# TRUSTline Dengue IgG/IgM Rapid Test - Cassette

REF AR0061C IVD

**for the detection and differentiation of IgG and IgM anti–dengue virus in human serum, plasma or whole blood.**



## INTENDED USE

The TRUSTline Dengue IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM anti–dengue virus (DEN1, 2, 3 and 4) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result 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(s) should be considered to confirm the test result obtained by this device.

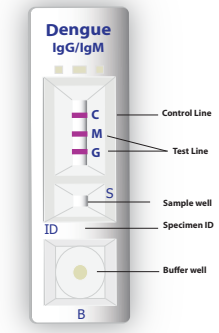
## SUMMARY AND EXPLANATION OF THE TEST

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (DEN1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting *Stegemyia* family, principally *Aedes aegypti* and *Aedes albopictus*. Today, more than 2.5 billion people living in 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 virus. IgM anti-dengue virus starts to appear 3 days after initial exposure and remains in circulation for about 30-60 days. IgG anti-dengue virus levels rise around 7 days, peak at 2-3 weeks and persist for the duration of life[4-6].

The TRUSTline Dengue IgG/IgM Rapid Test detects IgG and IgM anti-dengue virus in human serum, plasma or whole blood. It can be performed within 20-25 minutes by minimally skilled personnel without the use of laboratory equipment.

## TEST PRINCIPLE




The TRUSTline Dengue IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a burgundy colored conjugate pad containing dengue recombinant envelope antigens conjugated with colloidal gold (dengue Ag conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with antibodies for the detection of IgG anti-dengue virus, the M line is pre-coated with antibodies for the detection of IgM anti-dengue virus, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action along the cassette. IgG anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated anti-human IgG, forming a burgundy colored G line, indicating an IgG anti-dengue virus positive test result and suggesting a secondary or past infection with dengue virus.

IgM anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated anti-human IgM, forming a burgundy colored M line, indicating an IgM anti-dengue virus positive test result and suggesting either an acute primary or secondary dengue infection. An IgM and IgG positive result indicates a late primary or early secondary acute infection.

Absence of any G, M or T lines suggests a negative result. Each test contains an internal control (C line) which should exhibit a burgundy colored line of the control antibodies regardless of color development on any of the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

## REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (5 mL/bottle)
4. One package insert (instruction for use)

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Lancing device for whole blood test

## 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 of any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed 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 bio-hazardous waste.
11. Handle the Negative and Positive Controls in the same manner as patient specimens.
12. The testing results should be read 20-25 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 20-25 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. 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 1-30 °C. If stored at 1-8 °C, ensure that the test device is brought to room temperature before opening. The test device is stable until the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30 °C.

## SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

**Plasma**

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
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 venipuncture.
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. If not tested immediately, store specimens at 1-8 °C for up to 5 days. For longer storage, specimens should be kept frozen at -20 °C.

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.

**Whole Blood**

Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®). Do not use any hemolyzed blood for testing.

Whole blood specimens should be stored at 1-8 °C if not tested immediately. The specimens must be tested within 24 hours of collection.

## ASSAY PROCEDURE

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

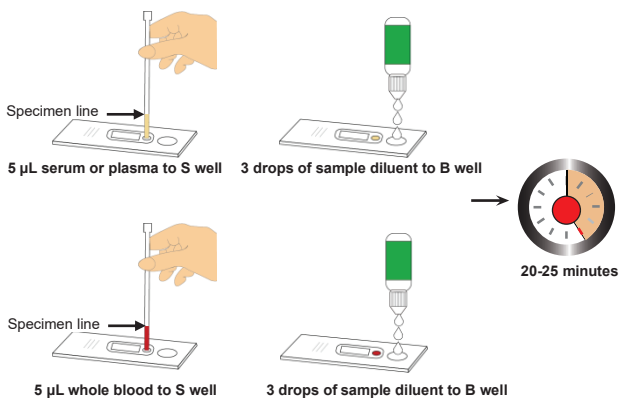
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat and dry surface.

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

Step 4: Fill the capillary tube with the serum, plasma or whole blood specimen not exceeding the specimen line as shown in the image below. The volume of the specimen is around 5 µL. ***For better precision, transfer the specimen by a pipette capable of delivering 5 µL of volume.***

Holding the capillary tube vertically, dispense the entire specimen (5 µL) into the center of the sample well **(S well)** making sure that there are no air bubbles.

Immediately add 3 drops (about 90-120 µL) of Sample Diluent into the buffer well **(B well)** with the bottle positioned vertically.




Step 5: Set up a timer.

Step 6: Read the result at 20-25 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 25 minutes only. ***Any results interpreted outside of the 20-25 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local laws governing the disposal of devices.***

## QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If there is no visible C line, review the whole procedure and repeat the test using a new device.

2. **External Control:** Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.

c. A new shipment of kits is used.
d. The temperature during storage of the kit falls outside of 1-30 °C
e. The temperature of the test area falls outside of 15-30 °C.
f. To verify a higher than expected frequency of positive or negative results.
g. To investigate the cause of repeated invalid results.

## INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C line is present, the absence of any burgundy color in both test lines (G and M) indicates that no anti-dengue virus antibodies are detected. The result is negative or non-reactive.


2. **POSITIVE RESULT**:

2.1 In addition to the presence of C line, if only the G line develops, the test result indicates that IgG anti-dengue virus is detected. The result is IgG anti-dengue virus positive or reactive.


2.2 In addition to the presence of C line, if only the M line develops, the test result indicates that IgM anti-dengue virus is detected. The result is IgM anti-dengue virus positive or reactive.


2.3 In addition to the presence of C line, if both G and M lines develop, the test result indicates that IgG and IgM anti-dengue virus are detected. The result is IgG and IgM anti-dengue virus positive or reactive.


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

3. **INVALID:** If no C line develops, the assay is invalid regardless of any burgundy color in the test lines (G and M) as indicated below. Repeat the assay with a new device.


## PERFORMANCE CHARACTERISTICS

1. **Clinical Performance for IgG Test**

A total of 327 specimens (serum, Plasma, Whole Blood) were collected from susceptible subjects, and tested with the TRUSTline Dengue IgG/IgM Rapid Test and by a commercial Dengue IgG EIA.Comparison for all subjects is shown in the following table:

| | TRUSTline Dengue IgG/IgM Rapid Test | | |
|---|---|---|---|
| **Reference Test** | **Positive** | **Negative** | **Total** |
| **Positive** | 37 | 0 | 37 |
| **Negative** | 3 | 287 | 290 |
| **Total** | 40 | 287 | 327 |

Relative Sensitivity: 100%; Relative Specificity: 98.97%; Overall Agreement: 99.08%

2. **Clinical Performance for IgM Test**

A total of 314 specimens (serum, Plasma, Whole Blood) were collected from susceptible subjects and tested with the TRUSTline Dengue IgG/IgM Rapid Test and by a commercial Dengue IgM EIA. Comparison for all subjects is shown in the following table:

| | TRUSTline Dengue IgG/IgM Rapid Test | | |
|---|---|---|---|
| **Reference Test** | **Positive** | **Negative** | **Total** |
| **Positive** | 32 | 0 | 32 |
| **Negative** | 3 | 279 | 282 |
| **Total** | 35 | 279 | 314 |

Relative Sensitivity: 100%; Relative Specificity: 98.94%; Overall Agreement: 99.04%

3. **Cross-Reactivity**

No false positive dengue IgG and IgM test results were observed on 1-10 positive specimens from the following disease states:

| | | | | |
|---|---|---|---|---|
| Chikungunya | CMV | HAV | HBV | HCV |
| HIV | hCG | *H.pylori* | HAMA | *T. gondii* |
| Typhoid | Rubella | ANA | RF (up to 8,400 IU/mL) | |

4. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the TRUSTline Dengue IgG/IgM Rapid Test.

This was studied by spiking these substances into negative and positive serum samples spiked with dengue IgG and IgM. The results demonstrate that, at the concentrations tested, the substances studied do not affect the performance of the TRUSTline Dengue IgG/IgM Rapid Test.

List of potentially interfering substances and concentrations tested:

| | | | |
|---|---|---|---|
| 1. Albumin | 60 g/L | 6. Heparin | 3,000 U/L |
| 2. Bilirubin | 20 mg/dL | 7. Salicylic acid | 4.34 mmol/L |
| 3. Creatinine | 442 µmol/L | 8. Sodium citrate | 3.8% |
| 4. EDTA | 3.4 µmol/L | 9. Human IgG | 1,000 mg/dL |
| 5. Glucose | 55 mmol/L | | |

## LIMITATIONS OF TEST

1. The Assay Procedure and the test result interpretation must be followed closely when testing for the presence of antibodies to dengue virus in serum, plasma and whole blood from individual subjects. Failure to follow the procedure may lead to an inaccurate test results.
2. The TRUSTline Dengue IgG/IgM Rapid Test is limited to the qualitative detection of IgG and IgM anti–dengue virus 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. Information about the dengue virus serotype(s) present in a specimen cannot be obtained from this test.
4. The TRUSTline Dengue IgG/IgM Rapid Test cannot differentiate primary or secondary infection.
5. Serological cross-reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile virus, yellow fever, etc.). Therefore, it is possible that patients who were exposed to these viruses may show some level of reactivity with this test.
6. A negative or non-reactive result for an individual subject indicates absence of detectable dengue virus antibodies. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with dengue virus
7. 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.
8. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
9. Infection may progress rapidly. If the symptoms persist, while the results from TRUSTline Dengue IgG/IgM Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
10. The results obtained with this test should be interpreted in conjunction with other diagnostic procedures and clinical findings.

## REFERENCES

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6. Anonymous. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: World Health Organization, 1997

**Index of Symbols**

| | | |
|---|---|---|
| Consult instructions for use | REF Catalogue number | Caution |
| IVD For *in vitro* diagnostic use only | LOT Batch code | If device is non-sterile |
| Temperature limit 1-30 °C | Do not re-use | Use-by date |
| Manufacturer | Date of manufacture | Tests per kit |
| | | Keep dry |

Do not use if package is damaged — Keep away from sunlight

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