TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test - Cassette REF AR0062C IVD

for the simultaneous detection and differentiation of IgG, IgM and dengue NS1 antigen in human serum, plasma or whole blood

INTENDED USE

The TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of anti-dengue virus IgG, IgM and dengue NS1 antigen (DEN1, 2, 3, 4) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals to aid in the diagnosis of infection with dengue virus.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and 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

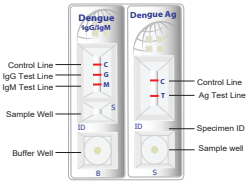
Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (DEN1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting Stegomyia family, principally Aedes aegypti and Aedes albopictus. Today, more than 2.5 billion people 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 virus. Anti-dengue virus IgM starts to appear 3 days after initial exposure and remains in circulation for about 30-60 days. Anti-dengue virus IgG rises around 7 days, peaks at 2-3 weeks and persists for the duration of life[4-6]. Detection of antigens, such as dengue NS1, released during virus replication in the infected patient show very promising results; 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, allowing early detection and prompt treatment[7].

The TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test detects anti-dengue virus IgG and IgM and circulating dengue NS1 antigen (DEN1, 2, 3, 4) in human serum, plasma or whole blood. It can be performed within 20-25 minutes by minimally skilled personnel and without the use of laboratory equipment.

TEST PRINCIPLE

The TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test contains two test strips (left side: Dengue IgG/IgM test; right side: Dengue Ag test). The Dengue IgG/IgM Rapid Test on the left-side is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing recombinant dengue 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 anti-dengue virus IgG, the M line is pre-coated with antibodies for the detection of anti-dengue virus IgM, and the C line is precoated with a control line antibody.



The Dengue Ag Rapid Test on the right-side is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing antibodies to dengue NS1 antigen, conjugated with colloidal gold (dengue Ab conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is precoated with antibodies to dengue NS1, and the C line is pre-coated with a control line antibody. The antibodies to dengue NS1 recognize the NS1 antigens from all four dengue virus serotypes.

When an adequate volume of specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Dengue NS1 antigen, if present in the specimen, will bind to the dengue Ab conjugates.

The immunocomplex is then captured on the membrane by the pre-coated antibodies to dengue NS1 antigen forming a colored T line. Anti-dengue virus IgG and/or IgM, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured by the pre-coated reagent forming a colored G and/or M line, respectively.

Dengue Ag positive result suggests an active infection. Dengue IgM positive result suggests a primary infection. Dengue IgG positive result suggests a secondary or past infection, and Dengue IgG and IgM positive result suggests late primary or early secondary infection. The results obtained with this test should be used in conjunction with other diagnostic procedures and clinical findings. Absence of any G, M or T lines suggests a negative result. Each test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies in each the left and right panels, regardless of color development on any of the test lines. If the C line does not develop in a panel, the test result is invalid and the specimen must be retested with another device. An invalid result in one panel does not invalidate the test result in the other panel.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes (for Dengue IgG/IgM test)
3. Plastic droppers (for Dengue Ag test)
4. Sample diluent (5mL/bottle)
5. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer.
2. Disposable Gloves
3. Lancing device for whole blood test
4. Alcohol Swab

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to 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 from any other type of test kit as a substitute for the components of this kit.
6. Do not use hemolyzed blood specimens 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. 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. Read the test results at 20 minutes after a specimen is applied to the sample well of the device. Any results interpreted after 25 minutes 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 2- 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 to temperatures above 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

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

Serum

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

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately, for 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 specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Drops of whole blood can be obtained by either finger tip 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 hemolyzed blood for testing. Capillary blood (fingertip puncture) can be used directly without anticoagulant. Collect blood with sample pipette and transfer it to sample well of device.

Whole blood specimens should be stored in refrigeration (2-8°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: Label the device with specimen ID number.

Step 4:

For detection of Dengue IgG/IgM

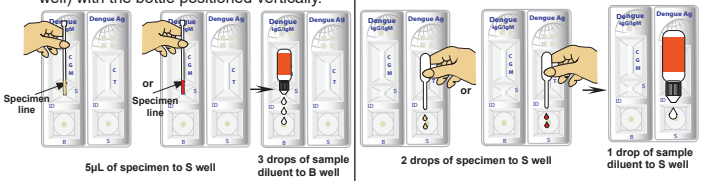
4.1 Fill the capillary tube with serum/plasma/whole blood specimen not to exceed the specimen line as shown in the images below.

4.2 Holding the capillary tube vertically, dispense the entire specimen (5 µL) into the center of the sample well (S well) ensuring 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.

For detection of Dengue Ag

4.1 Fill the plastic dropper with specimen.

4.2 Holding the dropper vertically, dispense 2 drops (about 60 µL) of serum/plasma or 2 drops of whole blood (about 70 µL) into the center of the sample well (S well), ensuring that there are no air bubbles.
Immediately add 1 drop (about 30-40 µL) of sample diluent to the sample well (S well) with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Read results at 20 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. **However, any results interpreted after 25 minutes should be considered invalid and must be repeated. Discard used device after interpreting the result following local laws governing the disposal of device.**

QUALITY CONTROL

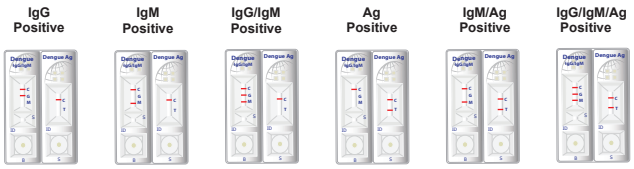
Internal Control: Each test panel contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C line is present, the absence of any color in the G, M or T lines indicates that neither anti-dengue virus antibodies nor dengue virus NS1 antigen are detected. The result is negative or non-reactive.

2. **INVALID:** If no C line develops, the assay is invalid regardless of any color in the G, M or T lines as indicated below. Repeat the assay with a new device.

3. POSITIVE RESULT:



Specimen with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic decision is made.

PERFORMANCE CHARACTERISTICS

1. Limit of Detection

The TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test was found to have a limit of detection of 0.25ng/mL as determined on recombinant dengue NS1 antigen from serotype 2 (DEN2)

2. Clinical Performance for Ag Test

A total of 353 specimens including serum, plasma and whole blood specimens were collected from susceptible subjects and normal healthy control subjects, and tested by the TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial Dengue Ag ELISA. Comparison for all subjects is shown in the following table:

	TRUSTline Duo Dengue Ag-IgG/IgM RapidTest		
Reference Test	**Positive**	**Negative**	**Total**
Positive	117	2	119
Negative	2	232	234
Total	119	234	353

Relative Sensitivity: 98.32%; Relative Specificity: 99.14%; Overall Agreement: 98.87%

3. Clinical Performance for IgG Test

A total of 327 specimens including serum, plasma and whole blood specimens were collected from susceptible subjects, and tested with the TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial Dengue IgG EIA. Comparison for all subjects is shown in the following table:

	TRUSTline Duo Dengue Ag-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%

4. Clinical Performance for IgM Test

A total of 314 specimens including serum, plasma and whole blood specimens were collected from susceptible subjects and tested with the TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test and by a commercial Dengue IgM EIA. Comparison for all subjects is shown in the following table:

	TRUSTline Duo Dengue Ag-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%

5. Cross reactivity

No cross reactivity was observed when tested the TRUSTline Duo Dengue Ag-IgG/IgM Rapid test with the following infectious diseases samples with the standard test procedure

Cross reactivity Specimen		Sample Size	Dengue IgG/IgM Reactivity	Dengue Ag Reactivity
Chikungunya Positive Specimen	Serum	5	Negative	Negative
	Plasma	3	Negative	Negative
	Whole Blood	2	Negative	Negative
HIV Positive specimen	Serum	5	Negative	Negative
	Plasma	2	Negative	Negative
	Whole Blood	3	Negative	Negative
HCV Positive Specimen	Serum	5	Negative	Negative
	Plasma	3	Negative	Negative
	Whole Blood	2	Negative	Negative
HBV Positive Specimen	Serum	3	Negative	Negative
	Plasma	1	Negative	Negative
	Whole Blood	1	Negative	Negative
Typhoid Positive Specimen	Serum	3	Negative	Negative
	Plasma	1	Negative	Negative
	Whole Blood	1	Negative	Negative
Malaria Positive Whole blood		10	Negative	Negative
Rubella Positive serum		5	Negative	Negative
ANA Positive Serum		5	Negative	Negative
HAMA Positive Serum		5	Negative	Negative
RF positive Serum (≤2,500 IU/ml)		5	Negative	Negative

6.Interference

Common substances (such as pain and fever medication, blood components) may affect the performance of the TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test. This was studied by spiking these substances into Negative and Positive Serum, Plasma and whole Blood samples spiked with dengue NS1 antigen, dengue IgG and IgM, respectively. The results demonstrate that, at the concentrations tested, the substances studied do not affect the performance of the TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin 3.5 g/dL and 5 g/dL
2. Bilirubin 1 mg/dL and 15 mg/dL
3. Creatinine 1.5 mg/dL and 5 mg/dL
4. EDTA 3.48 µmol/L
5. Glucose 80 mg/dL and 120 mg/dL
6. CRP 1 mg/dL and 4 mg/dL
7. Urea 9 mg/dL and 40 mg/dL
8. Bicarbonate 0.23 g/dL
9. Sodium citrate 3.8%

7. Dose Hook Effect

No hook effect was detected with dengue NS1 antigen concentration up to 200 µg/mL.

8. External Evaluation

The TRUSTline Duo Dengue Ag-IgG/IgM Rapid test was externally evaluated for sensitivity and specificity by District Public Health Laboratory, Government Headquarters Hospital, Manaparai The test results stated that the TRUSTline Duo Dengue Ag-IgG/IgM Rapid test showed 100% Sensitivity and 100% Specificity for Dengue Ag, 100% Sensitivity and 99.33% Specificity results for Dengue IgG and IgM.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of IgG and IgM antibodies to dengue virus and dengue NS1 antigen in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test is limited to the qualitative detection of IgG and IgM antibodies to dengue virus and dengue NS1 antigen in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the IgG and IgM antibodies and NS1 antigen titers in the specimen.
3. Information about the dengue virus serotype(s) present in a specimen cannot be obtained from this test.
4. A negative or non-reactive result for an individual subject indicates absence of detectable anti-dengue virus IgG or IgM antibodies or NS1 antigen. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with dengue virus.
5. A negative or non-reactive result can occur if the quantity of antibodies to dengue virus or dengue NS1 antigen present in the specimen is below the detection limits of the assay or the antibodies and antigen that are detected are not present during the stage of disease in which a sample is collected. For example, some patients may not produce detectable levels of IgM antibodies in early infection or repeat infection.
6. Infection may progress rapidly. If the symptoms persist while the result from TRUSTline Duo Dengue Ag-IgG/IgM Rapid Test is non-reactive, it is recommended to test with an alternative method such as PCR or ELISA.
7. Some specimens containing unusually high titers 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

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; 91:2395-400.
4. Price DD, Wilson SR, "Severe Dengue Infection." Medscape Reference Drugs, Diseases & Procedures, May 2011. Web. http://www.emedicine.com/EMERG/topic124.htm.
5. Innis BL, and Nisalak A, et al: An enzyme-linked immunosorbent assay to characterize dengue infections where denude and Japanese encephalitis co-circulate. Am J Trop Med Hygiene 1989: 40: 418-27.
6. Anonymous. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: World Health Organization, 1997.
7. 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. J Clin Microbiol, 2002, 40: 376–81.
8. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
Temperature limit 1-30 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry
		Keep away from sunlight
Do not use if package is damaged	Contains sufficient for <n> tests	

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