EZER™ Dengue IgG/IgM/NS1 Combo Rapid Test Device

INTENDED USE

The Dengue IgG/IgM/NS1 Combo Rapid Test Device is a lateral flow chromatographic immunoassay for the qualitative detection of Dengue IgG, IgM and NS1 in human whole blood, 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 Dengue IgG/IgM/NS1 Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

BACKGROUND

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 world-wide basis.

Serological detection of IgM antibodies is the most common method for the diagnosis of Dengue virus infection. Lately, the detection of antigens released during virus replication in the infected patient showed 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 treatment in place promptly.

The Dengue IgG/IgM Rapid Test is a rapid test that utilizes a combination of Dengue antigen-coated colored particles for the detection of IgG and IgM Dengue antibodies in the whole blood, human serum, or plasma.

The Dengue NS1 Rapid Test is developed to detect circulating Dengue antigen in the whole blood, serum, or plasma. The test can be performed by untrained or minimally skilled personnel, without laboratory equipment

PRINCIPLE

The Dengue IgG/IgM/NS1 Combo Rapid Test Device is a qualitative membrane-based immunoassay for the detection of Dengue antibodies and NS1 antigen in whole blood, serum, or plasma.

The IgG/IgM Rapid Test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in test line region 1 of the test. During testing, the specimen reacts with Dengue antigen-coated particles in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in test line region 1. If the specimen contains IgG antibodies to Dengue, a colored line will appear in test line region 1. In the IgM component, anti-ligand is coated in test line region 2 of the test. During testing, the specimen reacts with ligand anti-human IgM. Dengue IgM antibodies, if present in the specimen, reacts with the ligand anti-human IgM and the Dengue antigen-coated particles in the test strip, and this complex is captured by the anti-ligand, forming a colored line in test line region 2.

Therefore, if the specimen contains Dengue IgG antibodies, a colored line will appear in test line region 1. If the specimen contains Dengue IgM antibodies, a colored line will appear in test line region 2. If the specimen does not contain Dengue antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always change appeared in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

The NS1 Rapid Test is a lateral flow chromatographic immunoassay. The test cassette 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.

CONTENTS

Dengue IgG/IgM/NS1 test device	20	Tests
Sample buffer	1	Tube
IgG/IgM Plastic dropper	20	Pcs
NS1 Plastic dropper	20	Pcs.
Package Insert	1	Pc.

WARNINGS AND PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.

STORAGE CONDITIONS

All reagents are ready to use as supplied. Store unused test device 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.

SAMPLE COLLECTION AND PREPARATION

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

Whole blood:

Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.

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. Store specimens at 2°C to 8°C if not tested immediately, no longer than 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.

PROCEDURE

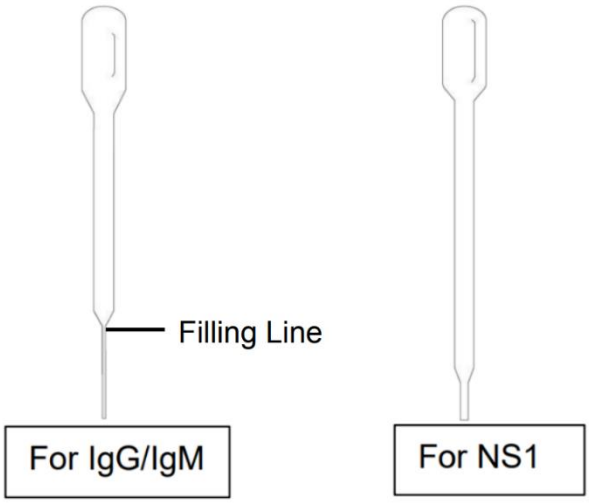
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

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: For whole blood Plasma or Serum samples:

Fill the plastic dropper with the specimen.* Holding the dropper vertically, dispense 10 μL of specimen and 2 drops of buffer into the IgG/ IgM sample well and 1 drop （approximately 30μl）of specimen and 2 drops of buffer into the NS1 sample well, making sure that there are no air bubbles.



*For specimen collection with provided dropper, draw specimen to fill entire length of the narrow tip for 10 μL volume. Release specimen in the sample well by squeezing the dropper. Repeat the same process to apply another 10 μL specimen.

Step 5: Set timer.

Step 6: Results can be read in 10 minutes.

Don't read results after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF RESULTS

Dengue IgG/ IgM Rapid Test:

POSITIVE RESULT:

IgM POSITIVE: * The colored line in the control line region (C) appears and a colored line appears in test line region 2 (M). The result is positive for Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.

IgG POSITIVE: * The colored line in the control line region (C) appears and a colored line appears in test line region 1 (G). The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.

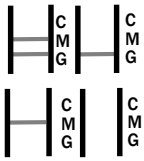
IgG AND IgM POSITIVE:* The colored line in the control line region (C) appears and two colored lines should appear in test line regions 1 and 2 (G and M). The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and is indicative of secondary Dengue infection.

*NOTE: The intensity of the color in the test line region(s) (G and/or M) will vary depending on the concentration of Dengue antibodies in the specimen. Therefore, any shade of color in the test line region(s) (G and/or M) should be considered positive.

NEGATIVE RESULT:

The colored line in the control line region (C) appears. No line appears in test line regions 1 or 2 (G or M).

INVALID RESULT:



Control band fails to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Dengue NS1 Rapid Test:

POSITIVE RESULT:

Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE RESULT:

Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

INVALID RESULT:

Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

QUALITY CONTROL

An internal procedural control is included in the test. A colored line appears in the control line region (C), confirming sufficient buffer volume and adequate membrane wicking.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

PERFORMANCE CHARACTERISTICS

1. Limit of Detection

The limit of detection (LOD) for the *EZER*™ Dengue IgG/IgM/NS1 Combo Rapid Test Device was 1:128 as the antibody titer for four type of Dengue IgM and IgG samples, and established for a total of 4 Dengue NS1 recombinant antigen：

Recombinant Antigen	mg/mL
Dengue NS1-I	3.5×10^{-3}
Dengue NS1-II	6.25×10^{-2}
Dengue NS1-III	4.5×10^{-2}
Dengue NS1-IV	1.13×10^{-2}

2. Cross Reactivity

No cross reactivity with the following specimens confirmed with related pathogen infections: Influenza A virus, Influenza B virus, Respiratory syncytial virus, Adenovirus, HBsAg, Syphilis, *H. Pylori*, HIV and HCV.

3. Interference substances

The *EZER*™ Dengue IgG/IgM/NS1 Combo Rapid Test Device was found no interference reaction with the following substances:

Substance	Concentration with no interference
Triglyceride	50 mg/dL
Ascorbic Acid	20mg/dL
Hemoglobin	1000mg/dL
Bilirubin	60mg/dL
Total cholesterol	6mmol/L

4. Clinical Studies

Dengue IgG/IgM Rapid Test:

The specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test.

Dengue IgG/IgM Rapid Test vs. ELISA

Dengue Infection	Result	IgM	IgG
Primary Infection	Positive	67	0
	Negative	2	69
	Total	69	69
Secondary Infection	Positive	43	47
	Negative	4	0
	Total	47	47
Non-Dengue Infection	Positive	0	0
	Negative	384	384
	Total	384	384

For the primary and secondary infection, the IgM Positive Percent Agreement is 94.8%, the IgG Positive Percent Agreement is 100%, and the Negative Percent Agreement is 100%.

Dengue NS1 Rapid Test:

A total of 500 patient samples from susceptible subjects were tested by the Dengue NS1 Rapid Test and by a

commercial EIA. Comparison for all subjects is showed in the following table:

		Commercial EIA		
		+	-	Total
Dengue NS1 Rapid Test	+	113	0	113
	-	3	384	387
	Total	116	384	500

Relative Sensitivity: 97.4%,
Relative Specificity: 100%,
Overall Agreement: 99.4%

LIMITATIONS OF THE PROCEDURE

1. The test for Dengue antibodies is limited to qualitative detection of Dengue IgM/IgG antibodies in human whole blood, serum or plasma. There is no linear relationship between the strength of the detection band and the concentration of antibodies in the sample.
2. 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.
3. The NS1 Test is limited to the qualitative detection of Dengue Ag in human whole blood, serum or plasma. The intensity of the test band does not linear correlate with Dengue Ag titer of the specimen.
4. A negative test result does not preclude the possibility of exposure to or infection with Dengue viruses.
5. A negative 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.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. If the symptom persists, while the result from Dengue IgG/IgM/NS1 Rapid Test is negative or non-reactive result, it is recommended to re-sample the patient few days late or test with an alternative test device such as PCR, ELISA.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

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Index of Symbols

	Attention, see instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between 2~30℃	LOT	Lot Number	REF	Catalog #

EC REP **Lotus NL B.V.**
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The Hague, Netherlands.

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