Dengue Dual Ag Ab Device (2–30°C)

CATALOGUE NUMBER	KIT SIZE (TESTS)
RADDEN1	20 Tests

Intended Use:
The Dengue Dual Ag Ab Device (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of NS1 antigen of and IgG and IgM antibodies to Dengue virus in human whole blood, serum, or plasma as an aid in the diagnosis of Dengue infection.

Summary:
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 IgG antibodies after or simultaneously with the IgM response. Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.
NS1 is one of the Dengue virus non-structural proteins which are thought to be involved in viral replication. NS1 is found either associated with intracellular organelles or associated with the plasma membrane or secreted as a soluble hexadimer. NS1 is essential for viral viability but its precise biological function is unknown. Antibodies raised in response to NS1 in viral infection can cross react with cell surface antigens on epithelial cells and platelets and this has been implicated in the development of Dengue Haemorrhagic fever.

Test Principle:
The Dengue Dual Ag Ab Device (Whole Blood/Serum/Plasma) is a qualitative membrane-based immunoassay for the detection of anti-Dengue IgG and IgM antibodies and Dengue NS1 antigen in whole blood, serum and plasma.
In the IgG/IgM antibody portion of the test, there is an IgG component and an IgM component which can separately determine the presence of anti-Dengue IgG and/or IgM. At the test lines anti-human IgG and anti-human IgM are coated on the membrane at the respective labelled areas. During testing, the sample reacts with Dengue antigen-coated particles coated near the sample well. The mixture migrates upward on the membrane by capillary action and interacts with the anti-human IgG at IgG test line and with the a human IgM at the IgM test line. If the sample contains IgG and/or IgM antibodie Dengue, a coloured line will appear in the respective test line region. If the sample does contain Dengue antibodies, no coloured line will appear at either of the test lines indicating a negative result. To serve as a procedural control, a coloured line should always appear at the control line, indicating that the proper volume of sample has been added and membrane wicking has occurred.
In the Dengue antigen portion of the test sample added to the sample well reacts with anti-NS1 antibody conjugated to particles and coated on the membrane there. The conjugate will bind any Dengue antigen present in the sample and then the mixture migrates upward on the membrane by capillary action where the antibody-antigen complexes will bind with anti-Dengue NS1 coated on the test line. Therefore, if NS1 antigen is present in the sample a coloured line will appear at the test line indicating a positive result. The intensity of the line will vary depending upon the amount of antigen present in the sample.

Reagents:
The Dengue Dual Ag Ab Device contains Dengue antigen conjugated gold colloid particles, anti-Dengue NS1 coated on the membrane and anti-human IgM, anti-human IgG coated on the membrane.

Materials Provided
Individually pouched test devices
Droppers
Buffer
Instructions for Use sheet

Materials not provided: Specimen collection containers, lancets, centrifuge, timer.

Precautions:
For professional in vitro diagnostic use only.
Follow Good Laboratory Practice procedures where samples and kits are handled and treat the device and all samples as if potentially infectious. Follow local regulations for correct disposal of samples.
Wear protective clothing including laboratory coat, disposable gloves and safety glasses when testing samples.
Humidity and temperature can adversely affect results.

Storage and Stability:
The kit can be stored at room temperature or refrigerated (2 - 30°C). The test device is stable up to the expiry date printed on the sealed pouch. The device must remain in the sealed pouch until use. DO NOT FREEZE. Do not use after the expiry date.

Sample Collection and Preparation:
The Dengue Dual Ag Ab Device can be performed using whole blood, serum, or plasma.
To collect Fingerprick Whole Blood Samples: Wash the patient's hand or clean with an alcohol swab. Allow to dry. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertips. Puncture the skin with a sterile lancet. Wipe away the first sign of blood. Gently squeeze the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site. Collect the blood drop in the dropper. Do not freeze whole blood samples. Whole blood collected by fingerprick must be tested immediately.
Separate serum and plasma from red blood cells as soon as possible to avoid haemolysis. Use only clear, non-haemolysed samples. Serum and plasma samples may be stored at 2 - 8°C for up to 3 days. For long term storage, samples should be frozen below -20°C. Frozen samples must be completely thawed and mixed well prior to testing. Samples should not be repeatedly thawed and re-frozen. If samples are to be shipped, they should be packed in compliance with local regulations for transportation of etiologic agents.

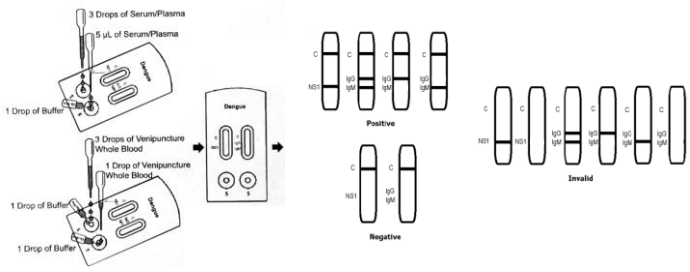
Assay Procedure:
Bring the device, samples, buffer and controls fully to room temperature (15 - 30°C) before starting any testing. Remove the test device from the sealed pouch, place it on a clean and level surface and use it immediately.

For **Serum or Plasma** samples:
For NS1 antigen: Hold the large dropper vertically and transfer 3 drops of serum or plasma (approximately 75 µl) to the sample well and start the timer. See illustration below.

For IgG/IgM antibodies: Hold the small dropper vertically, draw the sample up to the Fill Line (approximately 5 µl), and expel it into the sample well of the test device. Add 1 drop of buffer (approximately 40 µl) and start the timer. Avoid trapping air bubbles in the sample well.
Or: Pipette and dispense 5 µl of serum or plasma to the sample well of the test device, add 1 drop of buffer (approximately 40 µl) and start the timer.

For **Whole Blood** (Venipuncture/Fingerprick) samples:
For NS1 antigen: Hold the large dropper vertically and transfer 3 drops of whole blood (approximately 75 µl) to the sample well, add 1 drop of buffer (approximately 40 µl) and start the timer.
Using a capillary tube: Fill the tube and transfer approximately 75 µl of fingerprick whole blood sample to the sample well of test device, add 1 drop of buffer (approximately 40 µl) and start the timer.
Using hanging drops: Allow 3 hanging drops of fingerprick whole blood (approximately 75 µl) to fall into the sample well of test device, then add 1 drop of buffer (approximately 40 µl) and start the timer.
For IgG/IgM: Hold the small dropper vertically, draw whole blood to 1cm above the Fill Line, and transfer 1 drop of whole blood (approximately 10 µl) to the sample well, add 1 drop of buffer (approximately 40µl) and start the timer.
Using a micropipette: Pipette and dispense 10 µl of whole blood to the sample well of the test device, add 1 drop of buffer (approximately 40 µl) and start the timer.

Read the results at 10 minutes, do not interpret the results after 20 minutes.



Interpretation of Results:
NS1 Positive: Two coloured lines appear. One band appears at the control line (C) and another band develops at the test line (NS1).
IgG and IgM Positive: Three lines appear. One band appears at the control line (C), and bands develop at each of IgG test line and IgM test regions. The colour intensities of the lines do not have to match. The result is positive for IgG and IgM antibodies and is indicative of end stage of primary Dengue infection and early stage of secondary infection.
IgG Positive: Two lines appear. One band appears at the control line and another band develops at the IgG test line. The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.
IgM Positive: Two lines appear. One band appears at the control line and another band develops at the IgM test line. The result is positive for Dengue virus specific-IgM antibodies and is probably indicative of primary Dengue infection.
Note: The intensity of the colour of any of the lines in the test regions (NS1 and/or IgG and/or IgM) will vary depending on the concentration of Dengue NS1 antigen or IgG or IgM present in the sample. Therefore, any shade of colour at test lines should be considered positive.
Negative: One colour line appears in the control region (C). No bands appear in any of the test regions.
Invalid: A Control line fails to appear. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Quality Controls:
Internal procedural controls are included in the test. A colour line appearing at the control line is an internal control confirming that sufficient sample volume was added and correct wicking up the membrane occurred. 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:
- The Dengue Dual Ag Ab Device (Whole Blood/Serum/Plasma) will only indicate the presence of Dengue NS1 antigen and Dengue antibodies in the sample and should not be used as the sole criteria for the diagnosis of Dengue. Positive results should be confirmed by an alternative method.
- The assay procedure and the assay result interpretation must be closely adhered to when testing the presence of Dengue antigen in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
- A negative test result for Dengue NS1 does not preclude the possibility of exposure to or infection with Dengue virus. If test results are negative and clinical symptoms persist, additional testing using other clinical methods is recommended. Dengue NS1 and antibodies may be present but below the detection limit of the assay or that Dengue NS1 is not present during the stage of disease at which the sample was collected. Retesting a week later is recommended and testing with an alternative test device such as PCR, ELISA.
- Heterophilic antibodies and Rheumatoid Factor can interfere in test results.
- The results obtained with this test should not be used as the sole criteria for the diagnosis, only be interpreted in conjunction with other diagnostic procedures and clinical findings.
- Very early on during symptoms, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody ELISA assay 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 timeframe. For the secondary infection, a low fraction of anti-Dengue IgM and a high fraction of IgG that are broadly reactive to flaviviruses characterize the

antibodies. The IgM signal may be faint and some cross reactivity in the IgG line may appear.

- 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.

Performance Characteristics:

Expected Results:

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 an increase in dengue-specific IgG. In the majority of the cases, this is accompanied by elevated levels of IgM.

Correlation:

Sensitivity and Specificity

The Dengue Dual Ag Ab Device (Whole Blood/Serum/Plasma) was tested by sensitivity and specificity using a seroconversion panel and with samples from a population of symptomatic and asymptomatic subjects. Results were confirmed using a leading commercial Dengue Antigen EIA test for Dengue NS1 and leading commercial ELISA tests for anti-Dengue IgG and IgM.

The results showed that for NS1 the relative sensitivity of the Dengue Dual Ag Ab Device is 95.8%, and the relative specificity is 96.1%.

Dengue NS1

Method		Dengue Ag EIA Test		Total Result
	Results	Positive	Negative	
Dengue Dual Ag Ab Device	Positive	137	8	145
	Negative	6	200	206
Relative Sensitivity		143	208	351

Relative sensitivity: = 95.8% (95%CI* : 91.1% ~ 98.4%);
Relative specificity: = 96.1% (95%CI*:92.6% ~ 98.4%);
Accuracy: = 96.0% (95%CI*:93.4% ~ 97.8) *Confidence Intervals

The overall sensitivity for primary and secondary infection of the Dengue Dual Ag Ab Rapid Test Device is 94.3%, the relative specificity is 99.1% and the relative accuracy is 98.3%.

Dengue IgG/IgM

Dengue Primary Infection

Method			ELISA		
	Results		Positive		Negative
			IgM	IgG	
Dengue Dual Ag Ab Device	Positive	IgM	20	0	0
		IgG	4	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%		

Dengue Secondary Infection

Method			ELISA		
	Results		Positive		Negative
			IgM	IgG	
Dengue Dual Ag Ab Device	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			71.9%	98.4%	

Non-Dengue Infection

Method			ELISA		
	Results		Positive		Negative
			IgM	IgG	
Dengue Dual Ag Ab Device	Positive	IgM	0	0	1
		IgG	0	0	3
	Negative		0	0	429
Relative Sensitivity					99.1%

Relative sensitivity: = 94.3% (95%CI* : 87.2% ~ 98.1%);
Relative specificity: > 99.1% (95%CI* : 97.7% ~99.7%);
Accuracy: = 98.3% (95% CI* : 96.7% ~ 99.2%). * Confidence Intervals

Precision

Intra-Assay

Intra-Assay precision was determined using 15 replicates of four samples: a negative, a low positive, a middle positive and a high positive. The samples were correctly identified > 99% of the time.

Inter-Assay

Inter-Assay precision was determined by 15 independent assays using the same four samples. Three different lots of the Dengue Dual Ag Ab Device were tested using these samples. The samples were correctly identified > 99% of the time.

Cross-reactivity

The Dengue Dual Ag Ab Device has been tested using 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 of these samples in the test.

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 Creatine: 200mg/dl Caffeine: 20mg/dl
Gentisic Acid: 20mg/dl Haemoglobin: 1000mg/dl Albumin: 2g/dL
Oxalic Acid: 60mg/dl

None of the substances interfered in the Dual Ag Ab Device assay at the concentrations indicated.

References

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

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