Size : 137 x 218 mm

VioTEST™ Dengue Combo

Rapid test system for the detection of Dengue NS 1 antigen and IgG/IgM antibodies to Dengue virus in human serum/plasma

DEVICE

INTENDED USE

VioTEST™ Dengue Combo is a rapid, qualitative immunochromatographic test system for the detection of Dengue NS 1 (Dengue Non-Structural Protein-1) antigen and differential detection of IgG & IgM antibodies to Dengue virus in human serum or plasma. The test system can be used as a screening test for Dengue viral infection and as an aid for differential diagnosis of the self limiting primary Dengue infections and the potentially fatal secondary Dengue infections in conjunction with other criteria.

SUMMARY

Dengue virus (serotypes 1-4) belongs to the family of Flaviviridae, which is widely distributed in the epidemic and endemic areas throughout tropical and subtropical regions of the world. Dengue virus infection is considered significant in terms of morbidity, mortality and economic cost associated with it an estimated 100 million cases of dengue fever occurring throughout the world yearly. Dengue virus is transmitted in nature principally by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. The mosquito vector is highly domesticated and an urban species. Dengue presents typically as a fever of sudden onset with headache, retro-orbital pain, pain in the back and limbs (break-bone fever), lymphadenopathy and maculopapular rash. Primary dengue virus infection is characterized by elevation in dengue virus specific NS 1 antigen level in patient's blood stream from 1-6 days after onset of symptoms. Patients diagnosed with dengue infection in endemic areas generally have secondary infection, whereas patients in non-endemic areas are usually diagnosed with primary infection. Specific antibody response to Dengue virus enables serodiagnosis and differentiation between primary and secondary dengue infections and detection of potentially life threatening conditions such as DHF and DSS.

VioTEST™ Dengue Combo is a new generation rapid Immunochromatographic test system for detection of dengue virus infection in very early stage and differential diagnosis of dengue virus infection (primary or secondary), simultaneously.

PRINCIPLE

VioTEST™ Dengue Combo test kit consists of two devices, one device for detection of Dengue NS 1 antigen and second device for differential detection of IgG & IgM antibodies to dengue virus in human serum/ plasma specimen. Both the devices - Dengue NS 1 antigen and IgG/IgM to dengue virus, the detection system utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent.

In NS 1 detection device, as the test sample flows through the membrane assembly of the device, the highly specific Agglutinating sera for dengue NS 1 - colloidal gold conjugate complexes with dengue NS 1 antigen present in the sample and travels on the membrane due to capillary action. The complex moves further on the membrane to the test region (T) where it is immobilized by another specific Agglutinating sera for dengue NS 1 coated on the membrane leading to the formation of a pink-purple band. Absence of this colored band in the test region indicates a negative test result for dengue NS 1 antigen.

In IgG/IgM detection device, specific Agglutinating sera for human IgG and specific Agglutinating sera for human IgM are immobilized on the nitrocellulose membrane as two individual test bands (IgG and IgM) at region 'G' and region 'M' respectively. As the test sample flows through the membrane assembly within the test device, the Dengue specific antigen-colloidal gold conjugate complexes with specific antibodies (IgG and/ or IgM) to Dengue virus, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the specific Agglutinating sera for human IgG and/or Agglutinating sera for human IgM coated on the membrane leading to formation of colored band/s which confirms a positive test result. Absence of these colored bands in the test region indicates a negative test result for IgG & IgM antibodies to dengue virus.

In each NS 1 & IgG/IgM device; a built-in control band in the control area marked 'C' appears when the test has been performed correctly, regardless of the presence or absence of the dengue NS 1 antigen and/ or 'anti-Dengue virus' antibodies in the specimen. It serves to validate the test performance of each device.

REAGENT AND MATERIAL SUPPLIED

VioTEST™ Dengue Combo test system comprises of,

A. Individual NS 1 test Device pouch contains:

1. DEVICE Membrane pre-dispensed with Agglutinating sera for dengue NS 1- colloidal gold conjugate, Agglutinating sera for dengue NS 1 and Agglutinating sera for mouse globulin coated at the respective regions.
2. Desiccant pouch.
3. PIPETTE Disposable Plastic Sample Applicator.

B. Individual IgG/IgM test Device pouch contains:

1. DEVICE Membrane pre-dispensed with Dengue virus specific antigen colloidal gold conjugate, streptavidin gold conjugate, Agglutinating sera for human IgG at test region 'G', Agglutinating sera for human IgM at test region 'M' &

Colour	C	M	Y	K
Mauve	30	100	0	0
Black	0	0	0	100

Size : 137 x 218 mm

Biotinylated BSA at control region 'C'.
2. Desiccant pouch.

C. BUF Sample running buffer in a dropper bottle.
D. Package insert.

REF	702040010
Σ	10 T

ADDITIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 5 µl specimen accurately.
Stop watch.

STORAGE AND STABILITY

The test kit (including sealed pouches) may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch/ carton. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30°C for remaining duration of its shelf life. DO NOT FREEZE the kit or its components.

NOTE

1. For *in vitro* diagnostic use and for professional use only. NOT FOR MEDICINAL USE.
2. Do not use the kit beyond expiry date and do not re-use the test device.
3. Read the instruction carefully before performing the test.
4. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
5. Sample dispensing applicator provided with the kit should be used for NS 1 antigen testing only.
6. Colored block printed on the pouches of NS 1 Antigen test is 'Black' and IgG/IgM antibody test is 'Violet' for easy identification of specific test device.
7. Do not inter mix the reagent or devices from different lots.
8. Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
9. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.
10. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.

SPECIMEN COLLECTION PREPARATION

1. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
2. Though fresh serum/plasma is preferable, specimen may be stored at 2°C to 8°C for up to 24 hours in sterile condition, in case of delay in testing.
3. Do not use turbid, lipaemic, icteric and haemolysed serum or plasma specimen.
4. Freezing, thawing of the specimen should be avoided.
5. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.

TEST PROCEDURE

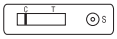
1. Bring the required number of NS 1 Antigen test pouches, IgG/IgM test pouches and buffer bottle of **VioTEST™ Dengue Combo** test system to room temperature before testing.
2. Open a foil pouch by tearing along the "notch". Remove both NS 1 antigen testing device, sample dispensing applicator and IgG/IgM testing device just prior to the testing.
3. Check the color of the desiccant pouches of both devices. It should be blue. If any of the desiccants has turned colorless or pink, discard that test device and use another device.
4. ***Once opened, the devices must be used immediately.***
5. Label both the devices with specimen identity.
6. Place both the devices on a flat horizontal surface and perform the test as below.

- **For dengue NS 1 antigen testing:**
  1. Holding the sample applicator (provided with the NS 1 test) vertically, carefully dispense exactly **3 drops** (75 µl) of the serum/plasma specimen into the specimen port (S). Alternatively, using a 75 µl precision micropipette, carefully dispense exactly 75 µl of the serum/plasma specimen into the specimen port (S).
  2. Immediately start the stopwatch and read the results at the end of **15 minutes**.
- **For IgG/IgM dengue antibody testing:**
  1. By using precision micropipette carefully add 5 µl serum or plasma specimen into the specimen port (S).
  2. Add **two drops** of sample running buffer into the same specimen port (S) and immediately start the stopwatch.
  3. Read the final result at the end of **15 minutes**.

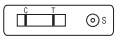
Size : 137 x 218 mm

INTERPRETATION OF RESULT

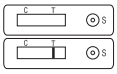
NS 1 Antigen test:



Negative Result: Only one pink / purple colored band appears at the Control Region © This indicates absence of dengue NS 1 antigen in the specimen.



Positive Result: Two pink / purple coloured bands appear at the Control Region (C) and Test Region (T). This indicates that the specimen contains detectable level of Dengue NS 1 antigen.



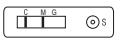
Invalid Result: The test result is invalid if no band appears on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

IgG/IgM antibody test:

Negative result: The presence of only single pink-purple coloured band in the control area marked 'C', indicates the absence of specific antibodies against Dengue virus or that the amount of antibodies is below the detection limit of the test.

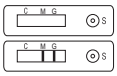
Positive Results:

In addition to the band in the control area marked 'C', appearance of two pink-purple coloured bands in the test region 'G' and region 'M', indicates the presence of Dengue virus specific IgM and IgG antibodies.



In addition to the control band in the control area marked 'C', appearance of a pink-purple coloured band in the test region 'M', indicates the presence of Dengue virus specific IgM antibodies.

In addition to the control band in the control area marked 'C', the appearance of a pink-purple coloured band in the test region 'G', indicates the presence of Dengue virus specific IgG antibodies.



Invalid Result: The test result is invalid if no band appears on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

In an in-house evaluation study, **VioTEST™ Dengue Combo** was evaluated with 80 known dengue positive samples (NS 1 Positive & IgM, IgG positive) and 60 known negative samples in comparison with a commercially available Dengue NS 1 & IgG/IgM detection test. 100% correlation in result for NS 1 and 98% correlation in result for IgG/IgM tests have been found.

LIMITATION OF THE TEST

1. **VioTEST™ Dengue Combo** test system detects the presence or absence of dengue NS 1 antigen and IgM and /or IgG antibodies to dengue virus in the human serum/plasma specimen. It should not be used as sole criteria for the diagnosis of dengue infection.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated.
3. Though **VioTEST™ Dengue Combo** does provide evidence to distinguish the past (secondary) infection from current (primary) ongoing infection, a negative result does not always preclude the sero-status of the infection of Dengue virus. Patient should be re tested after 3-4 days in case of clinically non-correlated result.
4. Serological cross reactivity across the other Flavi virus group may be occurred in certain cases.
5. It is a screening test, therefore isolation of virus, antigen detection in fixed tissue, RT-PCR; etc., or any other alternative diagnostic methods can be used for confirmation.
6. Various studies have reported interference due to presence of heterophile antibodies in patient's sample **VioTEST™ Dengue Combo** uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
7. IgM levels rise quickly and peak by one week after onset of symptoms and then fall to become undetectable over 2-3 months. IgG antibodies rise quickly and peak at about two weeks post infection and then decline slowly over 3-6 months.
8. Do not interpret the test results beyond 30 minutes.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Maria G. Guzman, Gustavo Kouri, Clinical and Diagnostic Laboratory Immunology, Advances in Dengue Diagnosis, Nov 1996, Vol. 3, No.6, p. 621-627.
2. Chew Thang Sang, Lim Siew Hoon, Andrea Cuzzubbo, Peter Devine, Clinical Evaluation of a rapid immunochromatographic test for the diagnosis of Dengue Virus Infection, May 1998, p 407-409.

Size : 137 x 218 mm

3. Dengue: Guideline for diagnosis, treatment, prevention and control. New edition. (WHO-TDR), Geneva: World Health Organization 2009.
4. Hematological observations as diagnostic markers in dengue hemorrhagic fever – a reappraisal, Sunil Gombe, K.N. Agarwal, P. Gupta, Piyush Gupta and D.K. Dewan, Indian Pediatrics 2001:38: 477-481.
5. Data on file: Zephyr Biomedicals.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Do not reuse
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	BUF — Sample Running Buffer
Use by	REF — Catalogue Number	DEVICE — Device	LOT — Batch Number / Lot Number
Σ — Contains sufficient for <n> tests		PIPETTE — Disposable Plastic Sample Applicator	

Manufactured by:

Zephyr Biomedicals

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