TOYO®

INSTRUCTION FOR USE
Dengue IgG/IgM Test
For Dengue Antibodies (IgG / IgM)
Detection in Whole Blood / Serum / Plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: TDG01

Test for the qualitative detection of antibodies (IgG and IgM) generated against Dengue virus in whole blood, serum, or plasma.

BACKGROUND INFORMATION

Dengue is a flavivirus, transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. It is widely distributed throughout the tropical and subtropical areas of the world, and causes up to 100 million infections annually. Classic Dengue infection is characterized by a sudden onset of fever, intense headache, myalgia, arthralgia and rash. Primary Dengue infection causes IgM antibodies to increase to a detectable level in 3 to 5 days after the onset of fever. IgM antibodies generally persist for 30 to 90 days. Most Dengue patients in endemic regions have secondary infections, resulting in high levels of specific IgG antibodies prior to or simultaneous with IgM response. Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections. The Dengue IgG/IgM Test Device is a rapid test that utilizes a combination of Dengue antigen coated colored particles for the detection of IgG and IgM Dengue antibodies in human whole blood, serum, or plasma.

INTENDED USE

Dengue IgG/IgM Test is a rapid immunoassay for qualitative detection of antibodies (IgG and IgM) generated against Dengue virus in human whole blood / serum / plasma as an aid in the diagnosis of primary and secondary Dengue infections.

REAGENTS

Dengue antigen-coated particles, ligand anti-human IgM, anti-human IgG and anti-ligand.

METHOD

Dengue IgG/IgM Test is a qualitative membrane-based immunoassay for the detection of Dengue antibodies in whole blood, serum, or plasma. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is immobilized in test line region 1 "IgG" of the test. During testing, the specimen reacts with Dengue antigen-coated particles in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in test line region 1 "IgG". If the specimen contains IgG antibodies generated against Dengue virus, a colored line will appear in test line region 1 "IgG". In the IgM component, anti-ligand is immobilized in test line region 2 "IgM" of the test. During testing, the specimen reacts with ligand anti-human IgM. if IgM antibodies generated against Dengue virus are present in the specimen, reacts with the ligand anti-human IgM and the Dengue antigen-coated particles in the test strip, and this complex is captured by the anti-ligand, forming a colored line in test line region 2 "IgM". Therefore, if the specimen contains Dengue IgG antibodies, a colored line will appear in test line region 1 "IgG". If the specimen contains Dengue IgM antibodies, a colored line will appear in test line region 2 "IgM". If the specimen does not contain Dengue antibodies, no colored line will appear in either of the test line regions, indicating a negative result. As a procedural control, colored line always appears in the "C" control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. 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.
4. Wear disposable gloves while performing the test.
5. Use a new micropipette tip for each sample.
6. 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.
7. In the early onset of fever, Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked immunosorbent assay (MACELISA) showed that 80% of the Dengue patients tested exhibited detectable levels of IgM antibody by the fifth day after infection, and 99% of the patients tested IgM positive by day 10. It is recommended that patients be tested within this time. For the secondary infection, a low molar fraction of Dengue IgM and a high molar fraction of Dengue IgG that is broadly reactive to flaviviruses characterize the antibodies. The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
8. Serological cross-reactivity across the flavivirus group (Dengue 1, 2, 3 & 4, St. Louis encephalitis, West Nile virus, Japanese encephalitis and yellow fever viruses) is common. Positive results should be confirmed by other means.
9. This test will indicate only the presence or absence of Dengue virus IgG and IgM antibodies 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, diluent and instructions for use.
Additional materials required but not provided: Micropipette, sample collection tube, centrifuge and timer, lancet (for only fingerstick whole blood), heparinized dispensing bulbs and capillary tubes (for only fingerstick whole blood).
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 Whole Blood Samples:** Draw whole blood into pipette and put 10 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.

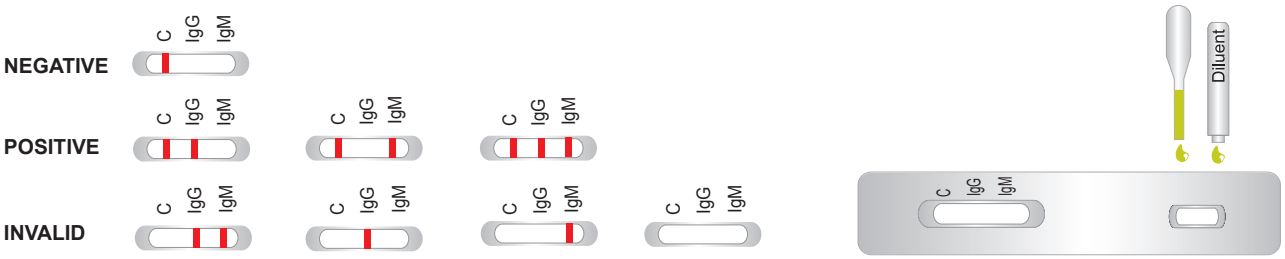
For Serum / Plasma Samples: Draw serum / plasma into pipette and put 5 μl into the sample well of the cassette. Immediately after, 1 drop of diluent is added into the sample well and allowed to soak in.

Avoid the formation of any air bubbles.

3. Depending on the Dengue IgG / IgM concentration in the sample, the test can react even in 5 minutes. 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, indicating that dengue antibody does not exist.
IgG POSITIVE: Two colored lines are visible in "C" and "IgG" areas. The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.
IgM POSITIVE: Two colored lines are visible in "C" and "IgM" areas. The result is positive for Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.
IgG AND IgM POSITIVE: Colored lines are visible in "C", "IgG" and "IgM" areas. The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and it is indicative of secondary Dengue infection.
The intensity of the color in the test line regions "IgG" and/or "IgM" will vary depending on the concentration of Dengue antibodies in the specimen. Therefore, any shade of color in the test line region(s) "IgG" and/or "IgM" should be considered 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 only a colored line in the "C" area of the test on negative samples and a colored line will appear in the "IgG" and/or "IgM" and "C" areas on positive samples. The color change 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

Sensitivity and Specificity

Dengue IgG/IgM Test has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test.

Dengue Infection	Result	IgM	IgG
Primary Infection	Positive	14	0
	Negative	3	17
	Total	17	17
	Sensitivity	**82,4%**	**0%**
Secondary Infection	Positive	39	55
	Negative	16	0
	Total	55	55
	Sensitivity	**70,9%**	**99,9%**
Non-Dengue Infection	Positive	0	0
	Negative	378	378
	Total	378	378
	Specificity	**99,9%**	**99,9%**

For the primary and secondary infection, the overall sensitivity is 95,8%, the overall specificity is 99,9%.

Intra-Assay

Within-run precision has been determined by using 10 replicates of four specimens: a negative, an IgG positive, an IgM positive and an IgG/IgM dual positive. The specimens were correctly identified.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same four specimens: a negative, an IgG positive, an IgM positive and an IgG/IgM dual positive. The Dengue IgG/IgM Test have been tested using these specimens. The specimens were correctly identified.

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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