Version-1.0

RAPITEST™

Dengue IgG/IgM

INTRODUCTION

Dengue IgG/IgM is a rapid immuno-chromatographic test for the qualitative and differential detection of IgG and IgM in human serum/plasma/whole blood. Dengue virus belongs to Flaviviridae family, common in tropical and sub-tropical regions, which is found in four distinct serotypes (1, 2, 3 and 4). Dengue fever is transmitted by mosquitoes, of the species Aedes aegypti and Aedes albopictus. Primary dengue infection results mostly in a self-limiting mild to high fever, while secondary infection is more common and potentially fatal resulting in severe conditions like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). The following table gives the duration for detectable rise in NS1 antigen, IgG and IgM antibodies in blood in cases of primary and secondary infections:

Ag/Ab	Primary Infection	Secondary Infection
NS1	1-9 days upto 15 days	1-9 days
IgM	5-10 days	4-5 days
IgG	14 days till lifetime	1-2 days

PRINCIPLE

The Dengue IgG/IgM device can simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test can also detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins. The device consists of a membrane with 3 pre-coated lines: a test line "G" of monoclonal anti-human IgG, a test line "M" of monoclonal anti-human IgM and a control line "C" of goat anti-dengue IgG. The sample moves along the membrane, complexing with the recombinant dengue virus envelope protein-gold conjugate. Pink/purple "G" and "M" lines will be visible in the result window if there are enough IgG and/or IgM antibodies respectively to dengue virus in the sample. Absence of either of the test lines indicates a negative result. The unreacted complexes move even further and are immobilized at the control region "C" and form a pink/purple colored band. Absence of control line "C" indicates an INVALID TEST.

KIT CONTENTS

Dengue IgG/IgM test is supplied with:

1. Dengue IgG/IgM device test cassettes – pouched with activated silica gel.
2. A running buffer vial.
3. Instructions manual.

STORAGE AND STABILITY

The test kit can be stored at temperatures between 2°C and 30°C in the sealed pouch to the date of expiration. The test should be kept away from direct sunlight, moisture and heat.

PRECAUTION

1. For in vitro diagnostic use only.
   For Professional use.
2. Do not use test kit beyond expiry date.
3. The test device should not be reused.
4. Do not intermix reagents of different lots.
5. Follow instructions carefully to get accurate results.
6. Not for medicinal use.
7. Follow standard guidelines for personal safety, handling and disposal of potentially infectious materials.
8. Testing of pooled samples is not recommended.

SPECIMEN COLLECTION & PREPARATION

The Dengue IgG/IgM test can be run on serum/plasma/whole blood samples. The test works best on fresh samples. For serum, collect blood into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. For plasma samples, collect blood into a container with anticoagulant and separate the plasma by centrifugation. For whole blood, use a lancet to deliver blood directly to the device or collect it into a container with anticoagulant. If the serum/plasma specimen cannot be tested on the day of collection, store the specimen in a refrigerator (2 to 4°C) for upto 3 days and at -20°C for longer storage. Blood samples should be used within 24 hours of collection. Bring the samples to room temperature before testing. Do not freeze and thaw the samples repeatedly.

TEST PROCEDURE

When you are ready to begin testing, open the sealed pouch by tearing diagonally along the notch. Remove the test device from the pouch and use it as soon as possible.

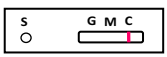
DENGUE IgG/IgM:

1.Add 5µl of serum/plasma OR 10µl whole blood sample into the sample well "S" using micropipette.
2.Add 2 drops of running buffer to the sample well "S".
3.Read test results within 5 - 20 minutes.

DO NOT READ RESULTS AFTER 20 MINUTES.
READING TOO LATE CAN GIVE FALSE RESULTS.

INTERPRETATION OF RESULTS:

DENGUE IgG/IgM NEGATIVE:



Only one colored band appears in the control (C) region. No apparent band in the test regions "G" or "M".

POSITIVE:

IgG POSITIVE

IgM POSITIVE

IgG/IgM POSITIVE

In addition to a pink colored control (C) band, a distinct pink colored band will also appear in the test region "G" for IgG positives, in the test region "M" for IgM positives and in both regions "G" and "M" for IgG/IgM positives.

INVALID:

A total absence of color in region "C" indicates procedure error and / or that the test reagent has deteriorated. Repeat with a new test. If problem persists discontinue using the kit immediately.

PERFORMANCE AND CHARACTERISTICS

In an in-house trial, four hundred and ten samples were tested in parallel with a licensed commercially available device and Dengue IgG/IgM. The results obtained are as follows:

Sample	No. of Samples	Licensed Kit	Dengue NS1	Dengue IgG/IgM
Negative	200	200	194	195
IgG Positive	47	47	-	47
IgM Positive	63	63	-	61
IgG/IgM Positive	44	44	-	43

Based on this evaluation, the sensitivity and specificity of Dengue NS1 and Dengue IgG/IgM have been found to be as follows:

Test Device	Sensitivity	Specificity
Dengue IgG/IgM	98.08%	97.56%

LIMITATIONS:

1.As with all diagnostic tests, all results must be compared with other clinical information available to the physician. A definite clinical interpretation should be made only by the physician after all clinical and laboratory findings have been evaluated.
2.The test should be used for the detection of Dengue IgG/IgM antibodies in human serum/plasma/whole blood samples.
3.If the test result is negative yet symptoms persist, confirmation using other clinical methods is recommended.A negative result at any time does not preclude the possibility of dengue infection.
4.Sample found to be positive by the above screening test must be confirmed by standard supplemental assay.
5.A negative result can occur if the quantity of Dengue virus NS1 antigen present in the specimen is below the detection limits of the assay, or the antigens are not present during the stage of disease in which the sample was collected.
6.Serological cross-reactivity across the Flavivirus group (Dengue virus, St. Louis encephalitis, Japanese encephalitis, West Nile and yellow fever virus) is common.
7.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. Where symptoms persist, patients should be re-tested 3-5 days after the first testing date.

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