

**AccuDiag™
Dengue IgM
ELISA Kit**

REF 8117-35



| Test | Dengue IgM ELISA |
|-----------------|--|
| Method | Enzyme Linked Immunosorbent Assay |
| Principle | Sandwich Complex |
| Detection Range | Semi-quantitative : Positive, Negative |
| Sample | 10 µL |
| Total Time | ~ 40 min. |
| Shelf Life | 12 Months from the manufacturing date |
| Specificity | 100% |
| Sensitivity | 85.9% |

INTENDED USE

The Diagnostics Automation Inc.(DAI) Dengue IgM ELISA Test is an enzyme linked immunosorbent assay (ELISA) for the qualitative identification of antibodies to dengue in human serum. The Dengue IgM ELISA kit is to be performed by trained medical technologies only.

SUMMARY AND EXPLANATION

Dengue fever, caused by any of the four serotypes of dengue virus, is endemic in Southeast Asia as well as South and Central America. Repeat infection with a second type of dengue virus is thought to cause dengue hemorrhagic fever in about 10 percent of infected people. Dengue antibodies do not confer immunity beyond 3-6 weeks to a second dengue type.

Epidemiological factors, clinical findings (including fever, tachycardia, thrombocytopenia, etc.), exposure in endemic regions, and other laboratory results should be considered in diagnosing acute disease. Acute disease diagnosis will also include a positive laboratory confirmation in many cases.

Infection with dengue virus can result in a wide disease spectrum, from a mild fever to life-threatening dengue hemorrhagic fever and dengue shock syndrome.¹ Symptoms of classical dengue fever, following a 5-8 day incubation period, include rash, severe headache, nausea, vomiting, chills, malaise, macular rash and may include lymphadenopathy. Hemorrhagic dengue fever involves increased blood vessel permeability which can lead to shock and death in about 10% of reported cases. Dengue fever can only be treated by supportive care and is prevented by mosquito control.

In primary infections, circulating IgM antibody to the viral coat proteins is detected 5-6 days after the onset of illness, and gradually decreases within 1-2 months of onset.^{6,7} IgG antibody to dengue virus is detected

approximately 14 days after onset in primary infections, and by day 2 in secondary infections. In secondary infections, IgM antibody may reappear but gradually diminishes, while IgG antibody persists, often at high titer. These patterns of dengue antibody development permit serological differentiation of primary and secondary infections. Characteristically, acute patients with primary infections have a higher IgM:IgG ratio than are found in secondary infections. Patients with secondary infections generally have higher IgG levels. Acute or recent infections are identified by a rise in antibody titer as well as high IgM levels.⁷

The *Flaviviridae* family includes the four serotypes of dengue virus as well as the yellow fever and Japanese encephalitis viruses. There is substantial cross reactivity among flaviviruses, due to the presence of common antigenic determinants. The four dengue serotypes cross react among themselves, but there are also unique determinants for each serotype.

Although infection with a given serotype confers durable protection against that serotype, dengue virus serotypes are not cross-protective, and reinfection with a second serotype has been linked to development of the more severe hemorrhagic form of dengue referred to as dengue hemorrhagic fever.^{1,6}

Determination of the infecting serotype may be important in gauging the potential severity of a dengue outbreak.¹ The most straightforward diagnosis of a recent infection is achieved by detection of the virus in patient's blood, either by isolating the virus in susceptible cell cultures or mosquitoes, or by identifying viral RNA with hybridization⁶ or PCR⁸ techniques. However, these methods are laborious and require specialized laboratory facilities. In addition, the level of circulating virus wanes as the antibody level rises, and these procedures are successful only when done within about 5 days of onset of illness.^{7,9}

Serological methods to detect dengue antibodies have been the most commonly used diagnostic procedures. The ELISA microwell method for the detection of IgM antibodies is currently the most useful procedure for providing a specific serological diagnosis of dengue infection. This method is reported to be as sensitive as the Hemagglutination Inhibition (HI) method.⁷ The IgM ELISA method is generally replacing other techniques for IgM determination because of its sensitivity, potential for automation and ability to accommodate large numbers of samples. For the determination of IgM antibody by the ELISA method, it has been recommended that measures be taken to eliminate IgG antibodies from current or previous flavivirus infections, since IgG antibodies may be present in excess and depress the sensitivity for the detection of IgM.¹⁰

TEST PRINCIPLE

The microwells are coated with purified dengue virus antigen from Vero cell cultured type 1-4 dengue. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

SPECIMEN COLLECTION AND PREPARATION

The DAI ELISA Dengue Fever test 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. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

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 patients 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.



Before use, bring all reagents and samples to room temperature (15-25 °C) and mix. (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature and mixed. **Ensure that (20X) Wash Concentrate is completely in solution before diluting to working concentration.** To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Test Strips:** Microwells containing dengue antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate:** One (1) bottle containing 11 ml of anti-human IgM (μ chain antibody) conjugated to peroxidase.
3. **Positive Control:** One (1) vial containing 1 ml of diluted positive human serum.
4. **Negative Control:** One (1) vial containing 1 ml of diluted negative human serum.
5. **TMB Substrate Solution (Chromogen):** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **RF Absorbent:** One (1) bottle containing 5 ml of goat anti-human IgG.
7. **Wash Concentrate (20X):** One (1) bottle containing 25 ml of concentrated buffer and surfactant.
8. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution.
9. **Stop Solution:** One (1) bottle containing 11 ml of 1.0 M phosphoric acid.

Materials required but not provided

1. Micropipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade (DI) water
4. Graduated cylinder
5. Tubes for sample dilution
6. Absorbent paper
7. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually)

PRECAUTIONS

1. **For Export Only**
2. **Do not deviate from the specified procedures when performing this assay.** All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
3. For In Vitro Diagnostic Use Only.
4. Do not interchange reagents between kits with different lot numbers.
5. Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
6. Unused microwells should be stored in the desiccated pouch to protect them from moisture.
7. Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
8. Do not add azides to the samples or any of the reagents.
9. Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
10. 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.
11. Treat all reagents and samples as potentially infectious materials. Controls have been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV be required test methods. Use care to prevent aerosols and decontaminate any spills of samples.
12. Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If

acid gets into the eyes, wash with copious amounts of water and seek medical attention.

13. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

ASSAY PROCEDURE

Notes:

- Ensure all samples and reagents are at room temperature (15-25 °C)
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each step should help to minimize bubbles in the wells.
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Dilute patient sera 1:40 using the Dilution Buffer (e.g. 10 μ l sera and 390 μ l dilution buffer).
3. Gather the required number of sample dilutions tubes (two for controls plus number of samples). Add **100 μ l** of negative control, positive control and diluted samples to the appropriate tubes. Add **40 μ l** RF Absorbent to each tube. Mix well.
4. Incubate at room temperature for **10 minutes**, then transfer all **140 μ l** of each tube to the appropriate wells.
5. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer. Add **2 drops** of Enzyme Conjugate to each well.
6. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
7. Add **2 drops** of the Chromogen to each well.
8. Incubate at room temperature for **10 minutes**.
9. Add **2 drops** of the Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**.
10. Read within one hour of adding Stop Solution.

* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

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 450/620-650 nm.

TROUBLE SHOOTING

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.

INTERPRATION OF THE RESULT

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 (0.3 - 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 0.3 - 1.0 OD, or +, ++, a second methodology (IgM) should be considered, or the sample may be interpreted as taken beyond rising titer (titer declining).

Initially Reactive: Samples interpreted as strongly reactive (>1.0 OD 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.

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.

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

PERFORMANCE CHARACTERISTICS

| | | Reference Method * | |
|----------------------------|---|--------------------|----|
| | | + | - |
| Diagnostic Automation, Inc | + | 55 | 0 |
| | - | 9 | 25 |

Positive Agreement: 85.9 % (55/64)

Negative Agreement: 100% (25/25)

*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

Diagnosis of dengue infection should not be made solely based on results of the DAI Dengue IgM ELISA Test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.

Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

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.

STORAGE

1. Reagents, strips and bottled components should be stored at 2-8 °C.
2. Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C).
3. All incubations are at room temperature (15-25 °C).

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