Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) Package Insert

REF IDEN-402 | English

A rapid test for the qualitative detection of antibodies (IgG and IgM) to Dengue virus in whole blood, serum, or plasma.

For professional in vitro diagnostic use only.

【INTENDED USE】

The Dengue Rapid Test Cassette (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. 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 Cassette (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 Cassette (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 IgG test line region. During testing, the specimen reacts with Dengue antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region. If the specimen contains IgG antibodies to Dengue, a colored line will appear in IgG test line region. In the IgM component, anti-human IgM is coated in IgM test line region. During testing, the specimen reacts with anti-human IgM. Dengue IgM antibodies, if present in the specimen, reacts with the anti-human IgM and the Dengue antigen-coated particles in the test cassette, and this complex is captured by the anti-human IgM, forming a colored line in IgM test line region.

Therefore, if the specimen contains Dengue IgG antibodies, a colored line will appear in IgG test line region. If the specimen contains Dengue IgM antibodies, a colored line will appear in IgM test line region. 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 appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

The test cassette contains Dengue antigen conjugated gold colloid particles and anti-human IgM, anti-human IgG coated on the membrane.

【PRECAUTIONS】

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure 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 assayed.
- 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 cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

【SPECIMEN COLLECTION AND PREPARATION】

- The Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood, serum, or plasma.
- To collect **Fingerstick Whole Blood Specimens:**
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Add the Fingerstick Whole Blood specimen to the test cassette by using a dropper or micropipette measuring 10 µL. The dropper provided with the test dispenses approximately 10 µL in one drop even if more blood is aspirated in the dropper.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens 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 specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.
- EDTA K2, Heparin sodium, Sodium citrate and Potassium oxalate can be used as the anticoagulant for collecting the specimen.

【MATERIALS 】

Materials provided

- Test cassettes
- Buffer
- Droppers
- Package insert

Materials required but not provided

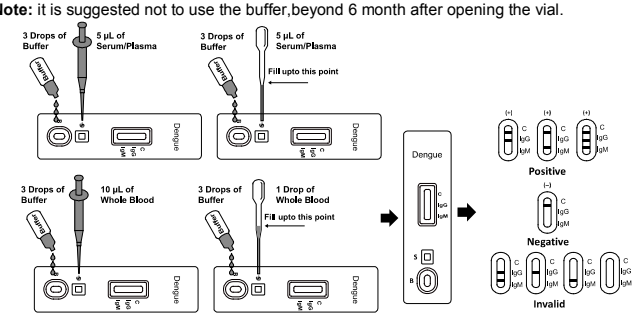
- Specimen collection containers
- Micropipette
- Lancets (for fingerstick whole blood only)
- Centrifuge (for plasma only)
- Timer

【DIRECTIONS FOR USE】

Allow the test cassette, specimen, 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 cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface.
   - For Serum or Plasma Specimens:
     - To use a dropper: Hold the dropper vertically, draw the specimen up to the Fill Line **(approximately 5 µL)**, and transfer the specimen to the specimen well **(S)** of the test cassette , then add **3 drops of buffer (approximately 120 µL)** into the buffer well **(B)** and start the timer. Avoid trapping air bubbles in the specimen well **(S)**.
     - To use a micropipette: Pipette and dispense **5 µL of specimen** to the specimen well **(S)** of the test cassette, then add **3 drops of buffer (approximately 120 µL**) into the buffer well **(B)** and start the timer.
   - For Whole Blood (Venipuncture/Fingerstick) Specimens:
     - To use a dropper: Hold the dropper vertically, draw the specimen about 1cm above the Fill Line, and transfer **1 drop of whole blood (approximately 10 µL)** to the specimen well **(S)** of the test cassette, then add **3 drops of buffer (approximately 120 µL)** into the buffer well **(B)** and start the timer.
     - To use a micropipette: Pipette and dispense **10 µL of whole blood** to the specimen well **(S)** of the test cassette, then add **3 drops of buffer (approximately 120 µL)** into the buffer well **(B)** and start the timer.
3. Wait for the colored line(s) to appear. The test result should be **read at 10 minutes**. Do not interpret the result after 20 minutes.

Note: it is suggested not to use the buffer,beyond 6 month after opening the vial.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

IgG and IgM POSITIVE:* Three colored lines appear. One colored line should be in the control line region (C), and two colored lines should appear in IgG test line region and IgM test line region. The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies indicated end stage of primary Dengue infection and early stage of secondary Dengue infection.

IgG POSITIVE:* Two colored lines appear. One colored line should be in the control line region (C), and a colored line appears in IgG test line region. The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.

IgM POSITIVE:* Two colored lines appear. One colored line should be in the control line region (C), and a colored line appears in IgM test line region. The result is positive for Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.

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

NEGATIVE: **One colored line should be in the control line region (C)**. No line appears in IgG and IgM test line region(s).

INVALID: **Control line fails to appear**. 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 cassette. 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 appearing in the control line region (C) is an internal valid procedural control, 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】

1. The Dengue Rapid Test Cassette (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 Cassette (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. It is recommended that patients be tested within this time. 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.
4. 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.
5. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
6. Results from immunosuppressed patients should be interpreted with caution.
7. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
8. 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.
9. The hematocrit of the whole blood should be between 25% and 65%.

【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 Cassette (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 Rapid Test Cassette (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.

The results show that the overall relative sensitivity for the primary and secondary infection of the Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) is 94.3%, and the relative specificity is 99.1%,and the relative accuracy is 98.3%.

Dengue Primary Infection for IgM/IgG test results

Method			ELISA		
Dengue Rapid Test Cassette (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 for IgM/IgG test results

Method			ELISA		
Dengue Rapid Test Cassette (Whole Blood/ Serum/Plasma)	Results		Positive		negative
			IgM	IgG	
	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			71.9%	98.4%	/

Non-Dengue Infection for IgM/IgG test results

Method			ELISA		
Dengue Rapid Test Cassette (Whole Blood/ Serum/Plasma)	Results		Positive		negative
			IgM	IgG	
	Positive	IgM	0	0	1
		IgG	0	0	3
	Negative		0	0	429
Relative Specificity			/	/	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 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 15 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 Cassette (Whole Blood/Serum/Plasma) have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

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

Interfering Substances

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

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

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

【BIBLIOGRAPHY】

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Index of Symbols

	Caution		Tests per kit		EU authorized representative
	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between 2-30°C		Lot number		Catalog #
	Do not use if package is damaged		Manufacturer		Consult instructions for use

Hangzhou AllTest Biotech Co.,Ltd.
#550,Yinhai Street
Hangzhou Economic & Technological Development Area
Hangzhou, 310018 P.R. China
Web: www.alltests.com.cn Email: info@alltests.com.cn

EC REP
MedNet EC-REP GmbH
Borkstrasse 10,
48163 Muenster,
Germany

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