Dengue Duo (WB)

Rapid Whole Blood Dengue Duo Test (IgG + IgM)

A Rapid qualitative Immunochromatographic test for the simultaneous detection of IgG and IgM Antibodies to Dengue Virus in human Whole Blood, Serum or plasma

For Professional In Vitro Diagnostic use only ***Read Instructions before use***

INTRODUCTION

Unimed ***FirstSign™ - Dengue Duo (WB)*** Test is a rapid immunochromatographic assay for simultaneous detection of IgG and IgM antibodies to Dengue virus in human whole blood, serum or plasma. The test is used as a screening test for Dengue viral infection and as an aid for differential diagnosis of primary and secondary infections in conjunction with other criteria.

SUMMARY

Dengue fever virus belongs to the group Flavivirus, which is widely distributed in the epidemic and endemic areas throughout tropical and subtropical regions of the world. Dengue fever virus is considered the most important in terms of morbidity, mortality and economic cost with an estimated about 100 million cases of dengue fever occurring throughout the world yearly.

Dengue viruses are transmitted in nature by day-biting Aeder mosquitoes. The most important mosquito vector is highly domesticated and urban species, *Aedes aegypti*. Dengue presents typically as a fever of sudden onset with headache, retrobullar pain, pain in the back and limbs (break-bone fever), lymphaderopathy and maculopaplar rash.

Patients diagnosed with dengue in endemic areas such as South East Asia generally have secondary infection, whereas patients in non-endemic areas are usually diagnosed with primary infection. Specific antibody responses to Dengue virus enable serodiagnosis and differentiation between primary and secondary dengue infections.

FirstSign™ - Dengue Duo (WB) Test is *a* new generation rapid Immunochromatographic test using highly specific and purified Recombinant Dengue Viral Antigens (all four Dengue Virus Serotypes) and is one of the simplest and fastest test for Differential Diagnosis of Dengue Fever.

TEST PRINCIPLE

FirstSign™ - Dengue Duo (WB) Test utilizes the principle of Immunochromatography, a unique two-site immunoassay on a membrane. Specific human IgM and human IgG binding proteins are immobilized on the nitrocellulose membrane respectively, as two individual test lines (IgM line and IgG line) in the test window **"T"** of the test device. The IgM line in the test window **"T"** is closer to the sample well and followed by IgG line in the test window (**T**). As the test sample flows through the membrane assembly within the test device, the colored–Dengue specific recombinant antigen-colloidal gold conjugate complexes with specific antibodies (IgM or IgG) of Dengue virus, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the Specific human IgM and/or human IgG binding proteins coated on the membrane leading to formation of a colored band, which confirms a positive test results. Absence of this colored band in the test window **"T"** indicates a negative test result. A built-in control line in the control window "**C"** appear when the test has performed properly, regardless of the presence or absence of anti-Dengue virus antibodies in the specimen.

REAGENTS AND MATERIALS SUPPLIED

Each kit contains:
A. 25 Individual pouches, each containing:
1. ***FirstSign™ - Dengue Duo (WB)*** (Test Card): Membrane test assembly predispensed with recombinant Dengue virus specific antigen colloidal gold conjugate, streptavidin gold conjugate, anti human IgM at test region 'M' Protein A at the test region 'G' and Biotin at the control region 'C'.
2. Desiccant pouch
3. Sample loop
B. Running Reagent – 1 Bottle
C. Product Insert

STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 4-30°C for the duration of the shelf life as indicated on the pouch.

PRECAUTIONS

1. This kit is for ***IN VITRO*** diagnostic use only. NOT FOR MEDICINAL USE.
2. This kit is for ***PROFESSIONAL*** use only.
3. Read the instructions carefully before performing the test.
4. This product does not contain any human source materials.
5. Do not use kit contents after the expiration date.
6. Handle all specimens as potentially infectious.
7. Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. When the assay procedure is complete, dispose specimens after autoclaving them at 121º C for at least 20 min. Alternatively, they can be treated with 0.5% Sodium Hypochlorite for 1-2 hours before disposal.
8. Do not pipette reagent by mouth and no smoking or eating while performing assays.
9. Wear gloves during the whole procedure.

SPECIMEN COLLECTION AND PREPARATION

1. No prior special preparation of the patient is required before sample collection by approved techniques.
2. Fresh serum / plasma is preferable. Serum / plasma may be stored at 2-8°C up to 3 days in case of delay in testing. For long-term storage, freeze the specimen at -20°C for 3 months or -70°C for longer periods.
3. The test works best on fresh whole blood samples. If testing cannot be done immediately, Blood samples collected with a suitable anticoagulant such as EDTA or Heparin or Oxalate should be stored at 2-8ºC up to 3 days. Blood samples should not be frozen.
4. Repeated freezing and thawing of the specimen should be avoided.
5. Do not use haemolysed, clotted, contaminated, lipamic and viscous/turbid specimen.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
7. Do not heat inactivate the sample.
8. Shipment of samples should comply with local regulations for transport of etiologic agents.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the kit components to room temperature before testing.
2. Open the pouch and remove the Card. Once opened, the test card must be used immediately.
3. Label the test card with patients identity.
4. Add 5µl of serum/ plasma or whole blood with a micropipette into the sample port “S”, OR using the 5µl sample loop provided with the kit. Dip the loop into the sample and then blot into the sample port ‘S’. Ensure that the loop does not retrieve clots or debris from the sample.
5. Add Five drops of Running Reagent in the well marked “R”.
6. At the end of 15 minutes read the results as follows.

RESULT INTERPRETATION

Negative Result:
The presence of a single pink-colored line in the control window **“C”** indicates the absence of specific antibodies against Dengue virus or the amount of antibodies are below the sensitivity level of the assay.

Positive Result:
1) The presence of three pink-colored lines: two test lines (IgM and IgG lines) within the test window **“T”** and one control line in the control window **“C”** indicates the presence of specific IgG and IgM antibodies against Dengue virus.

2) The presence of two pink-colored lines: one test line (IgM line) within the test window **“T”** and one control line in the control zone (C) indicates the present of specific IgM antibodies against Dengue virus.

3) The presence of two pink-colored lines: one test line (IgG line) within the test window **“T”** and one control line in the control window **“C”** indicates the present of specific IgG antibodies against Dengue virus.

Invalid Result:
If after 15 minutes no line is visible within the test or control window, the result is invalid. The test should be repeated with a new test card.

Performance Characteristics

1. In an in-house study, a panel of 320 patient sera was tested with the Unimed's ***FirstSign™ - Dengue Duo (WB)*** Test and a commercially available Reference IgG ELISA Test. The results are summarized in the following table:

		FirstSign™ - Dengue Duo (WB) Test		
		+	–	Total
Reference IgG ELISA Test	+	213	2*	215
	–	0	105	105
Total		213	107	320

* 2 Sera were borderline positive by Reference IgG ELISA (past exposure) and negative by ***FirstSign™ - Dengue Duo (WB)*** test.

FirstSign™ - Dengue Duo (WB) demonstrated 99.0% Sensitivity and 100% Specificity in the diagnosis of secondary dengue infection (IgG).

2. Another panel of 115 patient sera were tested with the ***FirstSign™ - Dengue Duo (WB)*** Test and a commercially available reference IgM ELISA Test. The results are summarized in the following table:

		FirstSign™ - Dengue Duo (WB) Test		
		+	–	Total
Reference IgM ELISA Test	+	79	1	80
	–	0	35	35
Total		79	36	115

FirstSign™ - Dengue Duo (WB) demonstrated 98.75% Sensitivity and 100% Specificity in the diagnosis of primary dengue infection (IgM).

3. In another in-house evaluation, fifty known positive and one hundred and ten known negative specimen were tested with ***FirstSign™ - Dengue Duo (WB)*** and compared with a licensed commercially available ELISA test. The results obtained are as follows:

Specimen Type	No. of Specimens Tested	Licensed Test	***FirstSign™ - Dengue Duo (WB)***
Negative for Ab. to Dengue	110	110	110
Positive for Ab. to Dengue	50	50	50

Based on the above study the specificity and sensitivity of ***FirstSign™ - Dengue Duo (WB)*** is 100%.

4. 25 samples were evaluated in an external study comprising of primary, secondary and negative Dengue sera, along with Japanese Encephelitis sera (JE) in parallel with Dengue IgM/ IgG Elisa and JE Elisa. ***FirstSign™ - Dengue Duo (WB)*** gave concordant results with all the samples with no cross reactivity with JE positive sera.

Overall ***FirstSign™ - Dengue Duo (WB)*** demonstrated excellent performance in the diagnosis of dengue infection and was able to distinguish between primary and secondary dengue infections through separate determinations of IgM and IgG.

Cross Reactivity: No cross reactivity with bilirubin (10 mg/dL), hemoglobin (18mg/dL) or triglycerides (up to 600 mg/dL).

Remarks

1. Do not use test kit beyond expiration date.
2. The test is limited to the detection of antibodies against Dengue viruses in whole blood, serum or plasma specimens.
3. Cases in which negative result is achieved while the clinical symptoms are indicative of Dengue virus infection, the test should be repeated with fresh collected samples and further investigation is needed.
2. While sample should be collected as soon as possible after onset of illness, it is recommended that follow up of testing should be done on day 10 after the first sample to allow seroconversion, especially when the test is negative and Dengue virus infection is clinically suspected.
3. Though ***FirstSign™ - Dengue Duo (WB)*** does provide evidence to distinguish the past (secondary) infection from current (primary) ongoing infection, a negative result does not preclude the possibility of infection with Dengue virus.
4. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated.
5. Repeatable reactive specimens should be conformed by another qualified test.
6. DHF is primarily the disease of children under 15 years in hyper endemic areas. Impending DSS symptoms include suspected abdominal pain, persistent vomiting, change in the level of consciousness, hypothermia and sudden decrease in platelet counts.
7. 80% of the patients may have detectable levels of IgM antibody by day 5 of illness and 99% by day 10.
8. IgM levels rise quickly and peak by two weeks after onset of symptoms and then fall to become undetectable over 2-3 months. IgG antibodies rise quickly and peak at about two weeks post infection and then decline slowly over 3-6 months.

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9. Data on file: Unimed International, Inc.

. Rev: 10/2003

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