Rapid Response™

Dengue IgG/IgM + NS1 Ag Combo Test Cassette

(Whole Blood/Serum/Plasma)

REF DENNS1MG-13C25 Product Insert

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Dengue virus (DENV) NS1 antigen, anti-DENV IgM and anti-Dengue IgG in human whole blood, serum, or plasma as an aid in the diagnosis of 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. In primary infections, IgG appears from the 14th day and persists for long times. Secondary infections show that IgG rises within 1~2 days after the onset of symptoms

The Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test Cassette (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 NS1 antigen coupled with colored particles and anti-human IgG/IgM antibody to detect anti-DENV IgM and IgG in human whole blood, serum, or plasma.

Principle

The Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test Cassette (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 Strip detects Dengue NS1 antigens through visual interpretation of color development. Anti-dengue NS1 antibodies are immobilized on the test region (T) of the membrane. During testing, Dengue NS1 antigens, if present in specimen, will bind to anti-dengue NS1 antibodies conjugated to colored particles on the conjugate 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 red band in the test region (T) indicates a positive result for the particular antigens, while its absence indicates a negative result. A red band at the control region (C) serves as a procedural control, indicating that membrane wicking is working.

The Dengue IgG/IgM Test Strip detects IgM and IgG 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 whole blood, serum, or plasma samples. When specimen is added to the sample well, specific IgM and/or IgG antibodies, if present, will bind to the DENV antigens conjugated to colored particles on the conjugate 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 IgM and/or anti-human IgG antibodies immobilized on the test region(s). Excess colored particle are captured at the internal control region.

The presence of a red band(s) on the test region(s) 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 membrane wicking is working.

Materials

Materials provided

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

Materials required but not provided

- Specimen collection container
- Centrifuge
- Micropipette
- 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 if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

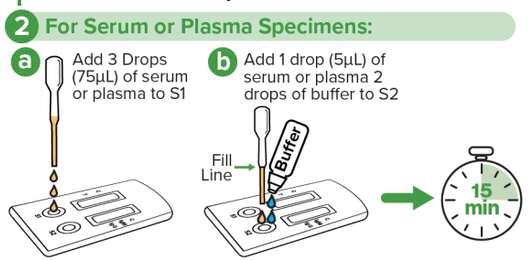
Specimen Collection and Storage

- The Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test Cassette (Whole Blood/Serum/Plasma) is intended for use with human whole blood, serum or plasma specimens only.
- 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.
- 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, serum or plasma 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 after collection. Do not freeze whole blood specimens.
- Containers containing anticoagulants such as EDTA, citrate, heparin or oxalate should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen serum or plasma specimens must be completely thawed and mixed well prior to testing. Avoid repeat-ed freezing and thawing of specimens.
- If specimens 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.

Procedure

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

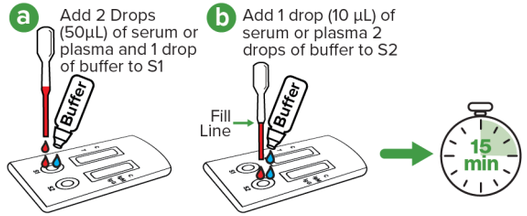
1. Bring the pouch to room temperature before opening. Remove the test device from the sealed pouch and use it as soon as possible. Place the test device on a clean and level surface. Label the test with patient or control identification.
2. **For Serum or Plasma Specimens:**



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 up to the Fill Line, 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 Whole Blood (Venipuncture/Fingerstick) Specimens:



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.

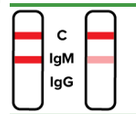
b. Using the provided 5µL disposable pipette, draw the specimen above the Fill Line (avoid the specimen entering the bubble of disposable pipette), and transfer 1 drop of whole blood (approximately 10 µL) to the specimen well (S2) of the test device, then add 2 drops of buffer and start the timer.

Note: Specimens can also be applied using a micropipette.

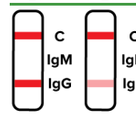
3. Wait for the colored line(s) to appear. Read results at 15 minutes. Do not interpret the result after 30 minutes.

Results Interpretation

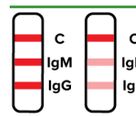
For Dengue IgG/IgMTest



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



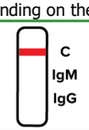
IgG Positive: *The colored line in the control region (C) changes from blue to red, and a colored line appears in the IgG test region. The result is positive for Dengue virus specific-IgG antibodies.



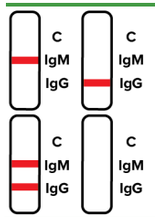
IgM and IgG Positive: *The colored line in the control region (C) changes from blue to red, and two colored lines should appear in IgG and IgM test regions. The color intensities of the lines do not have to match. The result is positive for IgM and IgG antibodies.

***NOTE:**

The intensity of the color in the IgG and/or IgM test region(s) will vary depending on the concentration of Dengue antibodies in the specimen.

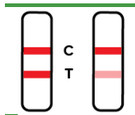


Negative: The colored line in the control region (C) changes from blue to red. No line appears in IgM or IgG test regions.



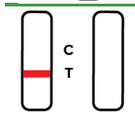
Invalid: Control line (C) is still completely or partially blue and fails to completely change from blue to red. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If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

For Dengue NS1 Test



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

Negative: Only one colored band appears, in the control region (C). No apparent colored band appears in 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:**
The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint pink line.

Quality Control

- An internal procedural control is included in the test. A colored line changes from blue to red in the control line region (C), confirming adequate membrane wicking.
- External controls 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. 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 Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo 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 Dengueanti-body concentration can be determined by this qualitative test.
4. The Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo 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.
5. In the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked im-munosorbent 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.
6. 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 anti-bodies.The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
7. 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.
8. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
9. Results from immunosuppressed 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 Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test Cassette (Whole Blood/Serum/Plasma) has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals.

For Dengue IgM test:

		Dengue IgM ELISA		
		+	-	Total
Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test	+	59	0	59
	-	1	140	141
	Total	60	140	200

Relative sensitivity: 98.3%(91.1%～99.7%)*
Relative specificity: >99.9%(97.3%～100.0%)*
Overall agreement: 99.5%(97.2%～99.9%)*
*95% Confidence Interval

For Dengue IgG test:

		Dengue IgG ELISA		
		+	-	Total
Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test	+	89	0	89
	-	1	150	151
	Total	90	150	240

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

For NS1 Ag test:

		Culture		
		+	-	Total
Rapid Response™ Dengue IgG/IgM + NS1 Ag Combo Test	+	104	3	107
	-	8	186	194
	Total	112	189	301

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

Bibliography

1. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue. Rev. Infect. Dis. 1984; 6:251-264
2. Halstead SB, Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239:476-481
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
5. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization
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. MicrobiolImmunol. 1994; 38(12): 951-5.

Glossary of Symbols

	Consult instructions for use	Σ	Test per Kit	REF	Catalogue #
35.6°F 2°C / 86°F 30°C	Store between 35.6°F to 86°F		Use by		Do Not Reuse
LOT	Lot Number	IVD	For *in vitro* diagnostic use only		Manufacturer
EC REP	Authorized Representative				

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

BTNX Inc.
722 Rosebank Road,
Pickering, ON L1W 4B2
Canada

Technical Support: 1-888-339-9964