Assay+ Dengue IgG / IgM Rapid Test User Instruction

REF Catalog No. Dengue-AB-20
IVD In Vitro Diagnostic

Intended use

The Dengue IgG/IgM Rapid Test is a lateral flow immunoassay to qualitatively to detect and differentiate IgM and IgG antibodies to all four serotype dengue viruses from whole blood, serum, plasma after 3-to-5-day infection from the suspected patients infected with dengue virus. It is intended to be used by professionals to aid in the diagnosis of infection with dengue viruses.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method should be considered to confirm the test result obtained by this device

Dengue infection and antibody test

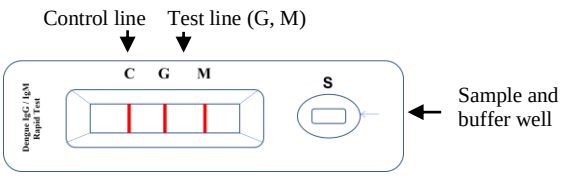
Dengue virus is a mosquito-borne flavivirus, consisting of four serotypes, it is considered as a major public health problem in tropical / subtropical areas. Dengue infection can be also classified into the primary and secondary infection. Due to four serotype dengue viruses, there is no cross-protection each other for each subtype, multiple infections are common in the infected patients.

Serological test is a common method for the diagnosis of infection with dengue virus. Dengue virus IgM starts to appear 3 days after initial exposure and remains for about 30-60 days. Anti-dengue virus IgG levels rise around 5 days, peak at 2-3 weeks and persist for the long time. Assay+ dengue IgM/IgG rapid test can be performed with 20 to 25 minutes by minimally trained personnel without the need of laboratory equipment.

Assay Principle

The assay+ Dengue IgM/IgG Rapid Test is a lateral flow chromatographic immunoassay device to detect the specific antibodies against dengue viruses. Dengue IgM and IgG antibodies bind to recombinant dengue antigens immobilized on the conjugate pad forming antibody - antigen conjugate complex, after following into nitrocellulose membrane, the complex can be captured by the antibodies to human IgM and IgG coated over the membrane forming visible red test line.

While immobilized rabbit IgG-conjugate will be captured by anti-rabbit IgG coated on nitrocellulose membrane forming red control line, following is the diagram of the test.



Kit Components

Contents	Quantity / kit	Description
Test cassette Catalogue #: Den100	20	Foil pouch contains one test cassette
Diluent buffer Catalogue #: DB100	2	Two 5ml extract buffer in one box
Package insert **Catalogue #:** IFU200	1	Instructions for use

Each test kit contains

1. A pouch
2. Pouch contains one cassette and plastic pipette
3. One box with 20 tests with two 5ml dropper bottle with diluent buffer

Stability and Storage Conditions

Dengue IgG/IgM test kit is stable at room temperature between 2-30ºC for two years in the unopened pouches.

General Precautions

- The test is for In Vitro diagnosis only.
- Appropriate infection control and handling procedures should be followed – do not smoke, eat or drink in the area where the test is to be performed. Use suitable clothing and gloves when handling samples and when performing the test.
- Do not pipette any samples or reagents by mouth.
- All materials should be considered as potentially infectious. To disinfect, either autoclave materials at 121.5ºC for 20 minutes or treat with sodium hypochlorite (1 percent solution).
- Do not use test beyond the expiration date indicated.

Sample Collection

Serum/plasma or whole blood is used for this device. The test works best on fresh samples. If testing cannot be done immediately, they should be stored at 2-8ºC after collection for up to 3 days. If testing cannot be done within 3 days, serum or plasma can be stored frozen at –20ºC or colder.

Test Procedure

1. Remove test cards from the pouches before the test, lay over a clean flat surface.
2. In case, the tested samples are in frozen status, the sample has to be sat in room temperature to completely thawing and mixed well.
3. Add 10ul serum/plasma or whole blood to the well over the test card using included pipette.
4. Follow adding sample, add two or three drops assay buffer from the dropper bottle.
5. Results are then read 15 to 30 minutes, make sure control line is always positive.

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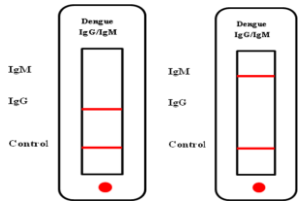
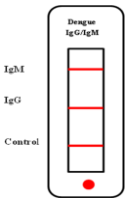
Reading test results

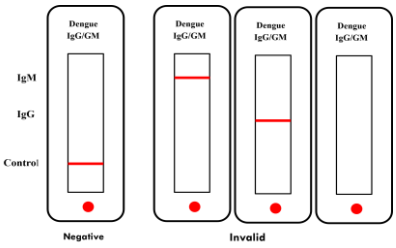
1. Dengue IgG or IgM positive



2. Dengue IgG and IgM positive



3. Dengue test negative and invalid



Quality Control

A known positive and negative control should be run to insure proper performance. All control tests should be handled in the same manner as patient samples.

Result analysis

1. Dengue IgM: positive or weak positive without dengue IgG after 3 to 5 days of symptom may indicate an early stage of dengue infection.
2. Positive dengue IgM and IgG may occur in primary dengue infection 5 -7 days after symptom.
3. Dengue IgG without dengue IgM may indicate at least 5 days after the infection.
4. Indeterminate, Control line should appear always If test line is positive or negative. without positive control line, it is recommended that a fresh device be used, and the test repeated carefully following the directions of the product insert.

Limitation of the Test

1. The assay procedure must be followed closely, failure to follow the procedure may lead to inaccurate results.

2. The Assay+ Dengue IgM/IgG Combo Rapid Test is limited to the qualitative detection of dengue virus IgG and IgM antibody in human serum, plasma, and whole blood. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.

3. This test cannot provide the serotype information of infected dengue virus.

4. The Assay+ Dengue IgM/IgG Combo Rapid Test cannot differentiate primary or secondary infection.

5. Serological cross-reactivity with other flaviviruses may occur (e.g., Japanese encephalitis, West Nile virus, yellow fever, etc.). Therefore, it is possible that patients previously exposed to these viruses may show some level of reactivity with this test.

6. A negative or non-reactive result can occur if the quantity of antibodies to dengue virus present in the specimen is below the limits of detection, or if the antibodies that are detected are not present during the stage of disease in which a sample is collected.

7. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.

8. The results obtained with this test should be interpreted in conjunction with other diagnostic procedures and clinical findings.

Performance Study:

1. Preclinical clinical study for dengue IgM and IgG:

Positive Dengue samples (43)	IgG positive	IgM positive
Dengue IgG and IgM (8)	8	6
Dengue IgM (25)	0	20
Dengue IgG (10)	9	0
Sensitivity for IgG or IgM	17/18=94.4%	26/33=81.8%

2. Preclinical clinical study for specificity

Sample number	Dengue IgG positive	Dengue IgM positive	Specificity	Reaction to Zika
50 negative specimens	0	0	100%	
10 Zika specimens	0	0		None

3. Preclinical clinical study for cross reactivity

Specimen	Result	Specimen	Result
HBV (4)	-	Anti-HBc (1)	-
HCV (5)	-	M. pneumonia (3)	-
HIV1/2 (5)	-	C. pneumonia (3)	-
Chikungunya (5)	-	HSV-1 (1)	-
Syphilis (3)	-	HSV-2 (1)	-
Anti-HBe (1)	-	WMV (5)	-
P. vivax (IgG) (5)	-	P. falciparum (5)	-

4. Antibody class specificity test

Assay+ dengue IgG / IgM rapid test can differentiate human IgG and IgM, in order to preclude the cross-reaction between anti-human IgG and IgM, the class specificity between IgG and IgM is included in our test to evaluate the potential the cross-reaction between anti-human IgM and IgG, producing false positive results for IgG, and the reverse, and the potential for IgM to compete with IgG and produce false negative results. In this test, five dengue positive IgG/IgM specimens were testes on Assay+ dengue IgG/IgM rapid test device in duplicate, then each specimen was incubated with 0.01M DTT for 15 minutes at 37ºC , DTT treated specimens re-tested on Assay+ Covid 19 antibody test device to observe the disappearance of IgM line. The results are included in the table below.

Sample Number	Dupli-cate	No DTT (IgM/IgG)	DTT treatment (IgM/IgG)	Expected result with DTT (IgM/IgG)
Dengue 3842	1	+/++	-/++	-/++
	2	+/++	-/++	-/++
Dengue 5765	1	+/++	-/++	-/++
	2	+/++	-/++	-/++
Dengue 8864	1	++/++	-/++	-/++
	2	++/++	-/++	-/++
Dengue 9351	1	+/++	-/++	-/++
	2	+/++	-/++	-/++
Dengue 0190	1	+/++	-/++	-/++
	2	+/++	-/++	-/++

5. Preclinical clinical study to recognize dengue serotypes 1, 2, 3, 4

Dengue serotype IgG	**Positivity**	**Intensity for IgG**
Dengue serotype 1	positive	+++
Dengue serotype 2	Positive	+++
Dengue serotype 3	Positive	++
Dengue serotype 4	Positive	+++

6. Clinical Performance study

Assay+ dengue IgG, IgM, or IgM/IgG rapid test	Dengue ELISA test (IgG, IgM or IgG/IgM)		
	Positive	Negative	Total
Positive	78	1	79
Negative	8	44	52
Total	86	45	131
Positive percent agreement (PPA)	90.69 % (95% CI: 84.55% - 96.29%)		
Negative percent agreement (NPA)	97.78% (95% CI: 93% - 100 %)		

INQUIRES AND GENERAL INFORMATION:

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