TOYO®

INSTRUCTION FOR USE
Dengue NS1 Test
For Dengue NS1 Antigen
Detection in Whole Blood / Serum / Plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: TDG03
Dengue NS1 Antigen Test

BACKGROUND INFORMATION

The global prevalence of dengue has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, the America, Eastern Mediterranean, Western Pacific, and particularly in South East Asia. The World Health Organization (WHO) estimates that more than 2.5 billion people are at risk of dengue infections with 50–100 million cases occurring annually.
One of the most important reasons for the increase in cases is most likely caused by rapid development and urbanization, which provide breeding sites for Aedes aegypti, a principal mosquito vector responsible for transmission of dengue virus (DENV). The spread of infection is also enhanced by modern air travel and international trade such as motor vehicle tires, which facilitates the transmission of infected individuals and mosquito larvae to non-infected areas, posing the treat of introducing both the virus and its vector. Therefore, the emerging pattern and the increasing trend in the incidence of dengue infection are of great concern as there is no specific treatment of dengue, and most forms of therapy are supportive in nature. Furthermore, a licensed vaccine is not available yet. Classical dengue fever (DF) is generally self-limited and is characterized by fever and a variety of non-specific signs and symptoms such as headache, malaise, weakness, rash and body aches. The DHF is distinguished from DF by the onset of plasma leakage, marked thrombocytopenia, and a bleeding diathesis. With the increasing incidence of dengue infection, the early diagnostic confirmation of dengue infection in patients allows for timely clinical intervention, etiological investigation, and disease control. Hence, diagnosis of dengue disease during the acute phase should be a priority and is a public health concern.
The NS1 is a highly conserved glycoprotein that is present at high concentrations in sera of dengue-infected patients during the early clinical phase of disease, and is found from Day 1 and up to Day 9 after onset of fever in sample of primary or secondary dengue-infected patients. The IgM become detectable on Day 3 to 5 of illness in case of primary dengue infection and persist for 2 to 3 months, whereas IgG appear by the fourteenth day and persist for life. Secondary infection shows that IgG rises within 1 to 2 days after onset of symptoms, simultaneously with IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result, usually, but not always with a positive IgM result.

INTENDED USE

Dengue NS1 Test is a rapid chromatographic immunoassay for the qualitative detection of Dengue NS1 antigen in human whole blood, serum or plasma.

REAGENTS

Dengue NS1 Test contains anti-Dengue Ag conjugated colloid particles, anti-Dengue Ag coated on the membrane.

METHOD

Dengue NS1 Test is a qualitative membrane-based immunoassay for the detection of Dengue NS1 antigen in whole blood, serum, or plasma. During testing, the specimen reacts with Dengue antibody-conjugate in the test cassette. The antibody conjugate will bind to Dengue antigen in the specimen sample which in turn will bind with Anti-Dengue NS1 coated on the membrane. As the reagent moves across the membrane, the Dengue NS1 antibody on the membrane will bind the antibody-antigen complex causing pale or dark pink line to form at the test line region of the test membrane. The intensity of the lines will vary depending upon the amount of antigen present in the sample. The appearance of pink line in the test region should be considered as positive result. If the specimen does not contain NS1 antigen at detectable level, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Read this insert completely and carefully prior to use of the test. Test must be performed in strict accordance with these instructions to obtain accurate results.
3. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
4. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
5. Wear disposable gloves while performing the test.
6. Use a new micropipette tip for each sample.
7. This test kit should be handled only by adequately qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
8. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
9. This test will indicate only the selectively total of Dengue virus NS1 antigen in the sample, and should not be used as the only basis for the diagnosis of Dengue infection.

As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

Kit components: Test cassettes, pipettes, diluent and instructions for use.
Additional materials required but not provided: Micropipettes, sample collection tube, centrifuge and timer.
Additional materials recommended but not provided: Negative and positive control materials.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.
For Whole Blood Samples : Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.
For Serum Samples : Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.
For Plasma Sample : Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.
Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

TEST PROCEDURE

1. Bring the tests and whole blood / serum /plasma samples to room temperature. Take the test out of its pouch.
2. **For Serum / Plasma Samples:** Draw serum / plasma into pipette and put 3 drops (75 µl) into the sample well of the cassette. Do not use diluent for serum / plasma samples.

For Whole Blood Samples: Draw whole blood into pipette and put 3 drops (75 µl) into the sample well of the cassette. Immediately after, 1 drop of diluent is added into the sample well and allowed to soak in.

Avoid the formation of any air bubbles.

3. Results should be read at 10 minutes as shown below. Do not interpret results beyond 20 minutes, results forming after 20 minutes should be regarded as invalid.

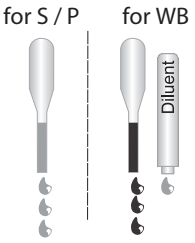
INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area, no apparent colored line appears in the test "T" area.

Positive: Two distinct colored lines appear. One color line should be in "C" area and another color line should be in "T" area.

The intensity of the color in the test line regions will vary depending on the concentration of Dengue NS1 antigen in the specimen. Therefore, any shade of color in the test line region should be considered as "positive".

Invalid: No colored line is visible in "C" area; test should be repeated using a new test device.



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Dengue NS1 test been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue Ag EIA and ELISA respectively.

Dengue NS1 Test

Method		Dengue Ag EIA		Total Results
Dengue NS1 Test	Results	Positive	Negative	
	Positive	70	4	74
	Negative	3	106	109
Total Results		73	110	183

Sensitivity of the Dengue NS1 Test is 95,8%, and the relative specificity is 96,3%.

Cross Reactivity

The Dengue NS1 Test was tested with HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, HCV, H. Pylori, MONO, CMV, Rubella and TOXO positive controls. No cross-reactivity has been observed.

Interferences

Following potentially interfering substances were tested with Dengue NS1 Test. No interference was observed.

Acetaminophen : 20 mg/dL	Acetylsalicylic Acid : 20 mg/dL	Ascorbic Acid : 2g/dL	Bilirubin : 1g/dL	Creatine : 200 mg/dL
Caffeine : 20 mg/dL	Genticic Acid : 20 mg/dL	Hemoglobin : 1000mg/dL	Albumin : 2 g/dL	Oxalic Acid : 60mg/dL

REFERENCES

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CE

Manufacturer
Consult instruction for use
Attention, see instruction for use
IVD *In vitro* diagnostic medical device
For single use only
Number of test
REF Catalog number
Storage temperature
LOT Lot number
Expiry date