Chuangxingwell

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

CE

A rapid test for a qualitative test for the detection of NS1 antigen, IgG and IgM antibodies of dengue virus in human whole blood, serum or plasma.
For professional in vitro diagnostic use only.

IVD

【INTENDED USE】

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

NS1 is one of 7 Dengue Virus non-structural proteins which are thought to be involved in viral replication. NS1 exists as a monomer in its immature form but is rapidly processed in the endoplasmic reticulum to form a stable dimer. A small amount of NS1 remains associated with intracellular organelles where it is thought to be involved in viral replication. The rest of NS1 is found either 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 Hemorrhagic fever.

The Dengue Combo Rapid Test Kit (Whole Blood/Serum/Plasma) is a rapid test that utilizes a combination of NS1 antigen, IgG and IgM antibodies of dengue virus in human whole blood, serum or plasma.

【PRINCIPLE】

The Dengue IgG/IgM Rapid Test (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 Kit. 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 Kit, 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 and/or IgM antibodies, a colored line will appear in IgG and/or 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.

The Dengue NS1 Rapid Test (Whole Blood/Serum/Plasma) 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 Dengue antibody-conjugate in the test Kit. The Gold-antibody conjugate will bind to Dengue antigen in the specimen sample which in turn will bind with Anti-Dengue NS1 coated on the membrane. As the reagent moves across the membrane, the Dengue NS1 antibody on the membrane will bind the antibody-antigen complex causing colored line to form at the test line region of the test membrane. The intensity of the lines will vary depending upon the amount of antigen present in the sample. The appearance of colored line in the test region should be considered as positive result.

【REAGENTS】

The Dengue IgG/IgM Rapid Test contains Dengue antigen conjugated gold colloid particles, anti-human IgM, anti-human IgG coated on the membrane.
The Dengue NS1 Rapid Test contains anti-Dengue conjugated colloid particles, anti-Dengue coated on the membrane.

【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.
- 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). with a valid period of 24 months. The test kit is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. Use the test kit within 1 hour once the foil pouch is opened. **DO NOT FREEZE**.

【SPECIMEN COLLECTION AND PREPARATION】

- The Dengue Combo Rapid Test Kit (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 Kit by using a 5 µL dropper or micropipette measuring 10 µL for IgG/IgM test and a 25 µL dropper or micropipette measuring 75 µL for NS1 test. The 5 µL or 25 µL droppers provided with the test dispenses approximately 10 µL or 25 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 local 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
- Droppers
- Buffer
- Package Insert

Materials Required But Not Provided

- Specimen Collection Containers
- Centrifuge
- Micropipette
- Timer
- Lancets (for fingerstick whole blood only)

【DIRECTIONS FOR USE】

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

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within 1 hour.
2. Place the cassette on a clean and level surface.

➢ For **Serum or Plasma specimen**:

For IgG/IgM:

- **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) to the **buffer well** (B) and start the timer. Avoid trapping air bubbles in the specimen well.
- **To use a micropipette:** Pipette and dispense **5 µL of serum or plasma** to the **specimen well** (S) of the test cassette, then add **3 drops of buffer** (approximately 120 µL) to the **buffer well** (B) and start the timer.

For NS1:

- Hold the dropper vertically and transfer **3 drops of serum or plasma** (approximately 75 µL) to the specimen well (S), and start the timer. See illustration below.

➢ For **Whole Blood(Venipuncture/Fingerstick)** specimen:

For IgG/IgM:

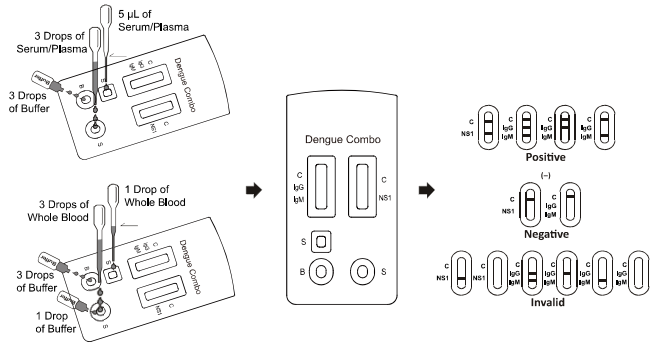
- **To use a dropper**: Hold the dropper vertically, draw the specimen up to about 1cm above the fill line, and transfer **1 drop of whole blood** (approximately 10 µL) to the **specimen well** (S) of the test cassettes, then add **3 drops of buffer** (approximately 120 µL) to the **buffer well** (B) 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 cassette, then add **3 drops of buffer** (approximately 120 µL) to the **buffer well** (B) and start the timer. See illustration below.

For NS1:

- **To use a dropper**: Hold the dropper vertically and transfer **3 drops of whole blood** (approximately 75 µL) to the specimen well (S), then add **1 drop of buffer** (approximately 40 µL) and start the timer. See illustration below.
- **To use a capillary tube**: Fill the capillary tube and transfer approximately 75 µL of fingerstick whole blood specimen to the specimen well (S) of test cassette, then add **1 drop of buffer** (approximately 40 µL) and start the timer. See illustration below.

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

Note: It is suggested not to use the buffer beyond 6 months after opening the vial.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

NS1 POSITIVE: * **Two colored lines appear.** One colored line should be in the control region (C) and another colored line should be in the test region (NS1).

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 test line region (NS1 and/or IgG and/or IgM) will vary depending on the concentration of Dengue NS1 antigen and/or IgG and/or IgG present in the specimen. Therefore, any shade of red in the test region should be

considered positive.

NEGATIVE: One colored line appears in the control region (C). No line appears in the test region (IgG/IgM/NS1).

INVALID: **Control line fails to appear.** Insufficient specimen 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 cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

【QUALITY CONTROL】

A procedural control is included in the test. A colored line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that a positive control and a negative control be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

1. The Dengue Combo Rapid Test Kit (Whole Blood/Serum/Plasma) will only indicate the presence of Dengue NS1 antigen and Dengue antibodies in the specimen and should not be used as the sole criteria for the diagnosis of Dengue.
2. The DIRECTIONS FOR USE and the INTERPRETATION OF RESULTS must be followed closely when testing the presence of dengue antibody/antigen in whole blood, serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
3. A negative test result for Dengue NS1 does not preclude the possibility of exposure to or infection with dengue viruses.
4. A negative result for Dengue NS1 can occur if the quantity of dengue antigen present in the specimen is below the detection limits of the assay, or the dengue antigen that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptom persists, while the result from Dengue NS1 Rapid Test is negative or non-reactive result, it is recommended to re-sample the patient few days later or test with an alternative test device such as PCR, ELISA.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.
8. In the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked immune sorbent assay (MACELISA) 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.
9. 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.
10. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
11. Results from immunosuppressed patients should be interpreted with caution.
12. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
13. 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.
14. The hematocrit of the whole blood should be between 25% and 65%.

【PERFORMANCE CHARACTERISTICS】

Sensitivity and Specificity

The Dengue Combo Rapid Test Kit (Whole Blood/Serum/Plasma) has passed a seroconversion panel and compared with a leading commercial Dengue Ag ELISA test using clinical specimens for Dengue NS1 and has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test for IgG and IgM. The results show that the relative sensitivity of the Dengue NS1 Rapid Test Kit (Whole Blood/Serum/Plasma) is 95.8%, and the relative specificity is 96.1%.And the overall relative sensitivity for the primary and secondary infection of the Dengue Rapid Test (Whole Blood/Serum/Plasma) is 94.3%, and the relative specificity is 99.1%,and the relative accuracy is 98.3%.

Dengue IgG/IgM

Dengue Primary Infection for IgM/IgG test results

Method			ELISA		
Dengue Rapid Test (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 (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 (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

Dengue NS1

Method		Dengue Ag ELISA Test		Total Results
Dengue NS1 Rapid Test (WholeBlood/Serum/Plasma)	Results	Positive	Negative	
	Positive	137	8	145
	Negative	6	200	206
Total Results		143	208	351

Relative sensitivity: 137/143*100%=95.8% (95%CI*: 91.1%~98.4%);
Relative specificity: 200/208*100%=96.1% (95%CI*: 92.6%~98.4%);
Accuracy: (137+200)/(137+6+8+200)*100%=96.0%(95%CI*:93.4%~97.8%).
*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of Dengue specimens. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same Dengue specimens. Three different lots of the Dengue Combo Rapid Test Kit (Whole Blood/Serum/Plasma) have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Dengue Combo Rapid Test Kit (Whole Blood/Serum/Plasma) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, anti-Syphilis, anti-HIV, HCV, *anti-H.Pylori*, anti-MONO, anti-CMV, anti-Rubella and anti-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	Acetylsalicylic Acid: 20 mg/dL	Ascorbic Acid: 2 g/dL
Bilirubin: 1 g/dL	Creatin: 200 mg/dL	Caffeine: 20 mg/dL
Gentisic Acid: 20 mg/dL	Hemoglobin 1000 mg/dL	Albumin: 2 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	IVD	In Vitro Diagnostic Medical Device
	Manufacturer		Date of Manufacture
	CE Marking		Do Not Re-use
	Keep Dry		Keep Away From Sunlight
LOT	Batch Code		Do Not Use if Package is Damaged
REF	Catalogue Number		Contains Sufficient for <n> Tests
	Use-By Date		Temperature Limit
EC REP	Authorized representative in the European Community		

Manufacturer
Jinan Chuangxingwell Biotech Co., Ltd.
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