



Dengue NS1 *Detect*TM Rapid Test

FOR RESEARCH USE ONLY

INTENDED USE

The Dengue NS1 *Detect*TM Rapid Test is an immunochromatographic strip assay for the qualitative detection of non-structural protein 1 (NS1) in human serum, and serves as an aid in the diagnosis of early Dengue infections. This test will aid in the rapid diagnosis of Dengue virus in human serum even prior to the presence of IgM or IgG antibodies. This test is intended for *research use only*.

SUMMARY AND EXPLANATION OF THE TEST

Dengue is an acute viral disease, which is transmitted by *Aedes aegypti* mosquitoes. Dengue is characterized clinically by biphasic fever, rash and hematopoietic depression, and by constitutional symptoms such as malaise, arthralgia, myalgia and headache (1). Infrequently, more severe disease is seen, manifested by hemorrhage fever which may progress to lethal shock (2, 3). It is endemic in the tropics and subtropics, worldwide, where an estimated 100,000,000 cases occur annually (4). It has been estimated that about 50 to 100 million cases of Dengue Fever (DF) occur every year with about 250,000 to 500,000 cases of Dengue Hemorrhagic Fever (DHF). During 2002, more than 30 Latin American countries reported over 10,000,000 (DF) cases with large number of DHF cases. This has been followed by extensive epidemics of DHF in several parts of India during 2003 through 2005. In the Americas, the reported incidence has more than tripled from 1996 to 2002. The incidence of Dengue outbreak has been reported in Hawaii (5), and in Laredo, Texas. The potential for the virus to cause a severe disease has also resulted in the inclusion of this pathogen on the CDC "category A" list for potential biological warfare and bioterrorism agents. Dengue NS1 (non-structural) protein is a multimeric secreted protein that is believed to play a role in viral RNA replication. It is strongly immunogenic eliciting antibodies with complement fixing activity. NS1 antigen can be detected in circulating blood during acute Dengue infection. The Dengue NS1 *Detect*TM Rapid Test detects NS1 antigen in serum samples following infection.

PRINCIPLE OF THE TEST

The Dengue NS1 *Detect*TM Rapid Test is a qualitative, membrane based immunoassay for the detection of NS1 antigen in human serum. The rapid test membrane is pre-coated with a NS1 specific antibody on the test line region and utilizes a separate control to assure assay flow and performance. During testing, the test sample is added directly to the sample region and the test is placed into a well containing 3 drops of buffer. The buffer and serum mix and interact with NS1-specific monoclonal antibodies conjugated to gold nanoparticles. The solution migrates upward on the membrane (via capillary action) to react with the anti-NS1 antibody on the membrane. If NS1 antigen is present, a red line will appear at the test line. The red line at the control region should always appear if the assay is performed correctly. The presence of this red line verifies that proper flow has occurred and catastrophic failure of the conjugate has not occurred.

The entire procedure takes approximately 30 minutes.

KIT CONTENTS AND STORAGE

1. Twenty-five (25) Dengue NS1 *Detect*TM Rapid Test dipsticks, individually pouched or 25 test strips in a vial with desiccant in the cap. Store at room temperature in vial.
2. Sixty-four (64) individual sample reservoir wells.
3. One (1) vial of Chase Buffer Type A, 6 ml. Store at room temperature.

Required but Not Provided

1. Pipettor and tips capable of measuring 5-50 µl of solution.

PRECAUTIONS

The sealed pouch or vial containing the test strip is designed to be stored at room temperature (22°C-30°C) for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used within 15 minutes after removal from the pouch or vial to prevent exposure to humidity (5 minutes in high humidity areas).

CHEMICAL HAZARD:

Material Safety Data Sheets (MSDS) are available for all components of this kit. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

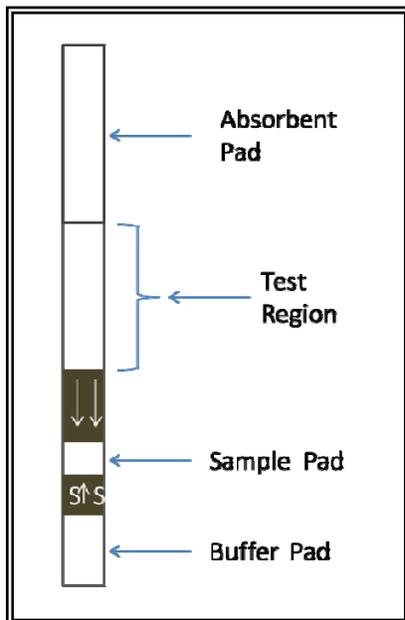
SPECIMEN COLLECTION AND PREPARATION

- Human serum must be used with this assay. Reagents have not been optimized, or tested with whole blood or plasma so they cannot be tested directly.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera if any indication of growth is observed.

TEST PROCEDURE

Before beginning, remove the Dengue NS1 *Detect* Rapid test from the foil pouch or vial and assure that all test serum samples are allowed to reach room temperature. Ensure that no physical damage (e.g., scratched membrane, torn pads, etc.) is apparent on the rapid test. Remove an individual reservoir well and SECURE the well in a microtube holder or, equivalently, a well from a 96-well ELISA plate may be used to run the assay. Never re-use reservoir wells. Always run the rapid test with a fresh well.

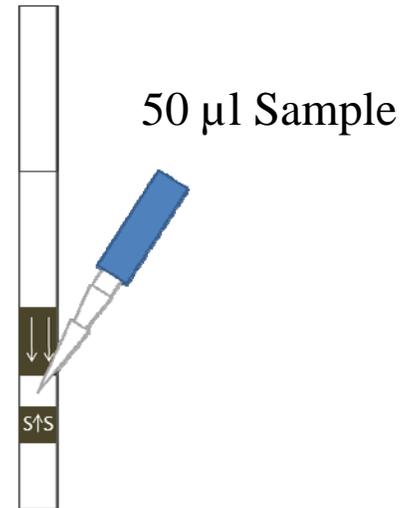
Dengue NS1 *Detect*TM Rapid Test



1. Add THREE (3) drops (approximately 120 μ l) of Chase Buffer Type A to the well.



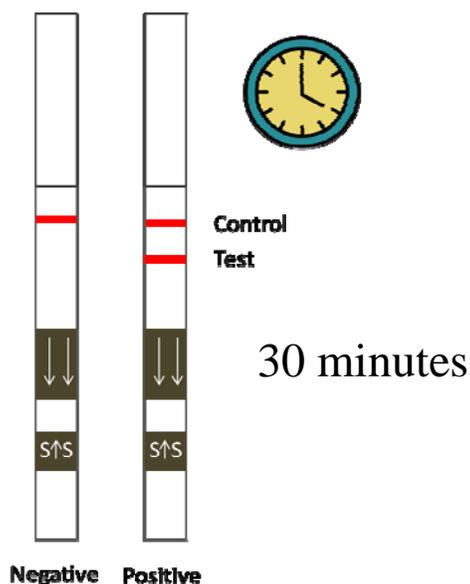
2. Carefully add 50 μ l of test sample to the Sample Pad. The Sample Pad is located between the arrows, as shown in the diagram. **DO NOT add the sample directly to the Buffer Pad or to the well shown in Step 1 above.**



3. Immediately place the rapid test in the well. Ensure that the 'Sample' side of the rapid test is facing downward into the well.

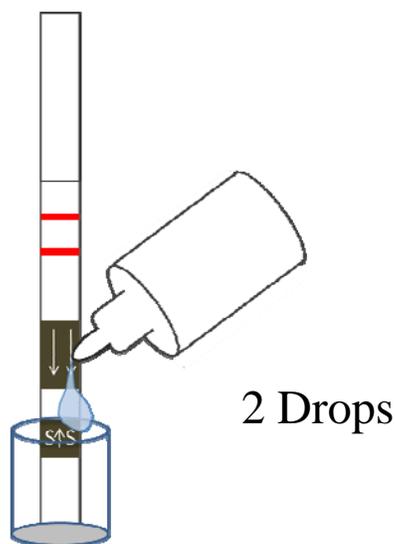


4. Read the rapid test after 30 minutes. Do NOT interpret results after 45 minutes, as this may lead to erroneous results.



OPTIONAL STEP

After completing the assay (30 minutes), transfer the rapid test strip to a new well and add 2 more drops of 'Chase Buffer Type A' to clean the test region.



Allow the test to dry in the well and store for reference. However, no further interpretation of the results should occur after the assay is completed. Doing so may lead to erroneous interpretations of the results.

INTERPRETATION OF RESULTS

A Positive Result

The test is positive for NS1 antigen when the control line (C) and the test line (T) appear in the test area. A faint line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of the NS1 antigen present. The test line for 'weakly positive' sera samples may show a weak positive but distinctly red line. The presence of a weak red test line should be considered a positive result.

A Negative Result

The test is negative when only the control line appears. No test line is present IN 30 MINUTES.

An Invalid Result

No lines appear at the control line areas. The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new Dengue NS1 rapid test and fresh serum.

Note: The red color in the test region will vary depending on the concentration of antibodies present. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

PERFORMANCE CHARACTERISTICS

The performance characteristics of this device have not been fully established.

REFERENCES

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