Fastep®

D-DEN-42

Dengue Rapid Test Device
(Whole Blood/Serum/Plasma)

INTENDED USE

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

INTRODUCTION

Dengue is a flavivirus, transmitted by Aedes aegypti and Aedes albopictus mosquitoes.[1] It is widely distributed throughout the tropical and subtropical areas of the world,[1] and causes up to 100 million infections annually.[2] 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.[3] Most Dengue patients in endemic regions have secondary infections,[4] resulting in high levels of specific IgG antibodies prior to or simultaneous with IgM response.[5] Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.

The Dengue Rapid Test Device (Whole Blood/Serum/Plasma) 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.

PRINCIPLE

The Dengue 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

The test device contains Dengue antigen-coated particles and ligand anti-human IgM. Anti-human IgG and anti-ligand are coated in the test line regions.

MATERIALS

Materials Provided

- Individually packed test devices
- Disposable pipettes
- 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.

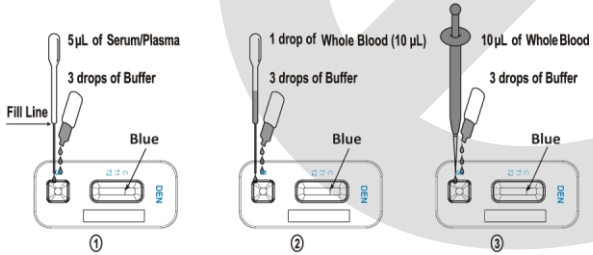
SPECIMEN COLLECTION AND STORAGE

- The Dengue Rapid 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 dropper vertically, draw the specimen up to the Fill Line (approximately 5 uL), and transfer the specimen to the specimen well (S) of the test device, then add 3 drops of buffer (approximately 90 µL) and start the timer. See illustration below. Avoid trapping air bubbles in the specimen well (S).
   For Whole Blood (Venipuncture/Fingerstick) Specimens:
   To use a 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 specimen well (S) of the test device, then add 3 drops of buffer (approximately 90 uL) and start the timer. See illustration below.
   To use a micropipette: Pipette and dispense 10 µL of whole blood to the specimen well (S) of the test device, then add 3 drops of buffer (approximately 90 µL) 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

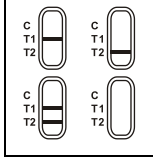
IgG POSITIVE:* The colored line in the control line region (C) changes from blue to red, and a colored line appears in test line region 1 (T1). The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.

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.

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. The Dengue Rapid Test Device (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. The test should be used for the detection of Dengue antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in Dengue antibody concentration can be determined by this qualitative test.
2. The Dengue Rapid Test Device (Whole Blood/Serum/Plasma) will only indicate the presence of Dengue antibodies in the specimen and should not be used as the sole criteria for the diagnosis of Dengue.
3. In the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) 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.[5] It is recommended that patients be tested within this time.
4. For the secondary infection, a low molar fraction of anti-Dengue IgM and a high molar fraction of IgG that is broadly reactive to flaviviruses characterize the antibodies.[5] The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
5. 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.[6,7,8] Positive results should be confirmed by other means.
6. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
7. Results from immunosuppressed patients should be interpreted with caution.
8. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
9. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Dengue infection.

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.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

The Dengue Rapid 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 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%.

Precision

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 >99% of the time.

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. 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

1. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue. Rev. Infect. Dis. 1984; 6:251-264
2. Halstead SB, Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239:476-481
3. Ruechusatsawat K, et al. Daily observation of antibody levels among dengue patients detected by enzyme-linked immunosorbent assay (ELISA). Japanese J. Trop. Med. Hygiene 1994; 22: 9-12
4. Lam SK. Dengue haemorrhagic fever. Rev. Med. Micro. 1995; 6:39-48
5. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization
6. Yamada K, et al. Antibody responses determined for Japanese dengue fever patients by neutralization and hemagglutination inhibition assays demonstrate cross-reactivity between dengue and Japanese encephalitis viruses. Clin Diagn Lab Immunol. 2003 Jul; 10(4): 725-8.
7. Dobler G, et al. Cross reactions of patients with acute dengue fever to tick-borne encephalitis. Wien Med Wochenschr (in German). 1997; 147(19-20): 463-4
8. Makino Y, et al. Studies on serological cross-reaction in sequential flavivirus infections. Microbiol Immunol. 1994; 38(12): 951-5.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marking according to IVD Medical Devices Directive 98/79/EC		

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