RDT Dengue IgA/IgG

Rapid assay for the simultaneous detection of IgA and IgG antibodies directed against Dengue virus in human serum, plasma or venous whole blood

IVD

CE

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1. INTENDED USE

RDT Dengue IgA/IgG is an individual test for qualitative and differential detection of IgA and IgG antibodies directed against Dengue virus NS1 antigen in human serum, plasma or venous whole blood as an aid in the diagnosis of acute dengue infection.

2. SUMMARY AND EXPLANATION OF THE TEST

In tropical and subtropical regions, dengue is the most important arbovirosis in terms of morbidity and mortality. Clinical course of the Dengue infection varies from asymptomatic or undifferentiated mild fever to Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) or even Dengue Shock Syndrome potentially fatal. Efficient and accurate diagnosis of dengue is of primary importance for clinical care, i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases. During the early stages of the diseases, virus isolation, nucleic acid or NS1 antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis (1, 2). Dengue IgA antibodies are detectable 3-6 days after onset of illness (3), at a period where circulating dengue viruses and NS1 antigen disappear from the blood. It is recommended to use RDT Dengue IgA/IgG rapid test in conjunction with dengue NS1 antigen detection assays to warrant an accurate diagnosis of the patient in the whole window of the acute phase of the disease. Recently, dengue specific IgA have been described to be efficient markers especially in secondary infections (4, 5) or in case of primary infections leading to severe outcome (6). Dengue IgG detection provides complementary information that, depending on the sampling time after fever onset, can help orientating the diagnosis towards infections with higher risk of complications (6).

3. PRINCIPLE OF THE PROCEDURE

RDT Dengue IgA/IgG is a unitary disposable test based on lateral flow immuno-chromatography principle on a strip in a cassette. 5 µl of specimen to be tested (serum or venous whole blood) are distributed in the sample identified well. Then 3 drops of migration buffer are added to buffer well and sample and conjugate are drawn along the membrane strip. Results are read after 20 minutes of migration. When present in the sample, Dengue specific IgA and/or IgG will complex with corresponding capture anti Immunoglobulin at the Test Line on the strip and with antigen associated to anti-NS1 antibodies coated to gold colloidal particles. There, a purple line will appear. Whatever is the patient status, the Control Line must turn from a light blue color to a purple color otherwise, the test is considered invalid and should be repeated using fresh sample and new cassette. For detailed procedure and results interpretation, refer to the scheme at the end of this insert.
4. REAGENTS

4.1. Description

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassette</td>
<td>Individually foil pouch test device with desiccant. Test strip includes one sample pad, one conjugate pad composed of Dengue Ag and antibody coated colloid gold and one celluloid membrane coated with anti-human IgG antibody (Test Line IgG) and anti-human IgA antibody (Test Line IgA) and anti-mouse IgG antibody (Control Line).</td>
<td>25 x 1 Ready-to-use</td>
</tr>
</tbody>
</table>
| Migration Buffer | Borate buffer
Preservative: ProClin™ 300                                                      | 1 x 3 ml Ready-to-use |

4.2. Storage and handling requirements

The RDT Dengue IgA/IgG should be stored at 2-30°C in a dry environment. Reagents can be used until expiry date mentioned on the package.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Conservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassette</td>
<td>In individual sealed pouch, until expiry date at 2-30°C</td>
</tr>
<tr>
<td>Migration buffer</td>
<td>Until expiry date at 2-30°C before opening, and 2 months at 2-30°C after opening</td>
</tr>
</tbody>
</table>

5. WARNING AND PRECAUTIONS

For in vitro diagnostic use. For healthcare professional use.

5.1. Health and Safety

- This kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately to with the Good Laboratory Practices.
- Handle patient samples and any material that comes directly in contact with them as potentially able to transmit infectious diseases.
• Avoid spilling samples. Spills must be rinsed with bleach diluted to 10%. The material used for cleaning must be discarded in a contaminated residue container.
• Patient samples and contaminated material should be discarded after decontamination.
• For hazard and precaution recommendation related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of the Instructions for Use. The safety Data Sheet is available on www.bio-rad.com.

5.2. Precautions related to the procedure
• For single use only.
• Do not use any part of the test beyond the expiration date.
• Always follow recommended storage conditions.
• Do not open the pouch until performing the assay.
• Do not touch the reaction zone with fingers.
• Strictly follow the assay procedure.
• DO NOT HEAT THE SAMPLE.
• Hold the pipette vertically when distributing the sample into the sample well ("S").
• Test should be performed at 18°C – 37°C.
• If stored refrigerated, wait 30 min before use for the reagents to stabilize at room temperature.
• Do not mix reagent from different lot number of kits.
• Do not use test device if the pouch is damaged.
• Do not use test device if desiccant is missing from the pouch.
• Do not process the test under direct ventilation that could dry the strip.

6. SPECIMENS
Serum, plasma (EDTA, heparin or citrate) or whole blood on anticoagulant (EDTA, heparin or citrate) is the recommended sample type. Observe the following recommendations for handling, processing and storage of blood samples:
• Collect all blood samples observing routine precaution for venipuncture.
• For whole blood, samples should be stored at 2-4°C and tested within 7 days. Do not freeze whole blood samples.
• For serum, allow samples to clot.
• Keep tubes stoppered at all times.
• After centrifugation, separate the serum from the clot or red cells in a tightly stoppered storage tube.
• Serum specimens can be stored at +2-8°C if test is performed within 7 days. If test is not completed within 7 days, or for shipment, freeze the samples at -20°C or colder.
• Do not freeze/thaw serum samples more than 3 times. Previously frozen specimens should be thoroughly mixed (vortex) after thawing prior to testing.
• Do not heat the samples.

7. PROCEDURE

7.1. Material required

7.1.1. Materials provided
Each RDT Dengue IgA/IgG kit contains:
• Twenty-five (25) individually foil pouches cassettes with desiccant for individual testing.
• One (1) migration buffer dropper (3 ml).
• One (1) instruction leaflet.

7.1.2. Materials required but not provided
• Automatic or semi-automatic, adjustable or preset, pipettes or multi-pipettes, to measure and dispense 5 µl.
• Disposable tips.
• Disposable gloves.
• Goggles or safety glasses.
• Sodium hypochlorite (bleach) and sodium bicarbonate.
• Container for biohazard waste.

7.2. Reagents preparation
All reagents are ready-to-use.

7.3. Assay procedure
Refer to figure at the end of the document.
• Remove the cassette from its pouch and place it on a flat surface.
• Label the test device with Patient ID or identification number.
• Using a laboratory pipette, distribute 5 µl of sample into the sample well (“S”) of the cassette holding the pipette vertically.
• Using Migration Buffer dropper bottle, immediately add 3 drops of Migration Buffer in round shape Diluent Well of the cassette.
• Read the results after 20 to 30 minutes of migration at room temperature (18-37°C).
• Do not read the results before 20 minutes of migration.
### 7.4. Interpretation of the Results
Consider all bands even faint as reactive.

- **REACTIVE RESULT**: presence of purple band at the Test Line A (IgA) and/or the Test Line G (IgG) and the Control Line (C).
- **NON REACTIVE RESULT**: presence of a purple band at the Control Line (C) only.

<table>
<thead>
<tr>
<th>NEGATIVE</th>
<th>Presence of C Control band and absence of G and A bands</th>
<th>No detection of IgA or IgG; In case of Dengue suspicion, retest after 3-5 days.</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>IgA: Presence of C Control and A bands and absence of G band</td>
<td>Presence of IgA against Dengue virus. Indicative of an acute primary or secondary Dengue Infection.</td>
</tr>
<tr>
<td></td>
<td>IgG: Presence of purple C Control and G bands and absence of purple A band</td>
<td>Presence of IgG against Dengue virus. Indicative of current or past Dengue Infection.</td>
</tr>
<tr>
<td></td>
<td>IgA and IgG: Presence of purple C Control, A and G bands</td>
<td>Presence of IgA and IgG against Dengue virus. Indicative of acute primary or secondary Dengue Infection.</td>
</tr>
<tr>
<td>INVALID</td>
<td>Absence of purple C Control band.</td>
<td>A wrong procedure is the most frequent cause of invalid result. Test should be repeated on fresh sample with a new cassette.</td>
</tr>
</tbody>
</table>

### 7.5. Test Validation Criteria
Test is considered INVALID if the Control Line (C) is absent, and should be repeated using a new cassette.
8. **TEST LIMITATIONS**

Diagnosis of recent infection by dengue virus can only be established on the basis of a combination of clinical and biological data.

- A negative test result cannot exclude a recent infection.
- Serological cross reactions with other Flaviviruses (Japanese encephalitis, West Nile, yellow fever viruses) have been described
- The RDT Dengue IgA/IgG is a qualitative test and does not indicate the amount of IgA or IgG antibody in the specimen.
- IgG and IgA antibodies can persist after the convalescence phase
- Results should not be interpreted before 20 minutes of migration.

9. **PERFORMANCE CHARACTERISTICS**

9.1. **Clinical performance**

9.1.1 **Relative specificity**

The relative specificity was evaluated on sera or venous whole blood samples from blood donors or sera from febrile patients clinically diagnosed as negative for Dengue infection. Calculation was done using clinical status as reference.

<table>
<thead>
<tr>
<th>Population</th>
<th>Total</th>
<th>Negative</th>
<th>Positive</th>
<th>Specificity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>French blood donors</td>
<td>115</td>
<td>115</td>
<td>0</td>
<td>100.0%</td>
<td>96.8%-100%</td>
<td>115</td>
<td>0</td>
</tr>
<tr>
<td>Healthy Indian donors</td>
<td>50</td>
<td>48</td>
<td>2</td>
<td>96.0%</td>
<td>86.3%-99.5%</td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td>Febrile Guiana patients</td>
<td>49</td>
<td>44</td>
<td>5</td>
<td>89.8%</td>
<td>77.7%-96.6%</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>214</td>
<td>207</td>
<td>7</td>
<td>96.7%</td>
<td>93.4%-98.7%</td>
<td>196</td>
<td>18</td>
</tr>
</tbody>
</table>

9.1.2. **Relative sensitivity**

The relative sensitivity was evaluated on sera from patients clinically diagnosed as positive for Dengue infection. Calculation was done using clinical status as reference.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>IgA positive</th>
<th>95% CI</th>
<th>IgG positive</th>
<th>95% CI</th>
<th>IgA or NS1 positive</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>31</td>
<td>100.0%</td>
<td>88.8%-100%</td>
<td>83.9%</td>
<td>66.3%-94.5%</td>
<td>100.0%</td>
<td>88.8%-100%</td>
</tr>
<tr>
<td>Singapore</td>
<td>50</td>
<td>50.0%</td>
<td>35.5%-64.5%</td>
<td>38.0%</td>
<td>24.6%-52.8%</td>
<td>94.0%</td>
<td>83.4%-98.7%</td>
</tr>
<tr>
<td>French Guiana</td>
<td>133</td>
<td>76.7%</td>
<td>68.5%-83.6%</td>
<td>64.7%</td>
<td>55.9%-72.7%</td>
<td>93.8%</td>
<td>88.1%-97.3%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>214</td>
<td>73.8%</td>
<td>67.4%-79.6%</td>
<td>61.2%</td>
<td>54.3%-67.8%</td>
<td>94.8%</td>
<td>90.8%-97.4%</td>
</tr>
</tbody>
</table>
9.2. Cross Reactivity studies
36 sera containing HAMA (12), ANA (12), or Rheumatoid Factor (12) were tested with RDT Dengue IgA IgG and did not show any non-specific reaction. A collection of 44 specimens from patients with infections other than Dengue, were tested with RDT Dengue IgA/IgG.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>IgA negative</th>
<th>IgG negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>25</td>
<td>24</td>
<td>96.0%</td>
</tr>
<tr>
<td>Yellow Fever</td>
<td>11</td>
<td>11</td>
<td>100.0%</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>8</td>
<td>7</td>
<td>87.5%</td>
</tr>
</tbody>
</table>

9.3. Precision
Precision was tested using 3 positive samples including 2 low positive for IgA, 1 low positive for IgG and 1 negative sample. Interpretation was 100% in agreement with the status except for one low positive point giving 98% and 95% agreement for IgG and IgA, respectively.

9.4. Interferences study
Samples containing 0.2 g/l of unconjugated bilirubin, hemolysed samples containing 2 g/l of hemoglobin, lipemic samples containing the equivalent of 30 g/l of triolein (triglyceride) or 5g/L of cholesterol, protein rich serums containing 120 g/l of total proteins do not affect the results.

10. QUALITY CONTROL OF THE MANUFACTURER
All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within Bio-Rad.
11. BIBLIOGRAPHY REFERENCES

1. World Health Organization and the Special Programme for Research and Training in Tropical Diseases (TDR); Dengue guidelines for diagnosis, treatment, prevention and control; new edition; 2009

2. Centers for Disease Control and Prevention; Dengue Laboratory Guidance and Diagnostic Testing; http://www.cdc.gov/dengue/clinicalab/laboratory.html


Remove the cassette from its pouch and place it on a flat surface.
Sacar el casete de su envase y colocarlo sobre una superficie plana.
Remova o cassete da bolsa e coloque-o numa superfície plana.

Using a laboratory pipette, distribute 5 µl of sample (serum, plasma or venous whole blood) into the sample well ("S") holding the pipette vertically.
Usando una pipeta de laboratorio, dispensar 5 µl de muestra (suero, plasma o sangre entera venosa) en el pocillo para muestras ("S") sujetando la pipeta verticalmente.
Utilizando uma pipeta de laboratório, distribua 5 µl da amostra (sangue total venoso, soro ou plasma) no poço de amostras ("S") mantendo a pipeta na posição vertical.
3. Using Migration Buffer dropper bottle, immediately add 3 drops of Migration Buffer into round shape diluent well of the cassette.

4. Read the results after 20 to 30 minutes of migration at room temperature (18 -37°C).

• Using Migration Buffer dropper bottle, immediately add 3 drops of Migration Buffer into round shape diluent well of the cassette.

• Utilizando el cuentagotas de solución de migración, añadir inmediatamente 3 gotas de solución de migración en el pocillo redondo de solución diluyente del casete.

• Utilizando o conta-gotas do tampão de migração, adicione imediatamente 3 gotas de tampão de migração no poço de diluente redondo do cassette.

• Leer los resultados transcurridos de 20 a 30 minutos de migración a temperatura ambiente (18-37°C).

• Leia os resultados 20 a 30 minutos após a migração e na temperatura ambiente (18 - 37 °C).
• This product contains human or animal components. Handle with care.
Warning
May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/ face protection. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.
o eruzione della pelle: consultare un medico. Smaltire il prodotto/recipiente in conformità con le disposizioni locali/regionali/nazionali/internazionali.

(LT)
Atsargiai
Gali sukelti alerginę odos reakciją.

(NL)
Waarschuwing
Kan een allergische huidreactie veroorzaken.

(PL)
Uwaga
Może powodować reakcję alergiczną skóry.

(PORT)
Atenção
Pode provocar uma reacção alérgica cutânea.

(RO)
Atenție
Poate provoca o reacție alergică a pielii. 
Purtăți mânusile de protecție/imbrăcăminte de protecție/echipament de protecție a ochilor/chipament de protecție a feței. ÎN CAZ DE CONTACT CU PIELEA: spălați cu multă apă și săpun. În caz de iritație a pielii sau de erupție cutanată: consultați medicul. Aruncăți conținutul/containerul în acord cu regulamentele locale/regionale/naționale/internaționale.