first the sample flow on the membrane and then only the assay buffer should be added in the buffer well for accurate results.

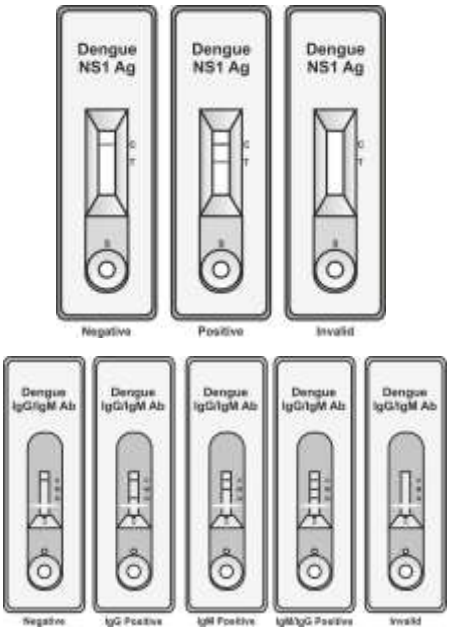


CE

Dengue Combi Pak
(NS1 Ag+IgG/IgM Ab Card)

IMMUNOPAK

INTERPRETATION OF RESULTS



Dengue NS1 Ag test

- **NEGATIVE**
  The presence of only one pink-purple line (control line) in the control region indicates a negative result.
- **POSITIVE**
  Two lines ("T" band and "C" band), one appears in the test line and another in the control line; it is suggested to do sample dilution and re-testing process. The re-test result is true; if the test still shows two lines, the result is positive for dengue NS1 Ag.
- **INVALID**
  If no line or only T line is visible within the window after performing the test, the result is considered invalid

Dengue IgG/IgM test

- **Negative**
  The control line is only visible on the test device. No Dengue-Specific IgG and IgM antibodies were detected. Retest within 3-5 days if dengue Infection is suspected.
- **IgM Positive**
  The control line (C) and IgM line (IgM) are visible on the test device. This is positive for IgM antibodies to dengue virus. This is indicative of a primary dengue infection.
- **IgG Positive**
  The control line (C) and IgG line (IgG) are visible on the test device. This is positive for IgG antibodies. This is indicative of a secondary or previous dengue infection.
- **IgG and IgM Positive**
  The control line (C), IgM (IgM) and IgG (IgG) are visible on the test device. This is positive for both IgM and IgG antibodies. This is indicative of a late primary or early secondary dengue infection.
- **INVALID**
  If no line or only T line is visible within the window after performing the test, the result is considered invalid. Some causes of invalid results are: Not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen should be retested using a new test cassette.

LIMITATIONS

1. A negative result can occur if the quantity of Dengue virus NS1 antigen present in the specimen is below the detection limits of the assay or the antigens that are detected are not present during the stage of disease in which a sample is collected.
2. A negative test result cannot exclude a recent infection.
3. The presence of detectable Dengue virus NS1 Ag may mean positive for early Dengue infection. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. In early infections and some secondary infections, detectable levels of IgM antibodies may be low where 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 3-4 days after the first specimen.
5. Serological cross-reactivity across the flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.

REFERENCES

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 Japanese encephalitis co-circulate. Am. J. Trap. 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.

An ISO 13485: 2012 & GMP Certified Company

3/7, B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com
Phone: +91·265·2281631
Email: mail@reckondiagnostics.com

RECKON DIAGNOSTICS P. LTD.