Dengue IgG/IgM ELISA Kit

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96 assays
Version: 02

Intended for research use only

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Introduction

Intended Use

The Dengue IgG/IgM ELISA Kit is an enzyme linked immunosorbent assay (ELISA) for the qualitative identification of antibodies to dengue in human serum.

Background

Dengue virus belongs to the Flaviviridae family, which includes four serotypes of dengue virus. Japanese encephalitis and Yellow fever are part of this flaviviridae family. Due to the presence of common antigenic determinants, cross reactivity occurs frequently. Cross reaction occurs among the four dengue serotypes themselves. During this reaction, distinctive determinants for each serotype also develop.

Dengue virus serotypes are not cross protective; thus an infection with one serotype does not offer protection against a reinfection with second serotype. This reinfection can then lead to a more serious form of dengue, commonly known as dengue hemorrhagic fever. This reinfection affects approximately ten percent of infected patients. Dengue antibodies do not extend immunity beyond 3-6 weeks.

It is important to know which infecting serotype is involved in relation to the potential severity of a dengue infection in those areas endemic to dengue, such as Southeast Asia and South and Central America. When considering diagnosis of acute dengue infection, factors need to be taken into account, such as exposure in endemic regions, epidemiological factors, clinical findings (i.e. fever, tachycardia, thrombocytopenia, etc.), and any other laboratory results.

Dengue virus infection can result in a broad scope of diseases, including mild fever to dengue hemorrhagic fever, and even dengue shock syndrome. Generally, the symptoms after a 5-8 incubation period can result in nausea, vomiting, chills, severe headache, rash, and lymphadenopathy. In approximately ten percent of dengue-infected patients, symptoms can lead to damage to blood vessels and subsequent death.

Infection periods differ in relation to appearance of antibodies to dengue infection. During a primary infection (5-6 days after onset of illness) what is detected first is the circulating IgM antibody to the viral coat proteins. This gradually decreases after one to two months. In secondary infections, IgG antibody to dengue virus is identified 14 days after onset of illness. Even though during this secondary infection IgM antibody reappears, it gradually dissipates. IgG antibody, however, continues on, even to a high titer. Generally, primary infection patients with acute dengue infection have a higher IgM/IgG ratio. IgG levels are higher typically in the secondary infection stage. An increase in antibody titer and high IgM levels indicate acute or recent infection.

Recent infection diagnosis has been achieved by identification of the virus in patient's blood. This is done...
either by identifying viral RNA with hybridization or PCR techniques, or by isolating the virus in cell cultures or mosquitoes. These methods have been found to be difficult, requiring specialized clinical lab equipment. Also, these procedures are effective when done within 5 days of onset of illness, and this is not as effective since the level of circulating virus decreases as the antibody level increases.

The most common method of diagnosis of dengue antibodies is that done serologically. What has been found to be the most useful method for bringing about an exact serological diagnosis of dengue infection has been the ELISA microwell procedure for detecting IgM antibodies. Because of its sensitivity, capability of handling many samples, and its potential for automation, the IgM ELISA technique is taking the place of other procedures that detect IgM antibodies. Even the Hemagglutination Inhibition (HI) method is not as sensitive as the ELISA test.

Caution is advised when using the ELISA procedure to determine IgM antibody. IgG antibodies should be eliminated from previous or current infections since the presence of these antibodies may reduce the sensitivity of the ELISA test.

- **Expected Values**
  
  The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

  Antibody levels are generally low or absent during very early infection. Symptomatic patients may have no antibody during the first 1-2 weeks after exposure and the antibody titer will rise with time.

**Principle of the Assay**

The principle of the Dengue IgG/IgM ELISA test involves three incubation steps. Before the first incubation, the microwells are coated with purified dengue virus antigen from Vero cell cultured type 1-4 dengue. The sera sample is added, and if there are any antibodies present, they will bind to the wells during the first incubation. Next, the wells must be washed of any test sample, and added to the wells at this point is the Enzyme Conjugate. During this second incubation, the Enzyme Conjugate will bind to any antibodies present. Before the third incubation step, more washings are necessary. Then a chromogen (tetramethylbenzidine or TMB) is added. With the presence of the Enzyme Conjugate and the peroxidase causing the consumption of peroxide, the chromogen changes to a blue color. The blue color turns to a bright yellow color after the addition of the stop solution, which ends the reaction. ELISA readers can be used to obtain results or the results can be read visually.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate: Microwells containing Dengue antigens (serotype 1-4) - 96 test wells in a test strip holder.</td>
<td>96 wells</td>
</tr>
<tr>
<td>Enzyme Conjugate: One bottle containing anti-human IgG + IgM (H+L) conjugated to peroxidase.</td>
<td>11 mL</td>
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<tr>
<td>Positive Control: One vial containing diluted positive human serum.</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control: One vial containing diluted negative human serum.</td>
<td>1 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution: One bottle containing the chromogen tetramethylbenzidine (TMB).</td>
<td>11 mL</td>
</tr>
<tr>
<td>Wash Concentrate (20X): One bottle containing concentrated buffer and surfactant.</td>
<td>25 mL</td>
</tr>
<tr>
<td>1% Dilution Buffer: Two bottles containing buffered protein solution.</td>
<td>30 mL x 2</td>
</tr>
<tr>
<td>Stop Solution: One bottle containing 1 M phosphoric acid.</td>
<td>11 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Reagents, strips and bottled components: Store between 2-8°C. Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25°C).

Materials Required but Not Supplied

- Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade water and graduated cylinder Tubes for sample dilution
- Absorbent paper
- Suggested Materials: ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually)

Precautions for Use

- Important note:
- For Export Only
- Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2-8°C. Crystallization will disappear after dilution to working strength.
- Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content.
Samples high in lipids should be clarified before use.

✓ Treat all sera as if capable of being infectious. Controls have been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods.

✓ This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

✓ Do not add azide to any of the kit reagents.

- Limitations of the Procedure

✓ Known cross reactions among dengue antigens must be considered during interpretation, since some epitopes are known to react with other flaviviruses. IgM testing will help to distinguish the cross-reactive samples.

✓ Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e. an alternative method that separately identifies IgM and IgG antibody) is recommended.
Assay Protocol

Reagent Preparation

✓ Wash Buffer - Remove cap and add contents of bottle to 475 mL of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

   Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.

✓ Automated Washing

  • Option A: Increased number if washes to five (5) per step with a 30 seconds dwell time between washes. After the last wash series (before addition of Chromogen), either rinse wells once with DI water or slap wells vigorously against an absorbant towel to remove all excess wash buffer.
  • Option B: Add one extra well (blank) to the run that does not contain a control or sample. After the last wash series (before addition of Chromogen), either rinse wells once with DI water or slap wells vigorously against an absorbant towel to remove all excess wash buffer. Subtract the OD value of this blank well from the same OD readings.

Sample Preparation

Dengue IgG/IgM ELISA Kit should be performed on serum. Serum may be stored at 2-8°C for up to five days. Serum may be frozen below -20°C for extended periods. Freezing whole blood samples is not advised.

Single specimens are used to assess exposure; two specimens collected at different times from the same individual are used to show sero-conversion. Paired specimens should be tested at the same time. It is recommended that a convalescent specimen be collected from individual showing either an initially non-reactive result or a weakly reactive result. Due to high cross-reactivity with other flaviviruses, an IgM test is recommended.

Coagulate blood and remove serum. Freeze sample at -20°C or lower if not used within 5 days. Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:40 dilution of human sera using the dilution buffer (e.g. 10 µL sera and 390 µL dilution buffer).
Assay Procedure

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µL (or two drops) of the negative control to well #1, 100 µL of the positive control to well #2 and
   100 µL of the diluted (1:40) test samples to the remaining wells.
   
   Note: Negative and positive controls are supplied pre-diluted. Do not dilute further.
3. Incubate at room temperature (15 to 25°C) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Slap wells against paper towels to remove all liquid.
9. Add 2 drops of the Chromogen to every well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of the Stop Solution and mix by tapping strip holder.
12. Read within one hour of adding Stop Solution.
Data Analysis

Calculation of Results

- Reading Results
  - Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.
  - ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650nm.
    If using Automated Washing Option B (Blank), set the ELISA reader to use the background correction option.

- Quality Control
  The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.

  Expected values for the controls are:
  - Negative - 0.0 to 0.3 OD units
  - Positive - 0.5 OD units and above

- Interpretation Of The Test
  - Initially Non-reactive: Samples interpreted as non-reactive (0.0-0.3 OD units, or zero color) indicate antibody is not present in the sample. Since antibody may not be present during early disease, (5-8 days incubation), confirmation 2-3 weeks later is indicated for laboratory diagnosis. At this later time, patients showing weak reactions (0.5 - ≤1.0 or +, ++) should be further tested by alternate methods or re-tested 10-14 days later. A convalescent serum with a significant reaction (>1.0 OD) indicates the formation of specific antibody against flavivirus. An initially negative result followed by a positive result implies seroconversion.
  - Initially Weakly Reactive: Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 2-3 weeks later (paired acute and convalescent sera), is recommended. >1.0 OD in the second sample confirms the presence of recent, specific antibody. [Caution: If this is a cross-reactive antibody, the convalescent serum sample may not show a higher antibody level than the acute sample.] If sample reading remains at ≥0.5 - ≤1.0 OD, or +, ++, a second methodology (IgM) should be considered, or the sample may be interpreted as taken beyond rising titer (titer declining). If a sample initially reads 0.3 -0.5, diagnosis is indeterminate then a second sample must be drawn. If the second draw is still within the 0.3 -0.5 range upon repeated testing, the result should be reported as “indeterminate” or an alternate methodology employed.
  - Initially Reactive: Samples interpreted as strongly reactive (>1.0 OD or +++ or >) may indicate the presence of specific antibody. Antibody presence alone cannot be used for diagnosis of acute infection, since antibodies from prior exposure may circulate for a prolonged period of time.
## Performance Characteristics

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<tr>
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<th>Reference Method</th>
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<td>+</td>
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<tr>
<td>Abnova</td>
<td>+</td>
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<td></td>
<td>-</td>
</tr>
</tbody>
</table>

- Positive Agreement: 100% (15/15)
- Negative Agreement: 100% (65/65)
Resources

Troubleshooting

Negative control has excessive color after development.

✓ Reason: inadequate washings
✓ Correction: wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

References
