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Dengue Virus IgM ELISA Kit

Name	Dengue Virus IgM ELISA Kit
INTENDED USE	The Dengue virus IgM ELISA Kit is intended for the detection of IgM antibody to Dengue virus in human serum or plasma.
SUMMARY AND EXPLANATION	The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in Third World tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. Secondary infection is the more common form of the disease in many parts of Southeast Asia and South America. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, hemorrhagic events, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 h if appropriate treatment is not administered. Primary dengue virus infection is characterized by elevations in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days. IgG levels also become elevated after 10 to 14 days and remain detectable for life. During secondary infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly from 1 to 2 days after the onset of symptoms

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PRINCIPLE OF THE TEST

Diluted patient serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS PROVIDED	96 Tests
Microwells coated with Dengue antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: yellow Cap. 1 Vial (ready to use)	1ml
Positive Control: Red Cap. 1 vial (ready to use)	1ml
Negative Control: Blue Cap. 1 vial (ready to use)	1ml
Enzyme conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

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STORAGE AND STABILITY	Store the kit at 2-8 C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun or strong light.
WARNINGS AND PRECAUTIONS	Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
SPECIMEN COLLECTION AND HANDLING	1. Collect blood specimens and separate the serum. 2. Specimens may be refrigerated at 2–8 C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
REAGENT PREPARATION	Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 C).
ASSAY PROCEDURE	Bring all specimens and kit reagents to room temperature (18-26 C) and gently mix.

Place the desired number of coated strips into the holder.
Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well.
Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel.
Dispense 100 l of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
Remove enzyme conjugate from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel.
Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature.
Add 100 L of stop solution.
Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.
Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3

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	Patient sample O.D. = 1.6 Ab Index = 1.6 / 0.4 = 4.0
LIMITATIONS OF THE TEST	To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures. Lipemic or hemolyzed samples may cause erroneous results.
QUALITY CONTROL	The test run may be considered valid provided the following criteria are met: The O.D. of the Calibrator should be greater than 0.250. The Ab index for Negative control should be less than 0.9. 3. The Ab Index for Positive control should fall within the range specified on the COA/label.
INTERPRETATION	The following is intended as a guide to interpretation of Dengue virus IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered. Antibody Index Interpretation 1.1 Detectable antibody to Dengue virus IgM by ELISA.
PERFORMANCE CHARACTERISTICS	Sensitivity and Specificity 118 patient sera were tested by this Dengue virus IgM ELISA and a reference ELISA method. 22 sera were positive and 93 were negative by both methods (97% agreement). The results are summarized below:

	Dengue virus IgM ELISA	Dengue virus IgM ELISA	Dengue virus IgM ELISA
	+		Total
Reference ELISA Kit +	22	2	24
	1	93	94
Total	23	95	118

2. Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation (%)
1	16	1.81	0.12	6.63
2	16	0.94	0.05	5.31
3	16	0.21	0.02	9.52

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation (%)
1	10	1.72	0.18	10.46
2	10	1.14	0.12	10.52
3	10	0.17	0.02	11.76

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