SHD
SCIENCE HEALTH DIAGNOSTICS

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25T

# SHD Dengue IgG/IgM & NS1 Combo Rapid Test Cassette

**(Whole Blood / Serum / Plasma)**

## INTENDED USE

SHD Dengue IgG/IgM & NS1 Combo Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of dengue virus NS1 antigen and of IgG and IgM antibodies to dengue virus in human whole blood, serum or plasma. The test is intended as an aid in the diagnosis of dengue virus infection and in the differentiation of primary and secondary infection. For professional in vitro diagnostic use only.

## PACKAGING SPECIFICATIONS

REF 960005 — 25T (25 individually pouched test cassettes per kit)

## SUMMARY AND EXPLANATION

Dengue virus 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 (Halstead, 1984; Halstead, 1988). Classic dengue infection is characterised by a sudden onset of fever, intense headache, myalgia, arthralgia and rash.

In primary dengue infection, IgM antibodies rise to a detectable level 3 to 5 days after the onset of fever and generally persist for 30 to 90 days (Ruechusatsawat et al., 1994; Lam, 1995). Most dengue patients in endemic regions have secondary infections, which result in high levels of specific IgG antibodies before or simultaneously with the IgM response (World Health Organization, 1997). The detection of specific anti-dengue IgM and IgG antibodies therefore also helps to distinguish primary from secondary infection.

NS1 is one of the seven non-structural proteins of dengue virus and is thought to be involved in viral replication. NS1 exists as a monomer in its immature form and is rapidly processed in the endoplasmic reticulum into a stable dimer. A small amount of NS1 remains associated with intracellular organelles; the rest is found either associated with the plasma membrane or secreted as a soluble hexamer. NS1 is essential for viral viability, although its precise biological function is unknown. Antibodies raised against NS1 during 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. NS1 antigen is common to all four dengue serotypes and is detectable from the first day of fever in both primary and secondary infection.

SHD Dengue IgG/IgM & NS1 Combo Rapid Test Cassette combines NS1 antigen detection with IgG and IgM antibody detection on a single cassette, covering both the early (antigen) and the later (antibody) phase of dengue infection.

## PRINCIPLE OF TESTING

**IgG/IgM component.** The IgG/IgM strip is a qualitative membrane-based immunoassay. Anti-human IgG is coated in the IgG test line region and anti-human IgM is coated in the IgM test line region. During testing, the specimen reacts with dengue antigen-coated colloidal gold particles in the cassette. The mixture migrates along the membrane by capillary action. Dengue-specific IgG in the specimen is captured by the anti-human IgG and forms a coloured line in the IgG test line region; dengue-specific IgM is captured by the anti-human IgM and forms a coloured line in the IgM test line region. If the specimen contains no dengue antibodies, no coloured line appears in either test line region.

**NS1 component.** The NS1 strip is a qualitative membrane-based immunoassay. During testing, NS1 antigen in the specimen binds to colloidal gold-conjugated anti-dengue NS1 antibody. The complex migrates along the membrane and is captured by anti-dengue NS1 antibody immobilised in the test line region (NS1), forming a coloured line. The intensity of the line varies with the amount of antigen present in the specimen.

**Procedural control.** A coloured line always appears in the control line region (C) of each strip, indicating that the proper volume of specimen has been added and that membrane wicking has occurred.

## MAIN COMPONENTS

| No. | Component | Description |
|---|---|---|
| 1 | Test cassette | 25 test cassettes, each sealed in a foil pouch with desiccant |
| 2 | Buffer | 1 vial |
| 3 | Droppers | Disposable specimen droppers (5 µL and 25 µL) |
| 4 | Instructions for use | 1 copy |

## STORAGE AND STABILITY

Store the kit at room temperature or refrigerated (2 – 30 °C). The test cassette is stable through the expiration date printed on the sealed pouch and must remain in the sealed pouch until use. **Do not freeze.** Do not use the kit beyond the expiration date. Do not use the buffer later than 6 months after first opening the vial.

## MATERIAL REQUIRED BUT NOT PROVIDED

| No. | Material |
|---|---|
| 1 | Specimen collection containers |
| 2 | Centrifuge |
| 3 | Micropipette |
| 4 | Lancets (for fingerstick whole blood only) |
| 5 | Capillary tubes (optional, for fingerstick whole blood) |
| 6 | Timer |

## SPECIMEN COLLECTION AND STORAGE

The test can be performed with whole blood (venipuncture or fingerstick), serum or plasma. EDTA K2, sodium heparin, sodium citrate and potassium oxalate can be used as anticoagulants.

**Fingerstick whole blood collection:**

- Wash the patient's hand with soap and warm water or clean it 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 drop of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Apply the specimen with the 5 µL dropper or a micropipette (10 µL) for the IgG/IgM test, and with the 25 µL dropper or a micropipette (75 µL) for the NS1 test. The provided droppers dispense approximately 10 µL or 25 µL per drop even if more blood is aspirated.

**Transportation and Storage:** Separate serum or plasma from blood as soon as possible to avoid haemolysis; use only clear, non-haemolysed specimens. Test specimens immediately after collection and do not leave them at room temperature for prolonged periods. Serum and plasma may be stored at 2 – 8 °C for up to 3 days; for long-term storage keep them below -20 °C. Venipuncture whole blood should be stored at 2 – 8 °C if the test is run within 2 days of collection; do not freeze whole blood. Fingerstick whole blood should be tested immediately. Bring specimens to room temperature before testing; thaw frozen specimens completely and mix well, and avoid repeated freeze-thaw cycles. For shipment, package and label specimens in accordance with local and international regulations for the transport of aetiological agents.

## TEST PROCEDURE

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

1. **Preparation.** Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within 1 hour. Place the cassette on a clean and level surface.
2. **Adding the specimen (IgG/IgM strip).** Serum or plasma: hold the 5 µL dropper vertically, draw the specimen up to the fill line (approximately 5 µL) and transfer it to the specimen well (S) below the IgG/IgM window, or dispense 5 µL with a micropipette. Whole blood: draw the specimen to about 1 cm above the fill line and transfer 1 drop (approximately 10 µL), or dispense 10 µL with a micropipette. Then add 3 drops of buffer (approximately 120 µL) to the same specimen well (S). Avoid trapping air bubbles in the specimen well.
3. **Adding the specimen (NS1 strip).** Serum or plasma: hold the 25 µL dropper vertically and transfer 3 drops (approximately 75 µL) to the specimen well (S) below the NS1 window; no buffer is added. Whole blood: transfer 3 drops (approximately 75 µL) with the dropper, or approximately 75 µL of fingerstick blood with a capillary tube, to the specimen well (S), then add 1 drop of buffer (approximately 40 µL).
4. **Reading.** Start the timer and read the results at 10 minutes. Do not interpret the results after 20 minutes.

| Specimen | Strip / well | Specimen volume | Buffer |
|---|---|---|---|
| Serum / plasma | IgG/IgM — right well S | 5 µL (1 dropper fill) | 3 drops (≈ 120 µL) to the same well |
| Serum / plasma | NS1 — left well S | 3 drops (≈ 75 µL) | None |
| Whole blood | IgG/IgM — right well S | 1 drop (≈ 10 µL) | 3 drops (≈ 120 µL) to the same well |
| Whole blood | NS1 — left well S | 3 drops (≈ 75 µL) | 1 drop (≈ 40 µL) to the same well |

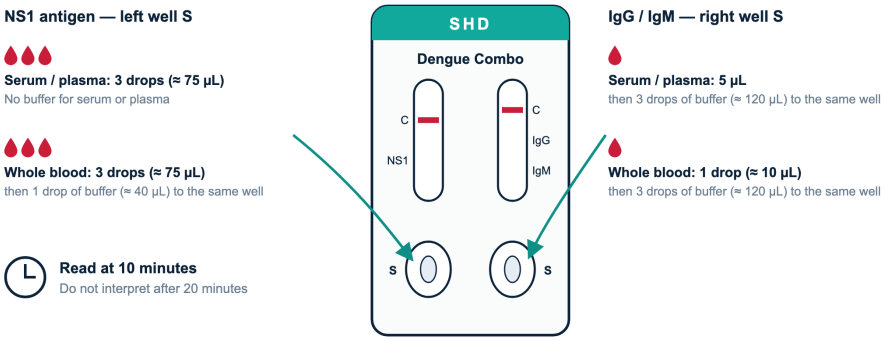



**Figure 1. Specimen and buffer application**

## QUALITY CONTROL

A procedural control is included in the test. A coloured line appearing in the control line region (C) of each strip is the internal procedural control; it confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this kit. Testing a positive and a negative control is good laboratory practice to confirm the test procedure and verify proper test performance.

## INTERPRETATION OF RESULTS

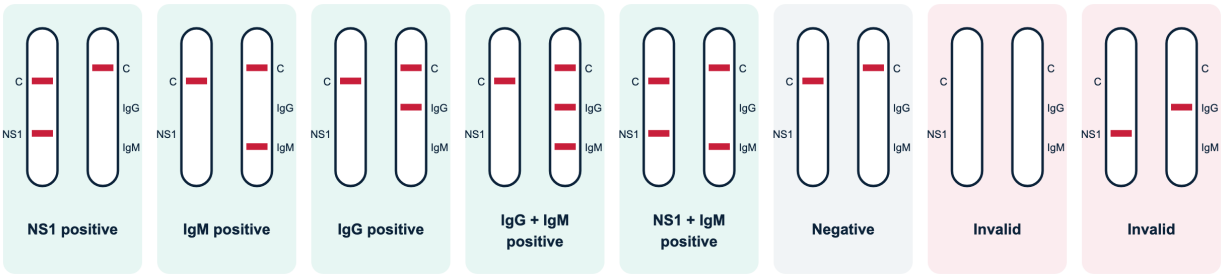



**Figure 2. Reading the NS1 strip (left) and the IgG/IgM strip (right)**

| Result | Lines | Interpretation |
|---|---|---|
| NS1 POSITIVE | C and NS1 on the NS1 strip | Dengue NS1 antigen detected; consistent with acute dengue infection. |
| IgG and IgM POSITIVE | C, IgG and IgM on the IgG/IgM strip | Dengue-specific IgG and IgM detected; indicates late primary or early secondary dengue infection. Line intensities need not match. |
| IgG POSITIVE | C and IgG on the IgG/IgM strip | Dengue-specific IgG detected; probably indicative of secondary dengue infection. |
| IgM POSITIVE | C and IgM on the IgG/IgM strip | Dengue-specific IgM detected; indicative of primary dengue infection. |
| NEGATIVE | C only on both strips | No NS1 antigen and no dengue IgG/IgM detected. |
| INVALID | No C line on a strip | Repeat with a new test cassette. |

The intensity of the colour in the test line regions (NS1, IgG, IgM) varies with the concentration of dengue NS1 antigen, IgG and IgM in the specimen. Any shade of red in a test line region should therefore be considered positive.

**Invalid results:** insufficient specimen volume or incorrect procedural technique 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 kit immediately and contact your local distributor.

## LIMITATIONS OF THE TEST

- SHD Dengue IgG/IgM & NS1 Combo Rapid Test Cassette indicates only the presence of dengue NS1 antigen and dengue antibodies in the specimen and should not be used as the sole criterion for the diagnosis of dengue.
- The test procedure and the interpretation of results must be followed closely when testing individual whole blood, serum or plasma specimens. Failure to follow the procedure may give inaccurate results.
- A negative NS1 result does not preclude exposure to or infection with dengue virus. A negative NS1 result can occur if the quantity of antigen in the specimen is below the detection limit of the assay, or if the antigen is not present at the stage of disease in which the specimen is collected.
- Specimens containing unusually high titres of heterophile antibodies or rheumatoid factor may affect expected results.

- If symptoms persist while the NS1 result is negative, re-sample the patient a few days later or test with an alternative method such as PCR or ELISA.
- In the early onset of fever, anti-dengue IgM concentrations may be below detectable levels. In primary infection, an IgM antibody-capture ELISA (MAC-ELISA) detected IgM in 80% of patients by the fifth day after infection and in 99% by day 10. For secondary infection, a low molar fraction of anti-dengue IgM and a high molar fraction of broadly flavivirus-reactive IgG characterise the antibody response (World Health Organization, 1997); the IgM signal may be faint and cross-reaction in the IgG line region may appear.
- Serological cross-reactivity across the flavivirus group (dengue 1, 2, 3 and 4, St. Louis encephalitis, West Nile, Japanese encephalitis and yellow fever viruses) is common (Makino et al., 1994; Dobler et al., 1997; Yamada et al., 2003). Positive results should be confirmed by other means.
- The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
- Results from immunosuppressed patients should be interpreted with caution.
- As with all diagnostic tests, results must be interpreted together with the other clinical information available to the physician. If the result is negative and clinical symptoms persist, additional testing with other clinical methods is recommended; a negative result does not at any time preclude the possibility of dengue infection.
- The haematocrit of whole blood specimens should be between 25% and 65%.

## PERFORMANCE CHARACTERISTICS

### Diagnostic sensitivity and specificity

The NS1 component was evaluated with a seroconversion panel and with clinical specimens against a commercial dengue antigen ELISA. The IgG/IgM component was evaluated with specimens from symptomatic and asymptomatic individuals and the results were confirmed with a commercial dengue IgG/IgM ELISA. The relative sensitivity and specificity of the NS1 component are 95.8% and 96.1%. The overall relative sensitivity of the IgG/IgM component for primary and secondary infection is 94.3%, its relative specificity is 99.1% and its relative accuracy is 98.3%.

### Dengue NS1

| Rapid test result | ELISA Positive | ELISA Negative | Total |
|---|---|---|---|
| Positive | 137 | 8 | 145 |
| Negative | 6 | 200 | 206 |
| Total | 143 | 208 | 351 |

Relative sensitivity: 137/143 = 95.8% (95% CI: 91.1% – 98.4%); relative specificity: 200/208 = 96.1% (95% CI: 92.6% – 98.4%); accuracy: (137 + 200)/351 = 96.0% (95% CI: 93.4% – 97.8%).

### Dengue IgG/IgM

| Population (ELISA reference) | Rapid IgM + | Rapid IgG + | Rapid negative | Relative performance |
|---|---|---|---|---|
| Primary infection — ELISA IgM positive (n = 24) | 20 | 4 | 0 | Sensitivity 83.3% |
| Secondary infection — ELISA IgM positive (n = 64) | 46 | 18 | 0 | IgM sensitivity 71.9% |
| Secondary infection — ELISA IgG positive (n = 64) | 1 | 63 | 0 | IgG sensitivity 98.4% |
| Non-dengue — ELISA negative (n = 433) | 1 | 3 | 429 | 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%).

### Precision

Within-run precision was determined with 15 measurements of each dengue specimen; the specimens were correctly identified more than 99% of the time. Between-run precision was determined by 15 independent assays on the same dengue specimens using three different lots; the specimens were correctly identified more than 99% of the time.

### Cross-reactivity

Specimens positive for HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, anti-syphilis, anti-HIV, anti-HCV, anti-H. pylori, anti-EBV (infectious mononucleosis), anti-CMV, anti-rubella and anti-Toxoplasma were tested. No cross-reactivity was observed.

### Interfering substances

The following substances were added to dengue-negative and dengue-positive specimens. None of them interfered with the assay at the concentrations tested.

| Substance | Concentration | Substance | Concentration |
|---|---|---|---|
| Acetaminophen | 20 mg/dL | Caffeine | 20 mg/dL |
| Acetylsalicylic acid | 20 mg/dL | Gentisic acid | 20 mg/dL |
| Ascorbic acid | 2 g/dL | Haemoglobin | 1,000 mg/dL |
| Bilirubin | 1 g/dL | Albumin | 2 g/dL |

| | | | |
|---|---|---|---|
| Creatine | 200 mg/dL | Oxalic acid | 60 mg/dL |

## WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- Do not use the test if the pouch is damaged. Each test cassette is for single use only.
- Do not eat, drink or smoke in the area where 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 standard procedures for the 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.

## WASTE MANAGEMENT

Used test cassettes, droppers and specimens should be considered potentially infectious and disposed of in accordance with local regulations.

## SDS

Safety Data Sheet could be obtained from www.shdtest.com//techfiles/RAPID/MSDS/744235

## ASSAY SPECIFICATIONS (Table 1)

| Parameter | Specification |
|---|---|
| Sample Type | Whole blood (venipuncture / fingerstick), serum, plasma |
| Sample Volume — IgG/IgM | 5 µL serum/plasma or 10 µL whole blood + 3 drops buffer |
| Sample Volume — NS1 | 75 µL serum/plasma; 75 µL whole blood + 1 drop buffer |
| Test Principle | Lateral flow immunochromatography, colloidal gold |
| Analytes | Dengue NS1 antigen; anti-dengue IgG; anti-dengue IgM |
| Result Type | Qualitative (positive / negative), visual reading |
| Reading Time | 10 minutes (do not read after 20 minutes) |
| Storage | 2 – 30 °C, in the sealed pouch; do not freeze |

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## KEY TO SYMBOLS USED

| Symbol | Title | Symbol | Title |
|---|---|---|---|
| IVD | In vitro diagnostic medical device | LOT | Batch code |
| REF | Catalogue number | ∑ | Contains sufficient for <n> tests |
| | Use-by date | | Biological risks |
| | Manufacturer | | Keep away from sunlight |
| | Temperature limit (2 – 30 °C) | ⊘ | Do not re-use |
| | Caution | i | Consult Instructions for Use |

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