1. **Explanation of the test**

**[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 (dengue viruses 1, 2, 3 and 4). In children, infection is often sub-clinical or causes a self-limited febrile disease. However, if the patient is infected a second time with a different serotype, a more severe disease, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur. Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes.

Traditionally, the serological diagnosis of an acute dengue virus infection has relied on showing a 4-fold or greater rise in anti-dengue virus antibody between paired acute- and convalescent-phase sera from a patient. The haemagglutination-inhibition test has been the most commonly used serological assay for dengue diagnosis.

Rapid and reliable tests for primary and secondary infections of dengue are essential for patient management. Primary dengue infection is associated with mild to high fever, headache, muscle pain and skin rash. Immune response includes IgM antibodies produced by 3rd-5th day of symptoms and persist for 30-60 days. IgGs appear the 14th day and persist for life. Secondary infections often result in high fever and in many cases with haemorrhagic events and circulatory failure. Secondary infections show that IgGs rise within 1-2 days after the onset of symptoms and induce IgM response after 20 days of infection.

**[INTENDED USE]** SD BIOLINE Dengue IgG/IgM Rapid Test is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test is intended for professional use as aid in the presumptive diagnosis between primary and secondary dengue infection. This test provides only a preliminary test result. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and serological test like haemagglutination-inhibition test, more specific alternative diagnosis method must be used in order to obtain a confirmation of dengue virus infection.

**[PRINCIPLE]** SD BIOLINE Dengue IgG/IgM Rapid Test is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test also can detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins.

**SD BIOLINE** Dengue IgG/IgM test device has 3 pre-coated lines, “G” (Dengue IgG Test Line), “M” (Dengue IgM Test Line) and “C” (Control Line) on the surface of the membrane. All three lines in result window are not visible before applying any samples. The “Control Line” is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple “G” and “M” lines will be visible in the result window if there are enough IgG and/or IgM antibodies to dengue virus in the sample. If IgG and/or IgM antibodies to dengue virus are not present in the sample, there is no color appearance in “G” and/or “M”. When a specimen is added to the sample well, anti-Dengue IgG and IgM s in the specimen will react with recombinant dengue virus envelope proteins-collodial gold conjugates and forms a complex of antibodies-antigen. As this complex migrates along the length of the test device by capillary action, it will be captured by the relevant anti-human IgG and anti-human IgM immobilized in two test lines across the test device and generate a colored line.

2. **Materials provided/Active ingredients of main components (25 Tests/kit)**

**SD BIOLINE** Dengue IgG/IgM kit contains following items to perform the assay.

1. **SD BIOLINE** Dengue IgG/IgM test device (individually foil packed with a desiccant)
   - 1 test strip includes Gold conjugates (as main component) 
   - Recombinant dengue virus envelope protein-gold colloidal (1 ± 0.2μg), Test Line “G” (as main component) 
   - Mouse monoclonal anti-human IgG (5 ± 1μg), Test Line “M” (as main component) 
   - Mouse monoclonal anti-human IgM (5 ± 1μg), Control Line (as main component) 
   - Rabbit anti-dengue IgG (2.5 ± 0.5μg)
2. Assay diluent; 100mM Phosphate buffer (5ml), Sodium azide (0.01% w/w)
3. 10ml Capillary pipette
4. Package insert

3. **Precautions/Kit storage and stability**

1. For best results, strict adherence to these instructions is required.
2. All specimens should be handled as being potentially infectious.
3. The test device should be stored at room temperature. Do not store at refrigerator.
4. The test device is sensitive to humidity as well as to heat.
5. Do not open or remove test device from individually sealed pouches until immediately before their use. Perform the test immediately after removing the test devices from the foil pouch.
6. Do not use it beyond the expiration date. The shelf-life of the kit is as indicated on the outer package.
7. Do not use the test kit if the pouch is damaged or the seal is broken.
8. The components (test device and assay diluent) in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.
9. The assay diluent contains low concentration of sodium azide as a preservative. Sodium azide is toxic and should be handled carefully to avoid ingestion and skin contact.

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### Test Procedure

1. **Using micropipette, add 10μl of serum, plasma or whole blood into the sample well marked “S”.**

2. **Put 3-4 drops of assay diluent into the round shaped well.**

3. **Interpret test results in 15-20 minutes.**

   **Do not read the results after 20 minutes. Reading too late can give false results.**

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### Interpretation

**Negative** (No dengue infection)
- One pink line “C” in result window at right

**Positive** (Primary dengue infection)
- Two pink lines “C” and “M” in result window.
- It is positive even if “M” line is weak.

**2. IgG positive** (Secondary or past dengue infection)
- Two pink lines “C” and “G” in result window.
- It is positive even if “G” line is weak.

**3. IgG and IgM positive** (Late primary or early secondary dengue infection)
- Three pink lines “C”, “M” and “G” in result window.

**Invalid**
- No control (C) line in result window.
- It is recommended that the specimen be re-tested.
4. Warnings
1) For in vitro diagnostic use only. DO NOT RE-USE test device.
2) The instructions must be followed exactly to obtain accurate results. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
3) Do not cut or smoke while handling specimens.
4) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
5) Avoid splashing or aerosol formation.
6) Clean up spills thoroughly using an appropriate disinfectant.
7) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
8) Do not mix and interchange with different specimen.

5. Specimen Collection, Storage and Precaution
1) Specimen Collection and Storage
   (1) Serum, plasma or whole blood samples may be used with this test.
   (2) Whole blood
   - [Collection by venipuncture]
     1. Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture.
     2. If blood specimens are not immediately tested, they should be refrigerated at 2−8°C.
     3. When stored at 2−8°C the blood specimens should be used within 3 days.
     4. For storage period longer than 3 days, freezing is recommended. They should be brought to room temperature prior to use.
     5. Using the blood specimens in the long-term keeping more than 3 days can cause non-specific reaction.
   - [Collection using a lancet]
     1. Clean the area to be lanced with an alcohol swab.
     2. Squeeze the end of the fingertip and pierce with a sterile lancet provided. Immerse the open end in the blood drop and then release the pressure to draw blood into the capillary pipette to black line.
   (3) Serum or Plasma
       [Serum] Collect the whole blood into the collection tube (NOT containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
       [Plasma] Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.
   (4) If serum or plasma specimens are not tested immediately, they should be refrigerated at 2−8°C. For storage period longer than 2 weeks, freezing is recommended. They should be brought to room temperature prior to use.
   (5) Serum or plasma specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

5. Precaution
(1) Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.
(2) Use separately disposable capillary pipettes or pipette tips for each sample in order to avoid cross-contamination of either samples which could cause erroneous results.

6. Procedure of the Test (Refer to figure)
1) Allow all kit components and specimen to room temperature prior to testing.
2) Remove the test device from foil pouch, place it on a flat, dry surface.
3) [Using a capillary pipette] With a 10μl capillary pipette provided, add 10μl of serum, plasma or whole blood specimen drawn to black line into the square sample well marked “S”. OR, [Using a micropipette] Add 1μl of serum, plasma or whole blood specimen into the square sample well marked “S”.
4) Put 3−4 drops (about 90−120μl) of assay diluent into the assay diluent well round shaped.
5) Interpret test results in 15−20 minutes.

Caution: Do not read test results after 20 minutes. Reading too late can give false results.

7. Interpretation of the test (Refer to figure)
1) Negative
   The control line is only visible on the test device. No IgG and IgM antibodies were detected. Re-test in 3−5 days if dengue infection is suspected.
2) IgM Positive
   The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Dengue virus. This is indicative of a primary dengue infection.
3) IgG Positive
   The control line (C) and IgG line (G) are visible on the test device. This is positive for IgG antibodies. This is indicative of secondary or previous dengue infection.
4) Invalid
   The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the likeliest reasons for control line failure. Repeat the test using a new test

8. Limitation of the test
1) This test detects the presence of antibodies to dengue virus in the specimen and should not be used as the sole criterion for the diagnosis of dengue virus infection.
2) In early infections and some secondary infections, detectable levels of IgM antibodies may be low. Some patients may not produce detectable levels of antibody within the first seven to ten days after infection. If clinical symptoms persist, patients should be re-tested in 3−4 days with the first specimen.
3) Serological cross-reactivity across the flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.
4) As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
5) If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of an early infection of dengue virus.
6) The test procedure, preparation and interpretation of results for this test must be followed strictly when testing.

9. Internal Quality Control
The “Control Line” is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. It confirms sufficient specimen volume and correct procedural technique. A clear background is also required.

10. Expected value
Primary dengue infection is characterized by the presence of detectable IgM 3−5 days after the onset of infection. Secondary dengue infection is characterized by the elevation of specific IgC 1−2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

11. Performance Characteristics
1) Sensitivity and Specificity
   The comparison results of SD BIOLINE Dengue IgG/IgM test with Haeumagglutination Inhibition (HI) test showed that SD BIOLINE Dengue IgG/IgM test had good correlation with HI test

<table>
<thead>
<tr>
<th>Reference assay</th>
<th>Index assay</th>
<th>Sample collecting timing or group</th>
<th>Dengue Infection status</th>
<th>Sensitivity</th>
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<tr>
<td>HI Test</td>
<td>SD BIOLINE Dengue IgG/IgM</td>
<td>Early acute</td>
<td>Primary</td>
<td>23</td>
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<tr>
<td></td>
<td></td>
<td>Early acute</td>
<td>Secondary</td>
<td>70</td>
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<td></td>
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<td>Negative group</td>
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<table>
<thead>
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<th>Diseases</th>
<th>Dengue IgM Negative/Total</th>
<th>Dengue IgG Negative/Total</th>
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<tr>
<td>Japanese Encephalitis</td>
<td>25/25</td>
<td>25/25</td>
</tr>
<tr>
<td>Yellow Fever</td>
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<td>Malaria P. falciparum</td>
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<td>Malaria P. vivax</td>
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<tr>
<td>Total</td>
<td>100/100</td>
<td>100/100</td>
</tr>
</tbody>
</table>

4) Precision: Within run and between run precisions have been determined by the testing of ten specimens three times: 4 of negative, 2 of low positive, 2 of medium positive and 2 of strong positive. All values were correctly identified 100% of the time.
5) To evaluate the interference of SD BIOLINE Dengue IgG/IgM rapid kit with known relevant interfering specimens, the hemolytic samples, rheumatoid factors-containing samples and lipemic, icteric samples were investigated. In these studies, those specimens did not interfere with this test kit.
6) Analytical Sensitivity: the limit of detection, the smallest amount of the target marker that can be precisely detected, have been equal or superior to a leading commercial dengue antibody detection rapid test kit.

12. Bibliography of suggested reading

Disclaimer:
While every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

Warning:
The Manufacturers and Distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative, in the use of this product.