DIAGNOCURE (INDIA)™ *Diagnocure* Dengue IgG/IgM Rapid Test (Serum/Plasma/Whole Blood)

In Vitro Diagnostic

INTENDED USE

Diagnocure OneStep Dengue IgG/IgM Rapid Test is a immunochromatographic assay for the simultaneous detection of IgG and IgM antibodies to Dengue virus in human whole blood, serum or plasma. The assay is used as a screening test for Dengue viral infection and as an aid for differential diagnosis of primary and secondary infections in conjunction with other criteria.

SUMMARY AND EXPLANATION OF THE TEST

Dengue fever is one of the most important mosquito-borne diseases inorld in the terms of morbidity, mortality. Dengue fever virus (serotypes 1 – 4) belongs to the group flavivirus, and is transmitted in nature by day-biting Aceder mosquitos. The most important mosquito vector is highly domesticated and urban species, *Aedes aegypti*. Primary Dengue infection, also known as Dengue Fever, is the most common type of dengue illness. It is associated with mild to high ffever, headache, muscle pain and skin rash. Secondary infection is known as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome, and ofter results in high fever and in many cases, with hemorrhagic events and circulatory failure. The fatality rate in patients with Dengue Shock Syndrome can be as high as 44%. Dengue presents typically as a fever of sudden onset with headache, retrobullar pain, pain in the back and limbs (break-bone fever), lymphaderopathy and maculopaplar rash. Patients diagnosed with dengue in endemic areas generally have secondary infection, whereas patients in non-endemic areas are usually diagnosed with primary infection. Specific antibody responses to Dengue virus enable serodiagnosis and differentiation between primary and secondary dengue infections. Dengue Rapid Test is *a* new generation Immunochromatographic test using recombinant dengue viral antigens of all four serotypes to detect specific antibody response.

TEST PRINCIPLE

Diagnocure OneStep Dengue IgG/IgM Rapid Test utilizes the principle of Immunochromatography. Mouse anti-human IgM and human IgG antibodies are immobilized on the nitrocellulose membrane respectively, as two individual test lines (IgM line and IgG line) in the test window of the test device. The IgG line in the test window is closer to the sample well and followed by IgM line. As the test sample flows through the membrane assembly within the test device, the colored–Dengue specific recombinant antigen-colloidal gold conjugate complexes with specific antibodies (IgM or IgG) of Dengue virus, if present in the sample.

This complex moves further on the membrane to the test region where it is immobilized by the anti-human IgM and/or human IgG binding proteins coated on the membrane leading to formation of a colored band, which confirms a positive test results. Absence of this colored band in the test window indicates a negative test result. A built-in control line will always appear in the test window when the test has performed properly, regardless of the presence or absence of anti-Dengue virus antibodies in the specimen.

REAGENTS AND MATERIALS PROVIDED

2. Individually sealed foil pouches containing:
   - One cassette device
   - One desiccant
3. Plastic droppers
4. Sample Diluent (1 bottle, 5 mL)
5. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

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°C - 30°C) before use.
5. Do not use components from any other type of test kit as a substitute for the components in this kit.
6. Do not use heamolized 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. 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 test results should be read within 20 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 20 minutes may give erroneous results.
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 4°C - 30°C**.** If stored at 4°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 bio-safety procedures.

Whole Blood

Consider any materials of human origin as infectious and handle them with standard biosafety procedures. Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Blood can be obtained by finger tip puncture as well.
Whole blood specimen should be stored in refrigeration (4°C-8°C) if not tested immediately for up to 3 days. The specimen should be frozen at -20°C for longer storage. Avoid repeat freeze and thaw cycles.

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by veinpuncture.
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 veinpuncture.
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 4°C-8°C if not tested immediately for up to 5 days. 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 the test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing assay.

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

Step 3: Label device with the specimen's ID number.

Step 4: Fill the blood transfer device (sample loop, mini plastic dropper or capillary tube) with the blood specimen not to exceed the specimen line as shown in the following images. The volume of the specimen is around 10 µL.

Note: Practice a few times prior to testing if you are not familiar with the blood transfer device. For better precision, transfer specimen by pipette capable of delivering a 10 µL volume.

Holding the blood transfer device (sample loop, mini plastic dropper or capillary tube) vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Then add 2 drops (about 50-70 µL) of Sample diluent immediately.

10 µL of specimen **2 drop of sample diluent**

Step 5: Set up timer.
Step 6: Results can be read in 20 minutes. Positive results can be visible in as soon as 1 minute.

Do not read results after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kits fall outside of 4°C - 30 C.
   e. The temperature of the test area falls outside of 15°C - 30 C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

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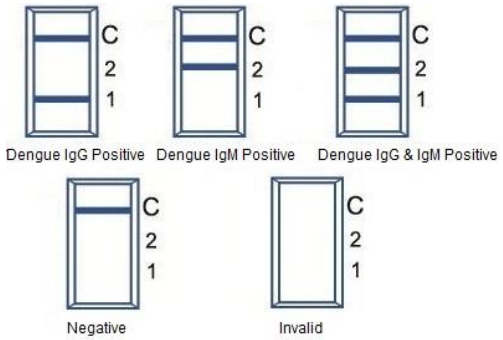
INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C line is present, the absence of any burgundy color in both test lines (1 and 2) indicates that no dengue specific antibody is detected. The result is nonreactive.
2. **POSITIVE RESULT**:
   - 2.1 In addition to the presence of the C line, if only the 1 line is developed, the test indicates the presence of IgG antibodies to Dengue virus. The result is reactive.
   - 2.2 In addition to the presence of the C line, if only the 2 line is developed, the test indicates the presence of IgM antibodies to Dengue virus. The result is reactive.
   - 2.3 In addition to the presence of the C line, if both the 1 and the 2 lines are developed, the test indicates the presence of IgG and IgM antibodies to Dengue virus. The result is also reactive.

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

3. INVALID: If no C line is developed, the assay is invalid regardless of any burgundy color in the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Accuracy**

A panel of 60 positive and 40 negative patient sera was tested with a reference *Diagnocure* Dengue Rapid Test. The results are summarized in the following table.

	Diagnocure **Dengue IgG/IgM Rapid Test**		
	Positive	**Negative**	**Total**
IgM Positive	35	0	35
IgG Positive	12	0	12
IgM/IgG Positive	13	0	13
Negative	0	40	40

Relative Sensitivity: 91.2% , Relative Specificity: 99.0%, Overall Agreement: 97.9%

2**. Cross Reactivity**

No cross reactivity with bilirubin (10 mg/dL), hemoglobin (18mg/dL) or triglycerides (up to 600 mg/dL).

LIMITATIONS OF TEST

1. The test is for qualitative detection of anti-Dengue antibody in human serum, plasma or blood sample and does not indicate the quantity of the antibodies.

2. The test is for *in vitro* diagnostic use only.

3. As in case of all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but should rather be made after all the clinical findings have been evaluated.

REFERENCES

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