Dengue Fever Virus IgM
ENZYME IMMUNOASSAY TEST KIT

SUMMARY OF ASSAY PROCEDURE

Step	(20-25ºC Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 = 5 µl / 200 µl		
2	Diluted samples, calibrator & controls	100 µl	30 minutes
3	Washing buffer (3 times)	350 µl	
4	Enzyme conjugate	100 µl	30 minutes
5	Washing buffer (3 times)	350 µl	
6	TMB Chromogenic Substrate	100 µl	15 minutes
7	Stop solution	100 µl	
8	Reading OD 450 nm		

NAME AND INTENDED USE

The Dengue Fever Virus IgM is intended for the detection of IgM antibody to Dengue fever virus in human serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

Dengue fever (DF) is an acute, self limiting, viral disease that is characterized by fever, headache, body pains, rash, lymphadenopathy, and prostration. In its most severe form, dengue hemorrhagic fever (DHF), infected patients will experience severe fever and renal failure leading to the often fatal dengue shock syndrome (DSS)1. It is estimated that approximately two billion people are at risk for DF world wide, and that over one million people per year are infected2. This, combined with the hundreds of thousands of cases of DSS, make dengue the most important arbovirus disease in the world. Dengue fever virus (DFV) is a flavivirus and is closely related to the yellow fever virus, Japanese encephalitis virus and other group B Arboviruses. Members of this group possess single stranded RNA which is surrounded by an icosahedral nucleocapsid covered with a 10 nm deep lipid envelope3. There are four strains of dengue fever virus, each serologically distinct. Infection with one strain does not protect the host from infection by the others. In fact, one report suggests DHF and DSS occurs most commonly in individuals that have been infected previously by another strain4. The presence of circulating, non-neutralizing, cross-reactive DFV antibody may act as an immune infection enhancement factor4. However, non-neutralizing, cross-reactive antibodies against other non-DFV flaviviruses are not associated with immune infection enhancement4. Dengue fever can be transmitted where ever the mosquito vectors, Aedes aegypti and Aedes albopictus, are found. A. aegypti is primarily localized to tropical and subtropical Americas and is indigenous to the southern part of the United States2. The primary vector for DF in Asia is A. albopictus. This mosquito has recently established itself in the United States as far north as central Illinois; however, DFV transmission has not been associated with it to date2. In most patients, suspected cases of DF are most rapidly diagnosed using serological methods. Traditionally, hemagglutination inhibition and plaque reduction neutralization have been used3. Hemagglutination inhibition antibody is usually detected in primary dengue fever cases by day 5 to 6 after the onset of fever. In secondary cases, rises in HI titer may be detected two- to-three-days after onset. By day 5 after onset, both primary and secondary patients have detectable IgM antibody, which may persist for as late as 90 days, a characteristic shared with other flaviviruses. Antiviral IgG usually appears by day 14 in primary dengue cases and by day 2 in secondary cases. IgG antibody has been detected as late as 60 years after exposure to the virus. In light of the remarkably durable IgM response of some flaviviruses, Gubler cautions that the presence of dengue-reactive IgM does not necessarily indicate a recent dengue virus infection (6). However, the IgG ELISA is useful as an epidemiological tool when used to establish sero-prevalence. This information can also be valuable in studies designed to determine the role immune enhancement plays in DSS.

PRINCIPLE OF THE TEST

Purified Dengue Fever Virus antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the Dengue Fever Virus IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell strips: Dengue Fever Virus antigen coated wells. (12 x 8 wells)

2. Absorbent Solution: 1 vial (22 ml)

3. Calibrator: Factor value (f) stated on label. 1 vial (150 µl)

4. Negative Control: Range stated on label. 1 vial (150 µl)

5. Positive Control: Range stated on label. 1 vial (150 µl)

6. Washing Concentrate (H) 20x: 1 bottle (50 ml)

7. Enzyme Conjugate: Red color solution. 1 vial (12 ml)

8. TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)

9. Stop Solution. 1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 – 8
2. 2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found nonreactive 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

2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.

4. This product contains components 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 - 8ºC for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25C) and gently mix.

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ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the sample , negative control, positive control, and calibrator to 200 µl of absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.

8. Add 100 µl of Stop Solution to stop reaction.
   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. To obtain Cut-off value: Multiply the OD450 of Calibrator by Factor (f) printed on label of Calibrator.

2. Calculate the IgM Index of each determination by dividing the OD values of each sample by obtained OD value of Cut-off.

NOTE*: This factor (f) is a variable for each kit*. For example:

If Factor (f) value on label = 0.35 Calculated Cut off Value equal
1.798 x 0.35 = 0.63

Sample	OD_{450}	Mean OD_{450} (A)	Calculated Cut off Value (B)	INDEX A/B	Interpretation
Calibrator f = 0.35	1.806 1.790	1.798	0.63		
Positive Control	1.643 1.662	1.653		2.62	Positive
Negative Control	0.023 0.022	0.023		0.04	Negative
Patient Sample 1	1.315 1.399	1.359		2.16	Positive
Patient Sample 2	0.206 0.212	0.209		0.33	Negative

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.

2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.

3. The IgM Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: IgM Index of 0.90 or less are seronegative for IgM antibody.
Equivocal: IgM Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: IgM Index of 1.00 or greater.

LIMITATIONS OF THE PROCEDURE

1. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgM antibody and render an IgM Index result negative.
2. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Precision:
The precision of the assay was evaluated by testing three different sera eight replicates on 3 days. The intra-assay and inter-assay C.V. are summarized below:

N = 8	Negative	Low positive	Positive
Intra-assay	11.8%	9.5%	6.9%
Inter-assay	14.3%	11.8%	10.1%

Cross-reactivity:
A study was performed to determine the cross-reactivity of the test to the following antibodie:
1. IgG and IgM of EBV, Mumps, Measle, and VZV.
2. IgG and IgM of Rubella, Toxo, CMV, HSV 1, and HSV 2.
3. IgM of RF.
4. IgG of ANA, anti- ds DNA.

All known positive samples tested give negative results to Dengue IgM test.

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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET048