Reliable
Reliable Pro-detect Biomedicals Pvt. Ltd.
In vitro Diagnostic

INTENDED USE

The *Reliable* one step Dengue IgG/IgM Rapid Test device is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM anti–dengue virus (DEN 1, DEN2, DEN3, and DEN4) in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with dengue viruses. Any reactive specimen with the *Reliable one step* Dengue IgG/IgM Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

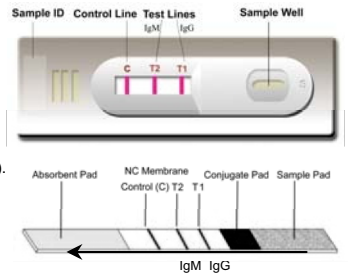
Dengue viruses, a family of four distinct serotypes of viruses (Den 1,2,3,4), are single-strained, enveloped, positive-sense RNA viruses. The viruses are transmitted by mosquitoes of the daytime-biting Stegemyia family, principally Aedes aegypti, and Aedes albopictus. Today, more than 2.5 billion people living in the areas of tropical Asia, Africa, Australia, and the Americas are at risk for dengue infection. An estimated 100 million cases of dengue fever and 250,000 cases of life-threatening dengue hemorrhagic fever occur annually on a worldwide basis[1-3].

Serological detection is a common method for the diagnosis of infection with dengue viruses. IgM anti-dengue virus starts to appear at 3 days after initial exposure and remain in the circulation for about 30-60 days. IgG anti-dengue virus raise at around 7 days, peak at 2-3 weeks, and persist for life[4-6].

The *Reliable one step* Dengue IgG/IgM Rapid Test detects IgG and IgM anti-dengue virus within 15 minutes. The test is user-friendly, without the need of cumbersome laboratory equipment.

TEST PRINCIPLE

The *Reliable one step* Dengue IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing dengue recombinant envelope antigens conjugated with colloid gold (dengue conjugates) and rabbit IgG-gold conjugates,2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with the antibody for the detection of IgG anti-dengue virus, T2 band is coated with antibody for the detection of IgM anti-dengue virus, and the C band is pre-coated with goat anti rabbit IgG.



When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. IgG anti-dengue virus if present in the specimen will bind to the dengue conjugates. The immunocomplex is then captured by the reagent coated on the T1 band, forming a burgundy colored T1 band, indicating a dengue virus IgG positive test result and suggesting a recent or repeat infection.

IgM anti-dengue virus, if present in the specimen, will bind to the dengue conjugates. The immunocomplex is then captured by the reagent pre-coated on the T2 band, forming a burgundy colored T2 band, indicating a dengue virus IgM positive test result and suggesting a fresh infection.

Absence of any T bands (T1 and T2) suggests a negative result.

The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One pipette dropper.
   c. One desiccant.
2. Sample Diluent (2 bottle, 5 mL)
3. One Package Insert (instruction for use).

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the procedure required in the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. The positive and negative controls should be kept at 2°C-8°C**.** If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately. Specimens can be stored at 2°C-8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. **Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.**

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to assay.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 5 drop (about 30-45 µL) of specimen into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result **Recommendation: Take a digital photograph for record keeping.**

QUALITY CONTROL

Using individual *Reliable one step* Dengue IgG/IgM Rapid Test Device as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

Expected results are as follows:

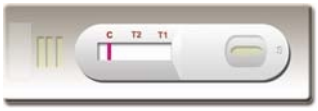
Negative Control
Only the C band shows color development, the two T bands (T1 and T2) show no color development.

Positive Control
The C band and two T bands (T1 and T2) show color development.

The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti- dengue virus antibodies are detected. The result is negative.



2. **POSITIVE RESULT**:

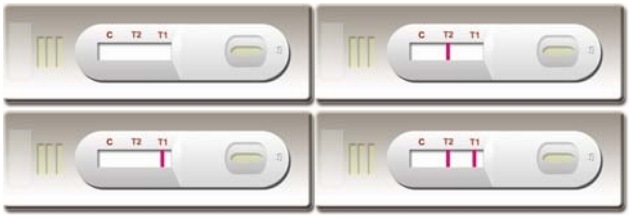
2.1 In addition to the presence of C band, if only T1 band is developed, indicates for the presence of IgG anti- dengue virus; the result is positive.

2.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of IgM anti-dengue virus. The result is positive.

2.3 In addition to the presence of C band, both T1 and T2 bands are developed, indicates for the presence of IgG and IgM anti-dengue virus. The result is also positive.

Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance For IgM Test**

A total of 224 patient samples from susceptible subjects were tested by the *Reliable one step* Dengue IgG/IgM Rapid Test and by a commercial EIA. Comparison for all subjects is showed in the following table:

	Reliable one step Dengue IgG/IgM Rapid Test		
IgM EIA Test	**Positive**	**Negative**	**Total**
Positive	22	2	24
Negative	5	195	200
Total	27	197	224

Relative Sensitivity: 91.6% , Relative Specificity: 97.5%, Overall Agreement: 96.9%

2. **Clinical Performance For IgG Test**

A total of 226 patient samples from susceptible subjects were tested by the *Reliable one step* Dengue IgG/IgM Rapid Test and by a commercial EIA. Comparison for all subjects is showed in the following table:

	Reliable one step Dengue IgG/IgM Rapid Test		
IgG EIA Test	**Positive**	**Negative**	**Total**
Positive	25	1	26
Negative	7	193	200
Total	32	194	226

Relative Sensitivity: 96.1% , Relative Specificity: 96.5%, Overall Agreement: 96.4%

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to dengue virus in serum or plasma from individual subjects. **Failure to follow the procedure may give inaccurate results**.
2. The *Reliable one step* Dengue IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to dengue virus in human serum or plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. The *Reliable one sep* Dengue IgG/IgM Rapid Test can not be used to differentiate if the infection is primary or secondary. No information of dengue serotypes can be provided with this test.
4. Serological cross reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile, yellow fever, etc.), therefore, it is possible that patients infected with these viruses may show some level of the reactivity with this test.
5. A negative result for an individual subject indicates absence of detectable dengue virus antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with dengue virus.
6. A negative result can occur if the quantity of the dengue virus antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected. Therefore, a follow up test or alternative tests such antigen test or PCR test method is recommended if the clinical findings strongly suggest an infection or when there is an outbreak.
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 only be interpreted in conjunction with other diagnostic procedures and clinical findings.**

REFERENCES

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