DENGUE Ag

For i*n Vitro* diagnostic use only

Immunochromatographic test for the qualitative detection of dengue virus antigen (Dengue Ag) in human serum, plasma or whole blood.

I. INTENDED USE

The Dengue Ag Test is a lateral flow chromatographic immunoassay for the qualitative detection of dengue virus antigen (Dengue Ag) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with dengue virus. Any reactive specimen with the Dengue Ag Test must be confirmed with an alternative testing method(s) and clinical findings.

II. SUMMARY AND EXPLANATION OF THE TEST

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (Den 1, 2, 3, 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 of IgM antibody is the most common method for the diagnosis of dengue virus infection. Lately, 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 to start promptly[4].

The Dengue Ag Rapid Test was developed to detect dengue antigen in serum, plasma or whole blood. The test can be performed by minimally trained personnel and without laboratory equipment.

III.PRINCIPLE

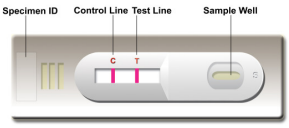
The Dengue Ag Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing mouse anti-dengue NS1 antigen conjugated with colloidal gold (Dengue Ab conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose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

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 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 mouse anti-dengue NS1 antibody forming a burgundy colored T line, indicating a Dengue Ag positive test result.

Absence of the T line suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies, regardless of the presence of a colored T line. Otherwise, the test result is invalid, and the specimen must be retested with another device.

IV. COMPOSITION OF KIT

1. Each kit contains 10 test devices, each sealed in a foil pouch with three items inside: one card (cassette) device, plastic dropper and one desiccant.
2. Sample Diluent (1 bottle,1,5 mL)
3. Instruction for use.



V. WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use and professional use only.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not open the sealed pouch, unless ready to conduct the assay.
4. Do not use expired devices.
5. Bring all reagents to room temperature (15℃-30℃) before use.
6. Do not use the components in any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolized blood specimen for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as biohazardous waste.
12. Handle the Negative and Positive Control in the same manner as patient specimens.
13. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

VI. REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2℃-30℃. The positive and negative controls should be kept at 2℃-8℃**.** If stored at 2℃-8℃, 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℃.

VII. SPECIMEN COLLECTION AND HANDLING

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

Siero

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

Sangue intero

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Do not use hemolyzed blood for testing.

Test specimens as soon as possible after collecting. Store specimens at 2℃-8℃ if not tested immediately, up to 5 days. The specimens should be frozen at -20℃ 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.**

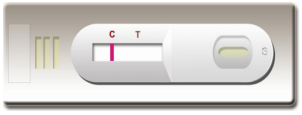
VIII. ASSAY PROCEDURE

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: Fill the pipette dropper with the specimen.
Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of serum/plasma or 2 drops (about 80-100 µL) of whole blood into the sample well making sure that there are no air bubbles.
Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

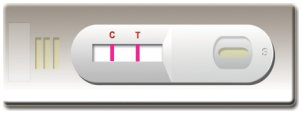
drop of specimen **1 drop of sample diluent** **20 minutes**

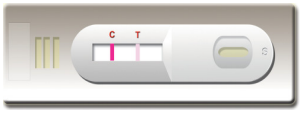
Step 5: Set up timer.
Step 6: Results can be read in 20 minutes. Positive results can be visible in as short as 1 minute.
Don't read result after 25 minutes. To avoid confusion, discard the test device after interpreting the result.

IX. READING TEST RESULTS

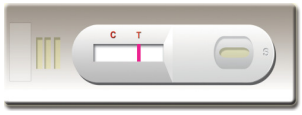


NEGATIVE RESULT: If only the C line is developed, the test indicates that the level of dengue antigen in the specimen is undetectable. The result is nonreactive or negative.





POSITIVE RESULT: If both the C and T lines are developed, the test indicates that the specimen contains dengue antigen. The result is reactive or positive.
Samples with reactive results should be confirmed with alternative testing method(s) such as PCR or ELISA and clinical findings before a positive determination is made.



INVALID: If no C line is developed, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

X. PERFORMANCES CHARACTERISTICS

1. Clinical Performance

A total of 114 patient samples from susceptible subjects were tested by the Dengue Ag Test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue Ag Rapid Test		
Dengue Ag EIA Test	**Positive**	**Negative**	**Total**
Positive	66	3	69
Negative	2	43	45
Total	68	46	114

Relative Sensitivity: 95.6%, Relative Specificity: 95.5%, Overall Agreement: 95.6%

2. Cross Reactivity

Specimens from other infectious diseases were tested for cross-reactivity with the Dengue Ag Test according to the standard procedure. The results showed that the following specimens did not cross-react with the Dengue Ag Test: HbsAg, HAV, HCV, Sifilide, TB, H.pylori

XI. LIMITATIONS

1. The Dengue Ag Test is limited to the qualitative detection of dengue antigen in human serum, plasma and whole blood. The intensity of the test line does not have a linear correlation with the dengue antigen titer of the specimen.
2. A nonreactive test result does not preclude the possibility of exposure to or infection with dengue viruses.
3. A nonreactive result can occur if the quantity of dengue antigen present in the specimen is below the detection limits of the assay or the dengue antigens that are detected are not present during the stage of disease in which a sample is collected.
4. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
5. If the symptoms persist while the result from Dengue Ag Rapid Test is nonreactive, it is recommended to re-sample the patient a few days later or to test with an alternative method such as PCR or ELISA.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

XII. REFERENCES (see Italian version)

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENTS **Cod. VQ84005 (10 test)**

Card (cassette) device	10 items
N.1 Vial with Sample Diluent	1,5 mL
Instruction for use	1 item

EDMA CODE 15 70 90 07 CE IVD