DENGUE ANTIBODY DEVICE (2-30°C)

CATALOGUE NUMBER	KIT SIZE (TESTS)
RADDEN2	20 Tests

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.

Summary:
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 uses a combination of Dengue antigen coated coloured particles for the detection of IgG and IgM Dengue antibodies in human whole blood, serum, or plasma.

Test 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 at the IgG test region. During testing, the sample reacts with Dengue antigen-coated particles loaded at the sample pad. The mixture then migrates upward on the membrane by capillary action where the sample anti-Dengue IgG–particle complexes are captured by the anti-human IgG at test region. If the sample contains IgG antibodies to Dengue, a coloured line will appear at the IgG test line region. For the IgM component, anti-human IgM is coated at IgM test region of the device. During testing, sample containing anti-Dengue IgM also reacts with Dengue antigen-coated particles. The mixture then migrates upward on the membrane by capillary action where the anti-Dengue IgM–particle complexes are captured by the anti-human IgM at test region. If the sample contains IgM antibodies to Dengue, a coloured line will appear at the IgM test line region.
If the sample does not contain Dengue antibodies, no coloured line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a coloured line should always appear in the control line region, indicating that the proper volume of sample has been added and membrane wicking has properly occurred.

Reagent:
The test device contains Dengue antigen-coated particles and anti-human IgM and anti-human IgG and in the test line regions.

Materials Provided
Individually pouched test devices
Disposable pipettes
Buffer
Instructions for Use sheet

Materials not provided: Sample collection container, centrifuge, micropipette, timer, lancets.

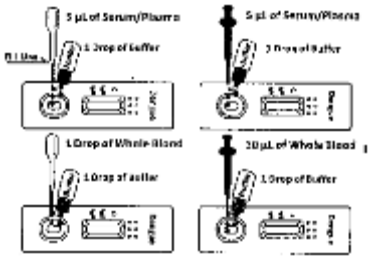
Precautions:
- For professional *in vitro* diagnostic use only. Do not use after the expiry date.
- Do not eat, drink or smoke in the area where the samples or kits are handled.
- Do not use the test if the pouch is damaged.
- Handle all samples as if they contain infectious agents. Observe established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

Reagent Preparation and Stability:

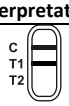
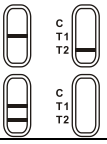
- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination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

Sample Collection and Storage:
- The Dengue Antibody Rapid Test Device (Whole Blood/Serum/Plasma) is intended for use with human whole blood, serum or plasma samples only.
- Only clear, non-hemolyzed samples are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after sample collection. Do not leave samples at room temperature for prolonged periods. Serum and plasma samples may be stored at 2-8°C for up to 3 days. For long term storage, samples should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood samples. Whole blood collected by fingerstick must be tested immediately.
- Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
- Bring samples to room temperature prior to testing. Frozen samples must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of samples.
- If samples 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.

Assay Procedure:
Allow the test device, sample, buffer, and/or controls to reach room temperature (15-30°C) 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 samples hold the dropper vertically, draw the sample up to the fill line (approximately 5 uL), and transfer the sample to the sample pad of the test device, then add 1 drop of buffer (approximately 40 µL) and start the timer. See illustration below. Avoid trapping air bubbles in the sample pad.
   For Whole Blood (Venipuncture/Fingerstick) samples hold the dropper vertically, draw the sample about 1 cm above the fill line, and transfer 1 drop of whole blood (approximately 10 µL) to the sample pad of the test device, then add 1 drop of buffer (approximately 40 uL) and start the timer. See illustration below.
   To use a micropipette: Pipette and dispense 10 µL of whole blood to the sample pad of the test device, then add 1 drops of buffer (approximately 40 µL) and start the timer. See illustration below.
3. Wait for the coloured line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.



Interpretation of Results:

	**IgG POSITIVE:*** A coloured line appears in the control line region (C) and a coloured line appears in the IgG test line region. The result is positive for specific-IgG antibody to Dengue virus and is indicative of secondary Dengue infection.
	**IgM POSITIVE:*** A coloured line appears in the control line region (C) and a coloured line appears in the IgM test line region. The result is positive for specific-IgM antibody to Dengue virus and is indicative of primary Dengue infection.
	**IgG AND IgM POSITIVE:*** A coloured line appears in the control line region (C) and two coloured lines appear in each of the test line regions. The colour intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and probably indicates the end of primary infection and also secondary Dengue infection.
***NOTE:** The intensity of the color in the test line region(s) will vary depending on the concentration of Dengue antibodies in the sample. Therefore, any shade of colour in the test line region(s) should be considered positive.	
	**NEGATIVE:** A coloured line appears in the control line region (C). No line appears in either test line regions.
	**INVALID:** No line appears in the Control line (C) region. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. 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 coloured line appears in the control line region (C), confirming sufficient buffer volume and adequate membrane wicking.
Quality Control 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 Antibody 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 samples 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 sample and the result should not be used as the sole criteria for the diagnosis of Dengue but used in conjunction with other clinical data.
3. During the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM enzyme-linked immunosorbent assay showed that 80% of 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.

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4. Secondary infection is characterized by a low fraction of anti-Dengue IgM and a high fraction of IgG that are reactive to flaviviruses. [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 Antibody Rapid Test Device (Whole Blood/Serum/Plasma) has been compared with a leading commercial Dengue ELISA test, demonstrating sensitivity of 83.3% for IgM in primary infection and 98.4% for IgG in secondary infection.

Performance Characteristics:

Sensitivity and Specificity

The Dengue Antibody Rapid Test Device (Whole Blood/Serum/Plasma) has been tested with samples obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test.

Dengue Primary Infection

Method			ELISA		
Dengue Antibody Rapid Test Device (Whole Blood/Serum/Plasma)	Results		Positive		Negative
			IgM	IgG	
	Positive	IgM	20	0	0
		IgG	4	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%	/	/

Dengue Secondary Infection

Method			ELISA		
Dengue Rapid Test Device (Whole Blood/Serum/Plasma)	Results		Positive		Negative
			IgM	IgG	
	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			73.4%	98.4%	/

Non-Dengue Infection

Method			ELISA		
Dengue Rapid Test Device (Whole Blood/Serum/Plasma)	Results		Positive		Negative
			IgM	IgG	
	Positive	IgM	0	0	1
		IgG	0	0	3
	Negative		0	0	429
Relative Sensitivity			/	/	99.1%

Relative sensitivity: (20+63)/(24+64) = 94.3% (95%CI* : 87.2% - 98.1%);
Relative specificity: 429/433 = 99.1% (95%CI* : 97.7% - 99.7%);
Accuracy: (20+63+429)/(24+64+433) = 98.3% (95% CI* : 96.7% - 99.2%). * Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of four samples, a negative, an IgG positive, an IgM positive and an IgG/IgM dual positive. The samples were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on four samples, a negative, an IgG positive, an IgM positive and an IgG/IgM dual positive. Three different lots of the Dengue Antibdy Rapid Test Device (Whole Blood/Serum/Plasma) have been tested using these samples. The samples were correctly identified >99% of the time.

Cross-reactivity

The Dengue Antibody Rapid Test Device (Whole Blood/Serum/Plasma) has been tested with samples positive for HAMA, RF, HBsAg, HBsAb, HBeAb, HBeAg, HBcAb, Syphilis, HIV, HCV, H.Pylori, MONO, CMV, Rubella and TOXO. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Dengue negative and positive samples.

Acetaminophen: 20mg/dl	Acetylsalicylic Acid: 20mg/dl	Ascorbic Acid: 2g/dl
Bilirubin: 1g/dl	Creatin: 200mg/dl	Caffeine: 20mg/dl
Gentisic Acid: 20mg/dl	Haemoglobin: 1000mg/dl	Albumin: 2g/dL
Oxalic Acid: 60mg/dl		

None of the substances at the concentration tested interfered in the assay.

References:

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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
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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		Do not reuse

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