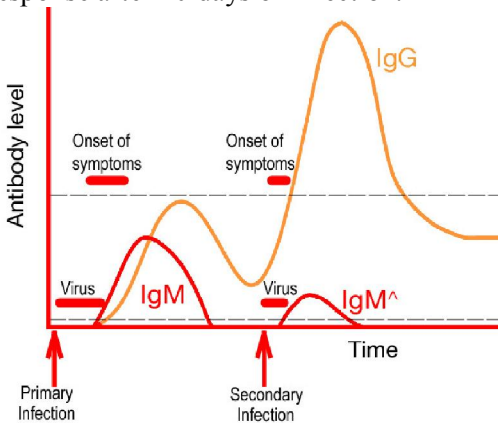
INTENDED USE

The Dengue IgG/IgM Rapid Test is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in **human serum, plasma or whole blood.** This test is intended for professional use as aid in the presumptive diagnosis between primary and secondary dengue infection. In the United States, this kit is intended for Research Use Only.
This test provides only a preliminary test result. Therefore, isolation of virus, antigen detection in fixed tissues, RT-PCR and serological test like haemagglutination-inhibition test, more specific alternative diagnosis method must be used in order to obtain a confirmation of dengue virus infection.

INTRODUCTION

Dengue viruses, transmitted by the mosquito, Aedes aegypti and Aedes albopictus mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world. There are four known distinct serotypes (dengue virus 1, 2, 3 and 4). In children, infection is often sub-clinical or causes a self-limited febrile disease. However, if the patient is infected a second time with a different serotype, a more severe disease, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur. Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes.
Traditionally, the serological diagnosis of an acute dengue virus infection has relied on showing a 4-fold or greater rise in anti-dengue virus antibody between paired acute- and convalescent-phase sera from a patient. The haemagglutination-inhibition test has been the most commonly used serological assay for dengue diagnosis. Rapid and reliable tests for primary and secondary infections of dengue are essential for patient management. Primary dengue infection is associated with mild to high fever, headache, muscle pain and skin rash. Immune response includes IgM antibodies produced by 3rd~5th day of symptoms and persist for 30~60 days. IgGs appear the 14th day and persist for life. Secondary infections often result in high fever and in many cases with haemorrhagic events and circulatory failure. Secondary infections show that IgGs rise within 1~2 days after the onset of symptoms and induce IgM response after 20 days of infection.



PRINCIPLE

The Dengue IgG/IgM Rapid Test is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in **human serum, plasma or whole blood.** This test also can detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins.
The Dengue IgG/IgM test device has 3 pre-coated lines, "G" (Dengue IgG Test Line), "M" (Dengue IgM Test Line) and "C" (Control Line) on the surface of the membrane. All three lines in result window are not visible before applying any samples. The "Control Line" is used for procedural control. Control line should always appear if the

test procedure is performed properly and the test reagents of control line are working. A purple "G" and "M" lines will be visible in the result window if there are enough IgG and/or IgM antibodies to dengue virus in the sample. If IgG and/or IgM antibodies to dengue virus are not present in the sample, there is no color appearance in "G" and/or "M".
When a specimen is added to the sample well, anti-Dengue IgGs and IgMs in the specimen will react with recombinant dengue virus envelope proteins-colloidal gold conjugates and forms a complex of antibodies-antigen. As this complex migrates along the length of the test device by capillary action, it will be captured by the relevant anti-human IgG and or anti-human IgM immobilized in two test lines across the test device and generate a colored line.

Materials provided

The Dengue IgG/IgM kit contains following items to perform the assay.

- Dengue IgG/IgM **test device** individually foil pouched with a desiccant
  1 **test strip** includes ;
  Gold conjugates (as main component) : Recombinant dengue virus envelope protein-gold colloid (1 ± 0.2 µg),
  Test Line "G" (as main component) : Mouse monoclonal anti-human IgG (5 ± 1 µg),
  Test Line "M" (as main component) : Mouse monoclonal anti-human IgM (5 ± 1 µg),
  Control Line (as main component) : Rabbit anti-dengue IgG (2.5 ± 0.5 µg)
- **Assay diluent**; 100 mM Phosphate buffer (5 mL), Sodium azide (0.01 % w/w)
- **Capillary pipette** 10 µL
- Package insert

ADDITIONAL NEEDED MATERIALS, NOT PROVIDED

- Clock
- Lancet
- Alcohol pad for disinfection

Precautions/Kit storage and stability

For best results, strict adherence to these instructions is required.
All specimens should be handled as being potentially infectious.
The test device should be stored at room temperature. Do not store at refrigerator.
The test device is sensitive to humidity as well as to heat.
Do not open or remove test device from individually sealed pouches until immediately before their use. Perform the test immediately after removing the test devices from the foil pouch.
Do not use it beyond the expiration date. The shelf-life of the kit is as indicated on the outer package.
Do not use the test kit if the pouch is damaged or the seal is broken.
The components (test device and assay diluent) in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.
The assay diluent contains low concentration of sodium azide as a preservative. Sodium azide is toxic and should be handled carefully to avoid ingestion and skin contact.

Warnings

- **For in vitro use only. In the United States, this kit is intended for Research Use Only.**
- **DO NOT RE-USE test device.**
- The instruction must be followed exactly to obtain accurate results. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
- Do not eat or smoke while handling specimens.
- Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
- Avoid splashing or aerosol formation.
- Clean up spills thoroughly using an appropriate disinfectant.
- Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
- Do not mix and interchange with different specimen.

Specimen Collection, Storage and Precaution

Sample collection with lancet:

1. Clean the area to be lanced with an alcohol swab.
2. Squeeze the end of the fingertip and pierce with a sterile lancet provided. Remove the first drop of blood with a sterile swab.
3. Take the 10 µL capillary pipette provided, immerse the open end in the blood drop and then release the pressure to draw blood into the capillary pipette to black line.

Collection by venipuncture

Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture.
If blood specimens are not immediately tested, they should be refrigerated at 2°C - 8°C.
When stored at 2°C - 8°C the blood specimens should be used within 3 days.
For storage period longer than 3 days, freezing is recommended. They should be brought to room temperature prior to use.
Using the blood specimens in the long-term keeping more than 3 days can cause nonspecific reaction.

Serum

Collect the whole blood into the collection tube (NOT containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.

Plasma

Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.

If serum or plasma specimens are not tested immediately, they should be refrigerated at 2°C - 8°C. For storage period longer than 2 weeks, freezing is recommended. They should be brought to room temperature prior to use. Serum or plasma specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

Precaution

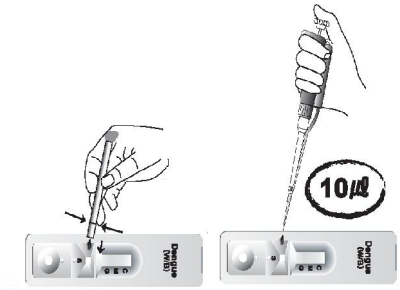
1. *Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.*
2. *As known relevant interference, hemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.*
3. *Use separately disposable capillary pipettes or pipette tips for each sample in order to avoid cross-contamination of either samples which could cause erroneous results.*

Procedure of the Test

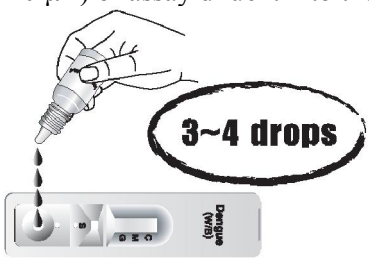
Allow all kit components and specimen to room temperature prior to testing.

1. Remove the test device from foil pouch; place it on a flat, dry surface.
2. [Using a capillary pipette]
   With a 10 µL capillary pipette provided, add 10 µL of serum, plasma or whole blood specimen drawn to black line into the square sample well marked "S". OR,

   [Using a micropipette]
   Add 10 µL of serum, plasma or whole blood specimen into the square sample well marked "S".



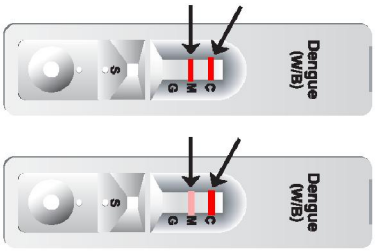
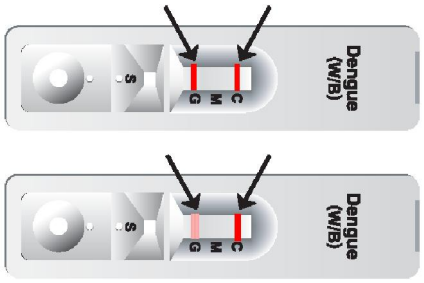
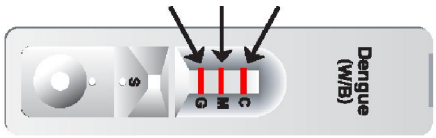
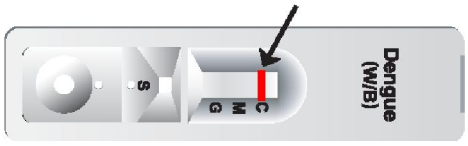
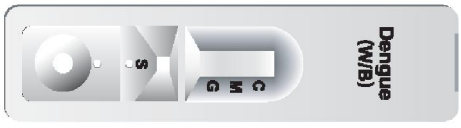
3. Put 3~4 drops (about 90 - 120 µL) of assay diluent into the assay diluent well round shaped.



4. Interpret test results in 15~20 minutes.

   Caution: Do not read test results after 20 minutes. Reading too late can give false results.

Interpretation of the test

Positive	
(1) Positive IgM – Dengue – Antibodies The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Dengue virus. This is indicative of a primary dengue infection.	
(2) <u>Positive IgG – Dengue – Antibodies</u> The control line (C) and IgG line (G) are visible on the test device. This is positive for IgG antibodies. This is indicative of secondary or previous dengue infection.	
(3) <u>Positive IgG – and IgM - Dengue - Antibodies</u> The control line (C), IgM (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies. This is indicative of a late primary or early secondary dengue infection.	
Negative	
The control line is only visible on the test device. No IgG and IgM antibodies were detected. Retest in 3-5 days if dengue infection is suspected.	
Invalid	
The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the likeliest reasons for control line failure. Repeat the test using a new test device.	

QUALITY CONTROL, INTERNAL QUALITY CONTROL

Good laboratory practice (GLP) advices to use controls to approving the correct performance of the tests.
The Dengue Test has two test lines, "M and G" and one control line "C" on the cassette. The lines can not be seen before adding the protein. The control line has to appear always if the test is processed right and the reagents are working.

Limitations

1. This test detects the presence of antibodies to dengue virus in the specimen and should not be used as the sole criterion for the diagnosis of dengue virus infection.
2. In early 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 ten days after infection. If clinical symptoms persist, patients should be re-tested in 3-4 days with the first specimen.
3. Serological cross-reactivity across the flavi virus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.
4. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
5. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of an early infection of dengue virus.
6. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.

The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. It confirms sufficient specimen volume and correct procedural technique. A clear background is also required

Expected value

Primary dengue infection is characterized by the presence of detectable IgM 3 - 5 days after the onset of infection. Secondary dengue infection is characterized by the elevation of specific IgG 1 - 2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM.

Performance Characteristics

Precision

Within-run and between-run precisions have been determined by the testing of ten specimens three times : 4 of negative, 2 of low positive, 2 of medium positive and 2 of strong positive. All values were correctly identified 100% of the time.

Cross reactivities

To evaluate the interference of Dengue IgG/IgM rapid kit with known relevant interfering specimens, the hemolytic samples, rheumatoid factors-containing samples and lipaemic, icteric samples were investigated. In these studies, those specimens did not interfere with this test kit.

Analytical Sensitivity

The limit of detection ; the smallest amount of the target marker that can be precisely detected ; have been equal or superior to a leading commercial dengue antibody detection rapid test.

Evaluation of the NADAL Dengue Tests

A. The comparison results of Dengue IgG/IgM Rapid test with Haemagglutination inhibition (HI) test showed that Dengue IgG/IgM Rapid test had good correlation with HI test

	102 Dengue IgG/IgM Positive Specimens (Reference assay : HI Test)		**Sensitivity**
	No. of Positive	No. of Negative	
Dengue IgG/IgM Rapid test	93	9	93/102x100% = 91.2%
	200 Dengue IgG/IgM Negative Specimens (Reference assay: HI Test)		**Specificity**
	No. of Positive	No. of Negative	
Dengue IgG/IgM Rapid test	20	180	180/200x100% = 90%

B. Sensitivity of the Dengue IgG/IgM Rapid test with specimen taken at the time of hospital discharge

Reference Assay	**Index Assay**	**Sample collecting timing or group**	**Dengue Infection status**	**Tp**	**Fp**	**Fn**	**Tn**
HI Test	Dengue IgG/IgM Rapid Test	Early / acute	Primary	23		5	
		Early / acute	Secondary	70		4	
		Negative group	No dengue infection		20		180

Tp : True positive, Fp : False positive, Fn : False negative, Tn : True negative

C. Cross-reactivity test with other flavi virus mediated and mosquitoes-borne disease, Dengue IgG/IgM Rapid Test showed no cross-reactivity with other flavi virus mediated disease and mosquitoes-borne disease like Malaria.

Disease	**Dengue IgM Negative/Total**	**Dengue IgG Negative/Total**
Japanese Encephalitis	25/25	25/25
Yellow Fever	25/25	25/25
Malaria *P. faciparum*	25/25	25/25
Malaria *P. vivax*	25/25	25/25
Total	100/100	100/100

Bibliography

1. Dengue haemorrhagic fever : Diagnosis, treatment, prevention and control. WHO 2nd Edition 1997
2. Songee L. ranch and Paul N. Levett. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. Clin. Diagn. Lab. Immunol. Vol 6(4) p 555-557, 1999
3. Jan Groen et al. Evaluation of six immunoassays for detection of dengue-virus specific immunoglobulin M and G Antibodies. Clin. Diagn. Lab. Immunol. Vol 7(6) p 867-871, 2000

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..