Fastep®

DEN-W23 **Dengue Combo test device (Whole Blood/Serum/Plasma)**

INTENDED USE

The Dengue Combo test device (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of dengue virus NS1 antigen and IgG and IgM antibodies to NS1 antigen in human whole blood, serum, or plasma as an aid in the diagnosis of primary and secondary Dengue infections.

INTRODUCTION

Dengue viruses, transmitted by the mosquito, Aedes aegypti and Aedes albopictus mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world. There are four known distinct serotypes (dengue virus 1, 2, 3 and 4). In children, infection is often subclinical or causes a self-limited febrile disease. However, if the patient is infected second times with a different serotype, a more severe disease, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur. Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes. 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 appear the 14th day and persist for life. Secondary infections show that IgGs rise within 1~2 days after the onset of symptoms and induce IgM response after 20 days of infection.

The Dengue Combo Test device (Whole Blood/Serum/Plasma) is composed of two test strips: 1) Dengue NS1 antigen Test strip and 2) Dengue antibodies Test strip. Dengue NS1 antigen Test strip utilizes specific monoclonal antibodies to detect Dengue NS1, and Dengue antibody Test Strip utilizes NS1 antigen coupled with colored particles and anti-human IgG/IgM antibody to detection of IgG and IgM antibodies in human whole blood, serum, or plasma.

PRINCIPLE

The Dengue Combo Test device (Whole Blood/Serum/Plasma) is composed of two test strips: 1) Dengue NS1 antigen Test strip and 2) Dengue antibodies Test strip.

The Dengue NS1 Ag Test Strip 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 anti-dengue NS1 -coated particles in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-dengue NS1 Ag in test line region. If the specimen contains Dengue virus NS1, a colored line will appear in test line region. If the specimen does not contain Dengue virus NS1, no colored line will appear in the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

The Dengue Antibody Rapid Test Device (Whole Blood/Serum/Plasma) 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 coated in test line region 1 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. If the specimen contains IgG antibodies to Dengue, a colored line will appear in test line region 1. In the IgM component, anti-ligand is coated in test line region 2 of the test. During testing, the specimen reacts with ligand anti-human IgM. Dengue IgM antibodies, if 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.

Therefore, if the specimen contains Dengue IgG antibodies, a colored line will appear in test line region 1. If the specimen contains Dengue IgM antibodies, a colored line will appear in test line region 2. If the specimen does not contain Dengue antibodies, 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 change from blue to red in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENT

For Dengue NS1 Ag Test Strip:
The test device contains Dengue NS1 antibody-coated particles and Another Dengue NS1 antibody is coated in the test line regions.
For Dengue antibody Test Strip:
The test device contains Dengue antigen-coated particles and anti-human IgM. Anti-human IgG are coated in the test line regions.

MATERIALS

Materials Provided

- Individually packed test devices
- 5ul Disposable pipettes for Dengue NS1
- 25ul Disposable pipettes for Dengue IgM/IgG
- Package insert
- Buffer

Materials Required but Not provided

- Specimen collection container
- Centrifuge
- Micropipette
- Timer
- Lancets

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use the test if the pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

- The kit should be stored at 2-30℃ until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The Dengue Combo test device (Whole Blood/Serum/Plasma) is intended for use with human whole blood, serum or plasma specimens only.
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8℃ for up to 3 days. For long term storage, specimens should be kept below -20℃. Whole blood collected by venipuncture should be stored at 2-8℃ if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results.

PROCEDURE

Allow the test device, specimen, buffer, and/or controls to reach room temperature (15-30℃) prior to testing.

1. Bring the pouch to room temperature before opening. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface.
   For Serum or Plasma Specimens:
   Hold the 5ul dropper vertically, draw the specimen up to the Fill Line (approximately 5 uL), and transfer the specimen to the Dengue IgM/IgG specimen well (S) of the test device, then add 3 drops of buffer (approximately 90 µL) and start the timer.
   Transfer 3 drops of serum/plasma (approximately 75 µL) to the Dengue NS1 specimen well (S) of the device with the provided disposable pipette and start the timer.
   For Whole Blood (Venipuncture/Fingerstick) Specimens:
   To use a 5ul dropper: Hold the dropper vertically, draw the specimen 0.5-1 cm above the Fill Line, and transfer 1 drop of whole blood (approximately 10 µL) to the Dengue IgM/IgG specimen well (S) of the test device, then add 3 drops of buffer (approximately 90 uL) and start the timer.
   Transfer 2 drops of whole blood (approximately 50 µL) to fall into the center of the Dengue NS1 specimen well(s) of the test device then add 1 drop of buffer, and start the timer.
   See illustration below.
3. Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

For Dengue IgM/IgG Test

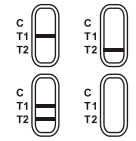
IgG POSITIVE:* The colored line in the control line region (C) changes from blue to red, and a colored line appears in test line region 1 (T1). The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.

IgM POSITIVE:* The colored line in the control line region (C) changes from blue to red, and a colored line appears in test line region 2 (T2). The result is positive for Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.

IgG AND IgM POSITIVE:* The colored line in the control line region (C) changes from blue to red, and two colored lines should appear in test line regions 1 and 2 (T1 and T2). The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and is indicative of secondary Dengue infection.

***NOTE:** The intensity of the color in the test line region(s) (T1 and/or T2) will vary depending on the concentration of Dengue antibodies in the specimen. Therefore, any shade of color in the test line region(s) (T1 and/or T2) should be considered positive.

NEGATIVE: The colored line in the control line region (C) changes from blue to red. No line appears in test line regions 1 or 2 (T1 or T2).



INVALID: Control line (C) is still completely or partially blue, and fails to completely change from blue to red. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

For Dengue NS1 Test

POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

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.

QUALITY CONTROL

An internal procedural control is included in the test. A colored line changes from blue to red in the control line region (C), confirming sufficient buffer volume and adequate membrane wicking.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

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. Some patients may not produce detectable levels of antibody within the first seven t en days after infection. Where symptoms persist, patients should be re-tested 3-4 days after the first specimen. Serological cross-reactivity across the Flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.

EXPECTED VALUES

Primary Dengue infection is characterized by the presence of detectable IgM antibodies 3-5 days after the onset of infection. Secondary Dengue infection is characterized by the elevation of Dengue-specific IgG. In the majority of the cases, this is accompanied by elevated levels of IgM.[5] The Dengue Rapid Test Device (Whole Blood/Serum/Plasma) has been compared with a leading commercial Dengue ELISA test,

demonstrating sensitivity of 82.4% for IgM in primary infection and >99.0% for IgG in secondary infection. The NS1 is expected to be detected 1 day after the onset of fever and persist up to 9days in both primary and secondary dengue infection. But if anti-NS1 antibodies produced, the detection of NS1 is inhibited.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

The Dengue Combo Test Device (Whole Blood/Serum/Plasma) 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 IgM/IgG Rapid Test Device vs. ELISA

Dengue Infection	Result	IgM	IgG
Primary Infection	Positive	14	0
	Negative	3	17
	Total	17	17
	Relative Sensitivity	**82.4%**	**0%**
Secondary Infection	Positive	39	55
	Negative	16	0
	Total	55	55
	Relative Sensitivity	**70.9%**	**>99.0%**
Non-Dengue Infection	Positive	0	0
	Negative	378	378
	Total	378	378
	Relative Specificity	**>99.0%**	**>99.0%**

For the primary and secondary infection, the overall sensitivity is 95.8%, the overall specificity is >99.0% and the overall accuracy is 99.3%.

Dengue NS1 Ag Test Device vs. Culture

Relative Sensitivity:92.8%

Relative Specificity:98.4%

Overall Agreement:96.3%

			Culture +	Culture -	Total
Dengue NS1 Ag	**NS1**	**+**	104	3	107
		-	8	186	194
			112	189	301

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of five specimens: a negative, an NS1 positive, an IgG positive, an IgM positive and an IgG/IgM dual positive. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same five specimens: a negative, an NS1 positive,,an IgG positive, an IgM positive and an IgG/IgM dual positive. Three different lots of the Dengue Rapid Test Device (Whole Blood/Serum/Plasma) have been tested using these specimens. The specimens were correctly identified >99% of the time.

LITERATURE REFERENCES

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GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		Do not reuse

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