Dengue IgM / IgG Rapid Test

Intended Use

The test is intended as an aid to diagnosis of dengue infection in humans. It is to be used to confirm infection in conjunction with clinical symptoms of dengue fever.

Summary and Explanation

Dengue fever, caused by any of the four serotypes of dengue virus, is endemic throughout many of the tropical and subtropical regions of the world. Like other types of flavivirus, dengue virus is transmitted to humans by mosquitoes (*Aedes aegypti* and *A. albopictus*). Repeat infection with a second type of dengue virus is thought to cause dengue hemorrhagic fever in about 10 percent of infected people. Antibodies to one type of dengue virus confer immunity to that type, but do not confer immunity to a second dengue type beyond 4-6 weeks.

Infection with dengue virus can result in a wide disease spectrum, from mild fever to life-threatening dengue hemorrhagic fever and dengue shock syndrome. Common symptoms include fever, chills, skin rash, severe headache, nausea, vomiting and malaise. In dengue hemorrhagic fever there is a significant depression of platelet levels and increased blood vessel permeability that can lead to shock and death. Dengue fever can only be treated by supportive care and is prevented by mosquito control.

In primary infections, the first immune response is production of IgM antibodies to the virus, which appear 5-6 days after the onset of symptoms and persist for 1-2 months. IgG antibodies appear approximately 14 days after onset of symptoms in primary infections and gradually decrease but persist at low levels for life. In secondary infections, IgG antibodies increase to high levels within 2 days of the onset of symptoms, while IgM antibodies may reappear but gradually diminish. These patterns of dengue antibody development permit serological differentiation of primary and secondary infections.

Biological Principles of the Procedure

Serum, plasma or whole blood samples may be used with this test. The sample is diluted in a buffer and added to the sample window of the test cassette. IgM and IgG antibodies, if present, will bind to red microparticles that are coated with dengue envelope proteins. As this complex migrates through the membrane the anti-IgM and/or anti-IgG lines on the membrane capture the complex, resulting in a pink to dark red line. The IgM and IgG lines are adjacent to the numbers 1 and 2, respectively, embossed in the bottom of the cassette. To ensure assay validity, a third control line is incorporated into the assay device and is adjacent to the letter C.

The appearance of any color in a specific test area (IgM or IgG) should be considered as positive for that particular antibody type. A red procedural control line should always develop to indicate that the test is performing properly.

Materials Provided

Dengue IgM/IgG Rapid Test kit contains the following components to perform the assay:

1. Test Device (cassette)
2. Sample loop (1 microliter)
3. Dilution tube holder
4. Assay buffer
5. Sample dilution tube
6. Instructions for use

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Accessories (required but not provided):

Timer Micropipette (if loop is not used) Lancet (if using finger prick blood)

Storage and Stability

The test devices are sealed in a moisture-proof foil laminate pouch containing a desiccant pack. These should be stored in the coolest and driest area available, preferably at 2-35°C. The kit has a shelf life of 15 months from the date of manufacture. The devices should not be frozen and must be protected from exposure to humidity: once a pouch is opened, the device must be used within one hour.

Precautions

1. Use disposable gloves while handling potentially infectious material and performing the assay.
2. Do not eat, smoke, or drink while handling specimens.
3. Clean up spills thoroughly using an appropriate disinfectant.
4. Do not use the kit beyond the expiration date.
5. Do not open the foil pouch until it attains room temperature to prevent formation of condensation.
6. For best results, follow the test procedure and storage instructions strictly.
7. Do not reuse the test devices, sample loops, or dilution tubes

Specimen Collection and Storage

1. Serum, plasma or whole blood may be used as sample. Fresh samples are preferred for testing. If samples are not immediately tested, they should be stored at 4-8°C for not more than 3 days. Samples may be frozen at
   –20°C, but should not be repeatedly frozen and thawed.
2. Fresh blood from a finger prick may also be used as a test sample.
3. Clotted samples or samples with microbial contamination must not be used.

Test Procedure

1. Bring the complete kit and samples to be tested to room temperature prior to testing. Once the device pouch is opened, it must be used within one hour.
2. Add 6 drops of assay buffer to a sample dilution tube. For convenience, a holder is provided for the tubes.
3. Using the sample loop provided, or a suitable micropipette, place one loop of sample (1 microliter) into the vial containing 6 drops of buffer. Mix the sample into the buffer by stirring with the loop
4. Pour the diluted sample into the open window of the cassette as indicated in the picture
5. After 20-30 seconds, the diluted sample plus dissolved conjugate will flow onto the nitrocellulose. Wait approximately 15 minutes to read the results. When using whole blood, there will be a slight coloration of the membrane. If objectionable, this background color can be eliminated by adding one additional drop of buffer to the sample window and waiting a additional 5-10 minutes.

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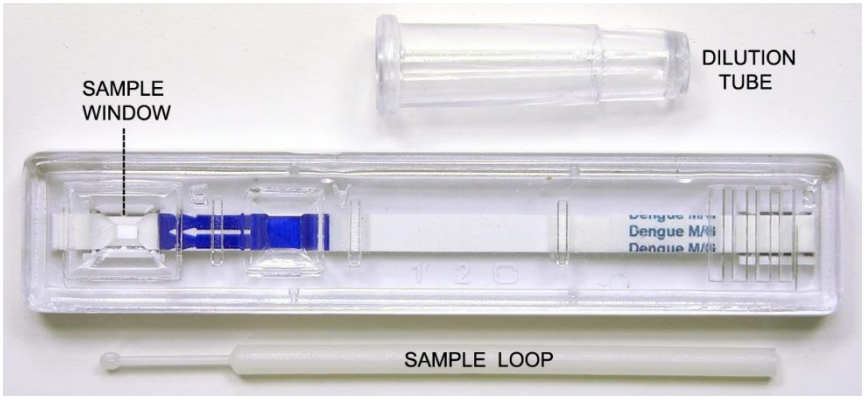
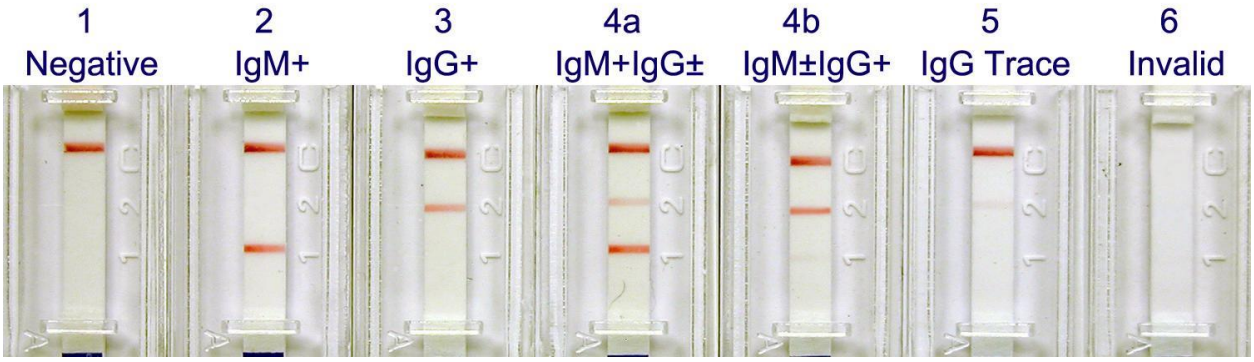


Interpretation of Results



As shown, a reactive test has one or two colored lines in the test area, and one in the control area. A single line adjacent to the embossed number 1 on the cassette indicates sample is IgM positive. A line adjacent to the number 2 indicates sample is IgG positive. Therefore, a test showing:

1. Only the control line is negative.
2. Only control and IgM lines indicate a primary dengue infection.
3. Only Control and IgG lines indicate a secondary dengue infection.
4. Control and both IgM and IgG lines:
    a. IgM line is strong and IgG line is weak indicates a primary infection.
    b. IgM line is weak and IgG line is strong indicates a secondary infection.
5. Control and very weak (trace) IgG line indicates prior exposure to flavivirus infection. See Limitations of Test.
6. Control line absent regardless of other lines indicates test is invalid and must be repeated.

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Performance Characteristics

A clinical study using a total of 100 samples was conducted using samples collected in India and Indonesia. The results of the **Dengue IgM/IgG Test** were compared with a commercially available ELISA test. The sensitivity and specificity of the IgM and IgG test results are given below:

IgM Results	ELISA Positive	ELISA Negative
Rapid Test Positive	41	1
Rapid Test Negative	1	57

Sensitivity = 97.6% Specificity = 98.3%

IgG Results	ELISA Positive	ELISA Negative
Rapid Test Positive	40	2
Rapid Test Negative	2	56

Sensitivity = 95.2% Specificity = 96.6%

Limitations and Interferences

1. The test procedure, precautions and interpretation of results for the test must be strictly followed.
2. As with all diagnostic tests, the test result must be consistent with clinical findings.
3. Results are to be interpreted within the epidemiological, clinical and therapeutic context.
4. In early primary 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 10 days of infection. If symptoms persist, retest the patient 3 - 5 days after the first testing date.
5. In endemic areas childhood infections with dengue or related flaviviruses are not uncommon and confer life-long levels of IgG antibodies. The sensitivity of the test has been adjusted to minimize detection of very low levels of IgG antibodies, nevertheless, very weak (trace) lines may appear, especially after 30 minutes from the time the test is started. In this case, retest the patient after 5-7 days to look for increased intensity of the IgG line. If there is no change, then dengue is not indicated.
6. The use of icteric or lipemic samples should be avoided.
7. This test should not be used on specimens from immunosuppressed individuals.
8. Negative results must be confirmed at 25 minutes.
9. Results should not be read after 35 minutes.

References

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3. Vorndam V., Kuno G, Laboratory diagnosis of dengue virus infections, Centers for Disease Control and Prevention, 1996.
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