Reliable

Reliable Pro-detect Biomedicals Pvt. Ltd.

In vitro Diagnostic

INTENDED USE

The ***Reliable one step*** Dengue Ag Rapid Test device is a lateral flow chromatographic immunoassay for the qualitative detection of dengue virus antigen (Dengue Ag) 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 Ag Rapid Test device must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

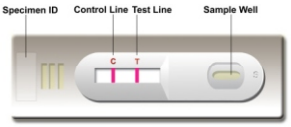
Dengue viruses, a family of four distinct serotypes of viruses (Den 1,2,3,4), are single-strained, enveloped, positive-sense RNA viruses. The viruses are transmitted by mosquitoes of the daytime-bitting Stegemyia family, principally Aedes aegypti, and Aedes albopictus. Today, more than 2.5 billion people 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 of IgM antibody is the most common method for the diagnosis of dengue virus infection. Lately, detection of antigens released during virus replication in the infected patient showed very promising result. It enables diagnosis from the first day after the onset of fever up to day 9, once the clinical phase of the disease is over, thus allows early treatment in placed promptly[4-].

The***Reliable one step*** Dengue Ag Rapid Test device is developed to detect circulating dengue antigen in serum or plasma. The test can be performed by untrained or minimally skilled personnel, without laboratory equipment.

TEST PRINCIPLE

The ***Reliable one step*** Dengue Ag Rapid Test device is a lateral flow chromatographic immunoassay. The test device consists of: 1) a burgundy colored conjugate pad containing mouse anti-dengue NS1 antigen conjugated with colloid gold (Dengue Ab conjugates), 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with rabbit anti-dengue NS1 antigen, and the C band is pre-coated with goat anti-mouse IgG antibody. The antibodies to dengue antigen recognize the antigens from all the four serotypes of the dengue virus.



When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the test cassette. Dengue NS1 Ag if present in the specimen will bind to the Dengue Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated rabbit anti-NS1 antibody, forming a burgundy colored T band, indicating a Dengue Ag positive test result.

Absence of the T band 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-mouse IgG mouse IgG-gold conjugate regardless of the presence of colored T band. 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 plastic 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 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℃-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 25 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 25 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2℃-30℃. The positive and negative controls should be kept at 2℃-8℃ . If stored at 2℃-8℃, 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℃.

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℃ to 8℃ if not tested immediately.

Store specimens at 2℃ to 8℃ up to 5 days. The spe cimens should be frozen at -20℃ 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.

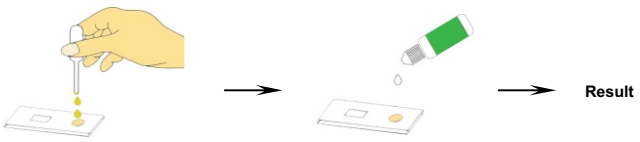
ASSAY PROCEDURE

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

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 7 drops (about 60-90 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.



7 drops of specimen **1 drop of sample diluents** **20-25 minutes**

Step 5: Set up the timer.

Step 6: Results can be read in 20-25 minutes. Positive results can be visible in as short as 1 minute.

Don't read results after 25 minutes. To avoid confusion, discard the test device after interpreting the result.

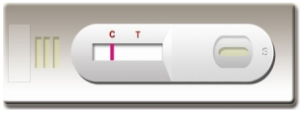
QUALITY CONTROL

Using individual *Reliable one step* Dengue Ag Rapid Test device as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) 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℃ -30℃.
5. The temperature of the test area falls outside of 15℃ -30℃

INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT: If only the C band is developed, the test indicates that the level of dengue Ag in the specimen is undetectable. The result is nonreactive.



2. POSITIVE RESULT: If both C and T bands are developed, the test indicates that the specimen contains dengue Ag. The result is reactive.

Samples with reactive results should be confirmed with alternative testing method(s) such as PCR or ELISA and clinical findings before a positive determination is made.

3. INVALID: If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 114 patient samples from susceptible subjects were tested by the *Reliable one step* Dengue Ag Rapid Test and by a commercial EIA. Comparison for all subjects is showed in the following table:

	Reliable one step Dengue (Ag)Rapid Test		
Dengue Ag EIA Test	**Positive**	**Negative**	**Total**
Positive	**66**	**3**	**69**
Negative	**2**	**43**	**45**
Total	**68**	**46**	**114**

Relative Sensitivity: 95.6%, Relative Specificity: 95.5%, Overall Agreement: 95.6%

LIMITATIONS OF TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of dengue Ag in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Reliable one step* Dengue Ag Rapid Test device is limited to the qualitativedetection of dengue Ag in human serum or plasma. The intensity of the test band does not linear correlate with dengue Ag titer of the specimen.
3. A nonreactive test result does not preclude the possibility of exposure to or infection with dengue viruses.
4. A nonreactive result can occur if the quantity of dengue Ag present in the specimen is below the detection limits of the assay, or the dengue Ag that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptom persists, while the result from *Reliable one step* Dengue Ag Rapid Test device is nonreactive result, it is recommended to re-sample the patient few days late or test with an alternative test device such as PCR, ELISA.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever. The emergence of a global health problem. Emerg Infect Dis 1995;1(2):55–57.
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3. Monath TP. Dengue: The risk to developed and developing countries. Proc Natl Acad Sci U S A 1994;91:2395–2400.
4. Alcon S, Talarmin A., Debruyne M., et al: Enzyme-Linked Immunosorbent Assay Specific to Dengue Virus Type 1 Nonstructural Protein NS1 Reveals Circulation of the Antigen in the Blood during the Acute Phase of Disease in Patients Experiencing Primary or Secondary Infections. Journal of Clinical Microbiology, 40: 376–381

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