Intended use:
The Cypress Diagnostics Dengue (IgG & IgM) Combo Card Test is a single use immunochromatographic screening test for the detection of IgG and IgM antibodies to Dengue virus in human serum or plasma. The test provides a differential detection of anti-dengue IgM and anti-dengue-IgG antibodies and can be used for the presumptive distinction between a primary and secondary Dengue infection.

Summary and explanation:
Dengue is a viral disease. Dengue viruses, members of flaviviridae, are transmitted principally in a cycle involving humans and mosquito vectors (Aedes aegypti and Aedes albopictus). Dengue virus infection presents as two clinical syndromes: Dengue fever (DF) and dengue hemorrhagic fever (DHF) or dengue shock syndrome. DF is a self-limited febrile disease and is most common type of dengue illness. It causes sudden fever with headache, retrobulbar pain, pain in the back and limbs (break-bone fever), lymphaderopathy, maculopapular rash and nausea. DHF is an immunopathogenic disease occurring after sequential dengue infections. DHF in its most severe form can threaten the patient’s life through increased vascular permeability and shock. The fatality rate in patients with Dengue shock syndrome can be as high as 44%.

The immune response to this virus includes the production of IgM antibodies by 3rd - 5th day of symptoms which remain in the sample. If IgG and/or IgM antibodies to Dengue virus are present in the sample, no coloured line appears in “G” and/or “M” regio. A purple “G” and “M” line 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, no coloured line appears in “G” and/or “M” regio.

When a sample is added to the sample well, anti-dengue IgG or IgM in the sample will react with colloidal gold conjugated with dengue virus envelope proteins and form 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 or anti-IgM antibody immobilized in two lines across the test membrane and generates a colored line.

Kit components:
Each kit contains following items:
- 25 Cypress Diagnostics Dengue Fever Combo Card Test devices
- 1 vial of diluent
- 25 capillary pipettes 5 µl
- 1 instruction leaflet.

Additional Materials Required but not provided:
- Timer

Storage and stability:
The Cypress Diagnostics Dengue Combo Card Test and diluent vial should be stored at room temperature between 2-30°C in the original sealed pouch. The test device is sensitive to humidity as well as to heat. Do not store at refrigerator or freeze test kits. Do not store the kit in direct sunlight. The kit is stable until the date imprinted on the box label and/or pouch. Do not use expired test kits.

Warnings and precautions:
1. The Cypress Diagnostics Dengue Fever Card Test is FOR IN VITRO DIAGNOSTIC USE only. For PROFESSIONAL USE only.
   Use the test only in accordance with instructions supplied with the kit.
2. Treat all materials in the test as if they were infectious. Observe established precautions against microbiological hazards while performing the procedure and follow the standard procedures for proper disposal of sera and used kits.
3. Do not use test devices if the pouch is damaged or the seal is broken.
4. Do not open or remove test devices from their individually sealed pouches until immediately before their use.
5. Do not mix components from different lot numbers.
6. The diluent contains low concentration of sodium azide as a preservative. Sodium azide is toxic and should, therefore, be handled carefully, avoiding ingestion or skin contact. It may react with lead or copper plumbing to form explosive metal azides. Flush with a liberal volume of water when disposing of unwanted reagent.

Sample collection:
The Cypress Diagnostics Dengue test is performed on serum or plasma.

Serial: Collect the whole blood into a clean tube without anticoagulant by venipuncture. Leave to settle for 30 minutes for blood coagulation and then centrifuge the blood to obtain the serum sample from the supernatant.

Plasma: Collect the whole blood into a clean tube containing anticoagulant (such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge the blood to obtain the serum sample from the supernatant.

Patient serum and plasma samples perform best when tested immediately after collection. If not to be tested immediately, the samples should be refrigerated immediately following collection at 2-8°C and can be used up to 2 weeks if testing within For storage period longer than 2 weeks, freezing is recommended. The samples should be brought to room temperature prior to use.

Samples containing precipitate may yield inconsistent test results. Such samples must be clarified prior to assay.

The use of hemolytic, lipemic, icteric or rheumatoid factors containing samples should be avoided because it can lead to impaired results.
Test Procedure:
1. Bring all test devices, reagents and samples to room temperature prior to performing the assay.
2. Use fresh test device for every sample. The device is not reusable.
3. Just prior to use, remove the required number of Dengue Combo Card test devices from their wrappers by tearing along the notched area and place on a flat surface area.
4. Label the test unit with patient name or identification number.
5. Add 5 µl of serum or plasma with a 5 µl capillary pipette (drawn to the black line) or a micropipette into the square sample well(s).
6. Add 3–4 drops (about 90–120 µl) of assay diluent to the round-shaped well.
7. Interpret test results at 15–20 minutes.
8. Do not interpret the test results after 20 minutes, this can give false results.

Interpretation of results:

**IgM Positive:**
The presence of a IgM line (M) and a control line (C) on the test device indicates the presence of specific IgM antibodies against Dengue virus. This is indicative of a primary Dengue infection.

**IgG Positive:**
The presence of a IgG line (G) and a control line (C) indicates the presence of specific IgG antibodies against Dengue virus. This is indicative of a secondary or past Dengue infection.

**IgG and IgM Positive:**
The presence of a IgM (M), a IgG (G) and a control line (C) on the test device indicates the presence of specific IgG and IgM antibodies against Dengue virus in the sample. This is indicative of a late primary or early secondary Dengue infection.

**Negative:**
The presence of only a control line (C) on the test device indicates a negative result. A negative test result does not exclude Dengue infection. If symptoms persist, a new sample should be drawn from the patient in 3-5 days and then should be retested.

**Inconclusive:**
A control line should always appear in the control zone, no matter if the IgM or IgG line appears. If there is no distinct control line visible, the test is inconclusive. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. The test should be repeated with a new device.

**Expected results:**
Primary Dengue is characterized by the presence of detectable IgM antibodies 3-5 days after the onset of infection. Secondary Dengue is characterized by the elevation of specific IgG 1-2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

**Quality control:**
A control line should always appear in the control area if the test has been performed correctly and the device is working properly. A clear background is also required.

**Limitations of the procedure:**
1. 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.
2. As in case of all diagnostic tests a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a physician after all the clinical findings have been evaluated.
3. 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 of infection. If symptoms persist, a fresh sample should be drawn from the patient 3-4 days after the first testing date and the new sample should be retested.
4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative serological result does not preclude the possibility of an early infection of Dengue virus.
5. Serological cross-reactivity across Flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.

**Performance Characteristics**

**Comparison study**
Cypress Dengue Combo Test showed good correlation with haemagglutination-inhibition (HI) test.

<table>
<thead>
<tr>
<th></th>
<th>HI</th>
<th>-</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Cypress Quick test</td>
<td>+</td>
<td>93</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>9</td>
<td>180</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>200</td>
<td>302</td>
</tr>
</tbody>
</table>

Sensitivity = 91,2% (93/102)
Specificity = 90,0% (180/200)

When classifying the samples according to their Dengue infection status, the following results were obtained with the Cypress Dengue Combo test (HI was taken as the reference test)

**Sample collection timing or group**

<table>
<thead>
<tr>
<th>Dengue infection status</th>
<th>True pos</th>
<th>False pos</th>
<th>False neg</th>
<th>True neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early acute Primary</td>
<td>23</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early acute Secondary</td>
<td>70</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative No Dengue infection</td>
<td>20</td>
<td></td>
<td></td>
<td>180</td>
</tr>
</tbody>
</table>

**Cross-reactivity test with other Flavivirus mediated and mosquitos-borne disease**
Cypress Dengue Combo Test showed no cross-reactivity with other Flavivirus mediated disease and mosquitos-borne disease like Malaria

**Precision**
Within-run and between-run precisions have been determined by a 3-fold testing of 10 samples of which 4 negative, 2 low positive, 2 medium positive and 2 strong positive. All values correlated for 100%.

**Interference**
To evaluate the interference of the Cypress Dengue Combo Test with known relevant interfering samples, haemolytic samples, rheumatoid factors-containing samples, lipaemic and icteric samples were investigated. In these studies, those samples didn’t interfere with this test kit.

**References**

Langdorp 09.2011