CE

Dengucheck™ 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

Dengucheck™ 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.

Dengucheck™ 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

Dengucheck™ Combo is a dual test device assembly comprising of Dengue NS 1 antigen detection and detection of IgG/IgM antibodies to dengue virus in human serum/ plasma specimen. 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. As the sample flows through the membrane assembly for NS 1 detection, 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. For IgG/IgM detection, as the test sample flows through the membrane assembly, 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 test assembly; 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

Dengucheck™ Combo kit contains

A. Individual pouch containing

1. Device assembly comprising of

DEVICE **NS 1 Test Assembly**

Membrane pre-dispensed with Agglutinating sera for dengue NS 1- colloidal gold conjugate, Agglutinating sera for dengue NS1 at test 'T' region and Agglutinating sera for mouse globulin coated at control 'C' band.

DEVICE **IgG/IgM Test Assembly**

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' & Biotinylated BSA at control region 'C'

2. Desiccant pouch.

B. PIPETTE Disposable Plastic Sample Applicator.

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

REF	502020010	502020025
	10 T	25 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 4ºC to 30ºC till the duration of the shelf life as indicated on the pouch/ carton. DO NOT FREEZE the kit or its components. After first opening of the sample running buffer bottle, it can be stored between 4ºC to 30ºC for remaining duration of its shelf life.

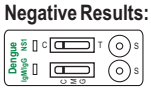
NOTE
1. For *in vitro* diagnostic use and for professional use only. NOT FOR MEDICINAL USE.
2. Do not use the kit beyond expiry date and do not re-use the test device.
3. Read the instruction carefully before performing the test.
4. Any modification to the given procedure and / or use of other reagents will invalidate the test procedure.
5. Sample applicator provided in the kit should be used for NS 1 antigen testing only.
6. Do not inter mix the reagent or devices from different lots.
7. 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.
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.

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. Refrigerated specimens must be brought to room temperature prior to testing.
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 pouch and buffer bottle of **Dengucheck™ Combo** to room temperature before testing.
2. Open a foil pouch by tearing along the “notch”. Remove the test device assembly just prior to the testing.
3. Check the colour of the desiccant pouch of device. It should be blue. If the desiccant has turned colorless or pink, discard the test device and use another device assembly.
4. **Once opened, the device must be used immediately.**
5. Label the device with specimen identity.
6. Place the device on a flat horizontal surface and perform the test as below.
   - **For dengue NS 1 antigen testing:**
   1. Holding the sample applicator (provided in the kit) 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'.
   - **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.
7. Immediately start the stopwatch and read the results at the end of **15 minutes**.

INTERPRETATION OF RESULT

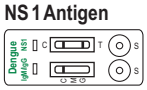
Negative Results:



Only one pink/purple colored band appears at Control Region 'C' of both Dengue NS 1 & IgG/IgM devices. This indicates absence of dengue NS 1 antigen and IgG/IgM antibodies to dengue virus in the specimen.

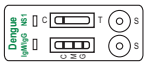
Positive results for:

NS 1 Antigen

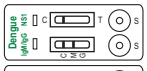


Two pink / purple coloured bands appear at the Control Region 'C' and Test Region 'T'. This indicates that the specimen contains detectable level of Dengue NS 1 antigen.

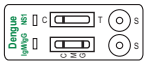
IgG/IgM Antibodies



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 IgG and IgM 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 results:

The test result is invalid if no bands appear 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, 100% co-relation in results were obtained when **Dengucheck™ Combo** was evaluated in comparison with commercial ELISA kit for detection of **Dengucheck™- NS 1** Antigen and Dengue IgG & IgM antibodies respectively.

The results are summarised in Table 1 & Table 2.

Table 1. NS 1 Antigen Detection

Specimens	Nos. Tested	Dengucheck™ Combo	Commercial ELISA	Dengucheck™ Combo	
				Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
Dengue NS 1 Negative	28	28	28	100% (87.66% to 100%)	-
Dengue NS 1 Positive	12	12	12	-	100% (73.54% to 100%)

Table 2. Dengue IgG/IgM Antibody Detection

Specimens	Nos. Tested	Dengucheck™ Combo	Commercial ELISA	Dengucheck™ Combo	
				Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
Dengue Negative	20	20	20	100% (83.16% to 100%)	-
Dengue IgG Positive	09	09	09	-	100% (66.37% to 100%)
Dengue IgM Positive	11	11	11	-	100% (71.51% to 100%)

LIMITATION OF THE TEST

1. **Dengucheck™ 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 **Dengucheck™ 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 occur 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 reported interference due to presence of heterophile antibodies in patient's sample **Dengucheck™ 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. Advances in Dengue Diagnosis, Maria G. Guzman, Gustavo Kouri, Clinical and Diagnostic Laboratory Immunology, Nov 1996, Vol. 3, No.6, p. 621-627.
2. Clinical Evaluation of a rapid immunochromatographic test for the diagnosis of Dengue Virus Infection, Chew Thang Sang, Lim Siew Hoon, Andrea Cuzzubbo, Peter Devine, May 1998, p 407-409.
3. Dengue and Dengue Hemorrhagic Fever, Duane J. Gubler, Clinical Microbiology Reviews, July 1998, Vol 11 No.3, p. 480-496/ (4) Dengue: Guideline for diagnosis, treatment, prevention and control. New edition. (WHO-TDR), Geneva: World Health Organization 2009.
5. 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.
6. Data on file: Viola Diagnostic Systems.

SYMBOL KEYS

Temperature Limitation	Manufacturer	DEVICE Device	EC REP Authorised Representative in the European Community
Use by	Consult Instructions for use	PIPETTE Disposable Plastic Sample Applicator	
Date of Manufacture	REF Catalogue Number	BUF Sample Running Buffer	Xn NaN., R22. S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
LOT Batch Number / Lot Number	IVD *In vitro* Diagnostic Medical Device	This side up	
Σ Contains sufficient for <n> tests	Do not reuse	Do not use if package is damaged	

Manufactured by:

Viola Diagnostic Systems

A Division of Tulip Diagnostics (P) Ltd.

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EC REP

CMC Medical Devices & Drugs S.L., Spain.

0923/VER-03