Windsor, Canada, www.immunoassay.ca

Duo Dengue IgM/IgG and NS1rapid test cassette Assayplus

Early detection of Dengue IgG/IgM and NS1 in serum / plasma or whole blood for *in vitro* diagnostic use

Introduction

Dengue virus is a mosquito-borne flavivirus, consisting of four serotypes, it is considered as a major public health problem in tropical / subtropical areas. 2.5-3.0 billion global population is estimated at risk in these regions. Annually about 50 million cases of dengue fever and more than 250,000 cases of dengue hemorrhagic fever occur based on WHO report.

Dengue virus infection includes 1. **dengue fever or simple dengue fever** usually seen with high fever, headache, myalgia, arthralgias, rashes and leucopenia; 2. **dengue haemorrhagic fever** is characterized with the acute onset, high fever, haemorrhagic diarrhea, thrombocytopenia, haemostasis and plasma leakage with a tendency to develop fatal shock (dengue shock syndrome).

Dengue infection can be also classified as primary and secondary infection. Due to four serotype dengue viruses, there is no cross-protection each other for each subtype, multiple infections are common in the infected patients. Infants or children dengue fever is usuauly present as primary infection;

Dengue NS1 antigen is an early mark appearing on the first day after symptom both in primary and secondary infection, the proportion of dengue NS1 antigen in blood is correlated with dengue virus load in patient. *Aassay+* dengue NS1 rapid test detects all four serotype dengue NS1, the sensitivity is over 80%. Dengue NS1 test has a narrow window for 1 to 6 days, a parallel test for dengue IgG/IgM test may need for testing together.

Dengue IgM is usually detectable in primary infection between 5 to 7 days after fever, while dengue IgG appears after 7 days. Therefore, the appearance of dengue IgM or IgM/IgG is mostly found in the primary infection depending on the time window of test; while dengue IgG usually appears in the secondary infection in the early days of infection with or without dengue IgM. Based on the tests from different geographic regions, our dengue IgG/IgM test has the sensitivity with clear signal over 90% for all four dengue serotypes, and equal sensitivity tested in the different geographic region, the specifity is over 95%.

Assay principle

For dengue NS1 test, 60 to 80 microliter serum / plasma sample or whole blood is used for the test. After specimen is loaded to the test device, dengue NS1 antigen in the specimen sample is captured by gold-conjugated monoclonal anti-dengue NS1 antibody, forming complex comprising of dengue NS1 antibody–conjugated gold and dengue NS1 antigen, it migrates along nitrocellulose membrane, the complex is captured by another dengue NS1 antibody on the test line forming a pink to deep pink band. The intensity of the band is variable depending on the amount of dengue NS1 antigen present in the blood sample. A red control line should always develop on the test strip indicating the proper performance.

For dengue IgG/IgM test, The recombinant dengue antigens are used in the test, the dengue antigens used for this test cover all four dengue serotypes. Serum / plasma or whole blood from dengue infection is loaded to the device, the specific dengue antibodies in specimen will bind to gold-conjugated dengue antigens forming antibody – collid gold-antigen complex. With complex migrating along nitrocellulose membrane, the complex is captured on the test lines coated with anti-human IgG or IgM causing a pink to deep pink band at the IgG or IgM test line. The signal intensity is variable depending on the amount of specific antibody present in specimen. A red control line should always develop over control line indicating the proper performance. This test device can detect all four dengue serotype infection. The sensitivity to detect dengue IgG and IgM is over 85% to 90%.

Kit Components

Each test kit contains:
1. A pouch
2. Pouch contains duo cassette test device
3. One 10 ul and 100 ul plastic pipette
4. Each 25 tests with one 3 ml dropper bottle with assay buffer

Stability and Storage Conditions

Duo dengue IgG/IgM and NS1 test kit is stable at room temperature between 10-28'C for one and half year in the unopened pouches.

DO NOT FREEZE the kit or expose to temperature extremes.

General Precautions

- The test is for *In Vitro* diagnosis only.
- Appropriate infection control and handling procedures should be followed – do not smoke, eat or drink in the area where the test is to be performed. Use suitable clothing and gloves when handling samples and when performing the test.
- Do not pipette any samples or reagents by mouth.
- All materials should be considered as potentially infectious. To disinfect, either autoclave

materials at 121.5'C for 1 hour or treat with Sodium hyprochlorite (1 percent solution).

- Do not use test beyond the expiration date indicated.

Sample Collection

Serum or plasma is used for this device. The test works best on fresh samples. If testing cannot be done immediately, they should be stored at 2-8'C after collection for up to 3 days. If testing can not be done within 3 days, serum can be stored frozen at –20'C or colder.

Test Procedure

1. Remove test cards from the pouches before your test, lay over a clean flat surface.

2. In case, the tested samples are in frozen status, the sample has to be sit in room temperature to completely thawing, and mixed well for test.

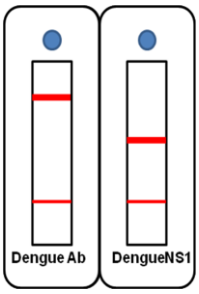
3. Add 10ul serum / plasma or whole blood to the well over the test card using included 10ul pipette. Follow adding sample, add two drops assay buffer from the dropper bottle.

4. Add 60 - 80ul serum / plasma or whole blood to NS1 well over the test card using included 100ul pipette. No assay buffer is used for NS1 test;

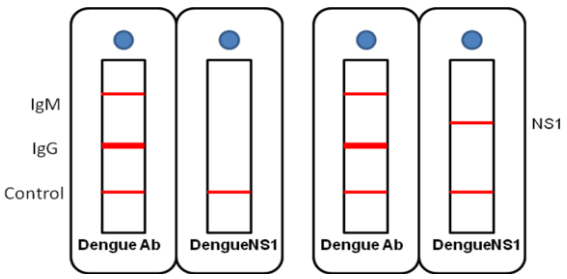
5. Results are then read 15 to 30 minutes, make sure control line is always positive.

Reading test results

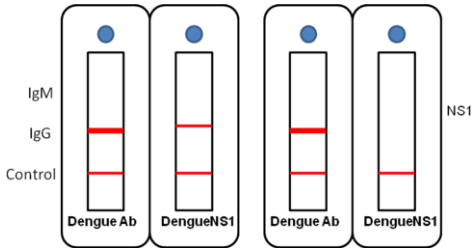
1. Dengue IgM negative, NS1 positive



2. Dengue IgM or IgG/IgM positive, NS1 negative



3. Dengue IgG positive, NS1 postive



4. Indeterminate

Control line should appear always If test line is positive or negative. without positive control line, it is recommended that a fresh device be used and the test repeated carefully following the directions of the product insert.

5. Quality Control:

A known positive and negative control should be run to insure proper performance. All control tests should be handled in the same manner as patient samples.

Result analysis:

1. Dengue IgM: positive or weak positive without dengue IgG between 2 to 5 days of symptom may indicate an early stage of primary dengue infection; in this stage, dengue NS1 may be detected as the rate of 60 to 70%.

2. Positive dengue IgM and IgG may occur in primary dengue infection 5 days after symptom; Most dengue NS1 has detectable concentration between 1 to 6 days, so after 5 days infection, NS1 may be positive or negative.

3. Strong dengue IgG without dengue IgM may indicate the secondary infection; Seconday infection has low rate with detectable NS1 due to presence of NS1 in patient blood.

Limitation of the test

This instruction should be followed carefully and performed properly.

Duo dengue IgM/IgG and NS1 test is designed to detect antibodies and NS1 antigen of dengue virus in serum or plasma. Testing of any other body fluids has not been validated and may not yield appropriate results.

A clinical evaluation of the patient's situation and history should also included with positive dengue IgG, IgM or NS1 test to establish the diagnosis.