AyoTes

Instruction For Use

Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma)

REF: IDAT-AS2023005

For professional *in vitro* diagnostic use only.

INTENDED USE

AyoTes Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Dengue virus (DENV) NS1 antigen, anti-DENV IgG and anti-Dengue IgM in human whole blood, serum, or plasma as an aid in the diagnosis of primary and secondary Dengue infections.

INTRODUCTION

Dengue viruses, transmitted by the mosquito, *Aedes aegypti* and *Aedes albopictus* mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world. There are four known distinct serotypes (DENV 1, 2, 3 and 4). In children, infection is often subclinical or causes a self-limited febrile disease. However, second infections with another serotype may lead to a more severe disease, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes.

NS1 is a highly conserved glycoprotein that is present at high concentrations in the sera of dengue-infected patients during the early clinical phase of the disease. NS1 antigen is found from the first day. and up to 9 days after onset of fever in sample of primary or secondary dengue infected patients. Usually IgM does not become detectable until 5 to 10 days after the onset of illness in cases of primary dengue infection and until 4 to 5 days after onset of illness in secondary infections. In primary infections, IgG appears from the 14th day and persist for life. Secondary infections show that IgG rises within 1~2 days after the onset of symptoms and induce IgM response after 20 days of infection.

The Dengue IgG/IgM + NS1 Combo Rapid Test device (Whole Blood/Serum/Plasma) is composed of two test strips: 1) Dengue NS1 Ag test strip and 2) Dengue IgG/IgM test strip. Dengue NS1 Ag test strip utilizes specific monoclonal antibodies to detect Dengue NS1, and Dengue IgG/IgM test strip utilizes Dengue viral antigen coupled with colored particles and anti-human IgG/IgM antibody to detect anti-DENV IgG and IgM in human whole blood, serum, or plasma.

PRINCIPLE

AyoTes Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma) is composed of two test strips: 1) Dengue NS1 Ag test strip and 2) Dengue IgG/IgM test strip.

The Dengue NS1 Ag Test detects Dengue NS1 antigens through visual interpretation of color development. Anti-dengue NS1 antibodies are immobilized at the test region (T) on the membrane. During testing, Dengue NS1 antigens, if present in the sample of whole blood/serum/plasma, will bind to anti-dengue NS1 antibodies conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-dengue NS1 antibodies at the test region. Excess colored particles are captured at the internal control zone.

The presence of a colored band at the test region (T) indicates a positive result for the particular antigens, while its absence indicates a negative result. A colored band at the control region (C) serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

The Dengue IgG/IgM Test detects IgG and IgM to DENV through visual interpretation of color development. DENV antigens, anti-human IgG and anti-human IgM are used to detect specific antibodies in the human blood, serum, or plasma samples. When a sample is added to the sample well on the test panel, specific IgG and/or IgM antibodies, if present, will bind to the DENV antigens conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-human IgG and/or anti-human IgM antibodies immobilized at the detection zone.

The presence of a colored band(s) in the test region indicates a positive result for the particular IgG and/or IgM antibodies, while its absence indicates a negative result. A red band at the control region (C) serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

MATERIALS

Materials Provided

- Individually packed test devices
- 5μLdisposable pipettes for Dengue IgG/IgM
- 25μLdisposable pipettes for Dengue NS1
- Package insert
- Buffer

Materials Required but Not provided

- Specimen collection container
- Centrifuge
- Timer
- Lancets

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use the kit beyond 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。

STORAGE 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 it if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- AyoTes Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma) is intended for use with human whole blood, serum or plasma specimens only.
- Collect specimens according to safe phlebotomy procedure.
- 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.
- Icteric, lipemic, hemolysed, heat treated and contaminated specimens may cause erroneous results.
- 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°C for up to 7 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 3 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, oxalate, or heparin may be used for plasma preparation.
- Bring specimens to room temperature prior to testing. Frozen serum or plasma specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of serum or plasma specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

PROCEDURE

Allow the test device, 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 device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface.

For <u>Serum or Plasma Specimens</u>:

a) Using the provided 25μL disposable pipette, transfer 3 drops of serum or plasma to the specimen well (S1), then start the timer.

b) Using the provided 5μL disposable pipette, draw the specimen <u>up to the Fill Line</u>, and transfer all the specimen (approximately 5 μL), to the specimen well (S2) of the test device, then add 2 drops of buffer and start the timer.

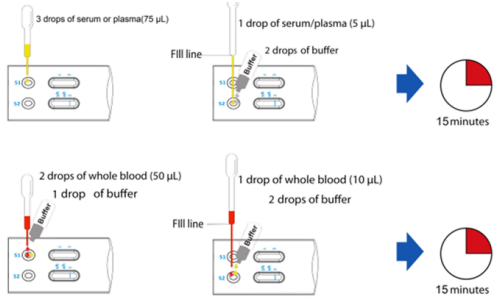
For <u>Whole Blood (Venipuncture/Fingerstick) Specimens</u>:

a) Using the provided 25μL disposable pipette, transfer 2 drops of whole blood to the specimen well (S1), and add 1 drop of buffer, then start the timer.

b) Using the provided 5μL disposable pipette, draw the specimen <u>above the Fill Line</u> (avoid the specimen entering the bubble of disposable pipette), and transfer 1 drop of whole blood (approximately 10 μL) to the specimen well (S2) of the test device, then add 2 drops of buffer and start the timer.

Note: Specimens can also be applied using a micropipette.

3 Wait for the colored line(s) to appear. Read results at 15 minutes. Do not interpret the result after 30 minutes.



INTERPRETATION OF RESULTS

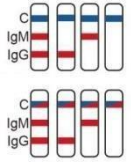
For Dengue IgG/IgM Test

**IgM POSITIVE: ***The colored line in the control region (C) changes from blue to red, and a colored line appears at the IgM test region. The result is positive for Dengue virus specific-IgM antibodies.

**IgG POSITIVE: ***The colored line in the control region (C) changes from blue to red, and a colored line appears at the IgG test region. The result is positive for Dengue virus specific-IgG antibodies.

IgM AND IgG POSITIVE: * The colored line in the control region (C) changes from blue to red, and two-colored lines should appear at the IgM and IgG test regions. The result is positive for both IgM and IgG antibodies.

NEGATIVE: The colored line in the control region (C) changes from blue to red. No line appears at the IgM or IgG test regions.

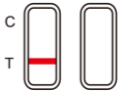


INVALID: Control line (C) is still completely or partially blue and fails to completely change from blue to red. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

For Dengue NS1 Ag Test

POSITIVE: Two colored bands appear on the membrane. One band appears at the control region (C) and another band appears at the test region (T).

NEGATIVE: Only one colored band appears at the control region (C). No colored band appears at the test region (T).



INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in the test line region(s) will vary depending on the concentration of Dengue antibodies or Dengue virus NS1 antigen in the specimen. Therefore, any shade of color in the test line region(s) should be considered positive.
2. A negative result can occur if the quantity of target antigen or antibody present in the specimen is below the detection limits of the assay, or the target antigen or antibody are not present during the stage of disease in which a sample is collected.
3. A negative test result cannot exclude a recent infection.
4. The presence of detectable Dengue virus NS1 Ag may mean positive for early Dengue infection. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

QUALITY CONTROL

An internal procedural control is included in the test.

For Dengue IgG/IgM antibody test, acolored line changes from blue to red in the control line region (C), confirming sufficient buffer volume and adequate membrane wicking.

For Dengue virus NS1 antigen test, a colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural

technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. A negative result for NS1 Ag test can occur if the quantity of Dengue virus NS1 antigen present in the specimen is below the detection limits of the assay, or the antigens are not present during the stage of disease in which a sample is collected.
2. The presence of detectable Dengue virus NS1 Ag may mean positive for early Dengue infection. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
3. The NS1 is expected to be detected 1 day after the onset of fever and persist up to 9 days in both primary and secondary dengue infection. But if anti-NS1 antibodies are produced, the detection of NS1 is inhibited.
4. The AyoTes Dengue IgG/IgM & NS1 Rapid Test (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.
5. The AyoTes Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma) will only indicate the presence of Dengue antibodies or antigen in the specimen and should not be used as the sole criteria for the diagnosis of Dengue.
6. 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 tenth day. It is recommended that patients be tested within this time.
7. 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. The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
8. Serological cross-reactivity with other virus species of the *Flavivirus* group (e.g., St. Louis, encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common. Positive results should be confirmed by other means.
9. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy. Results from immune-suppressed patients should be interpreted with caution.
10. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
11. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity and Specificity
The AyoTes Dengue IgG/IgM & NS1 Rapid Test (Whole Blood/Serum/Plasma) has been evaluated with clinical specimens. Results for anti-Dengue IgG or IgM detection were compared with ELISA, and results for Dengue NS1 Ag detection were compared with cell culture.

For Dengue IgM detection, :

			Dengue IgM ELISA		
			Positive	Negative	Total
Relative sensitivity: 98.8%(93.4%～99.8%)* Relative specificity: 100.0%(97.6%～100.0%)* Overall agreement: 99.6%(97.7%～99.9%) *95% Confidence Interval	**Dengue IgG/IgM + NS1 Ag Combo Rapid Test**	Positive	81	0	81
		Negative	1	158	159
	Total		82	158	240

For Dengue IgG detection:

			Dengue IgG ELISA		
			Positive	Negative	Total
Relative sensitivity: 98.9%(94.0%～99.8%)* Relative specificity: >99.9%(97.5%～100.0%)* Overall agreement: 99.6%(97.7%～99.9%)* *95% Confidence Interval	**Dengue IgG/IgM + NS1 Ag Combo Rapid Test**	Positive	89	0	89
		Negative	1	150	151
	Total		90	150	240

For Dengue NS1 Ag detection:

			Culture		
			Positive	Negative	Total
Relative sensitivity: 92.9%(86.5%～96.3%)* Relative specificity: 98.4%(95.4%～99.5%)* Overall agreement: 96.3%(93.6%～97.9%)* *95% Confidence Interval	**Dengue IgG/IgM + NS1 Ag Combo Rapid Test**	Positive	104	3	107
		Negative	8	186	194
	Total		112	189	301

Precision
Repeatability-Assay
Repeatability has been determined by using 10 replicates of specimens below: a negative, NS1 positive, IgG positive, IgM positive. The positive specimen includes low positive and high positive. The specimens were correctly identified >99% of the time.
Reproducibility-Assay
Reproducibility has been determined by three independent assays on specimens below: a negative, NS1 positive, IgG positive and IgM positive. The positive specimen includes low positive and high positive. Three different lots were tested using these specimens by 3 operators at three sites over 3 days. The specimens were correctly identified >99% of the time.

LITERATURE REFERENCES

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APPROVAL/REVISION DATE OF PRODUCT INSERT

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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