Size : 137 x 218 mm

CE

Dengucheck™ WB

Rapid test for the detection of IgM & IgG antibodies to Dengue Virus

DEVICE

INTENDED USE

Dengucheck-WB™ is a rapid, qualitative immunochromatographic test for simultaneous detection of IgM & IgG antibodies to Dengue virus in human serum/plasma/ whole blood. The test system can be used for screening of 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. 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-WB™ is a new generation rapid Immunochromatographic test using highly purified and specific Dengue antigens. It is a simple test for the differential diagnosis of dengue virus infection.

PRINCIPLE

Dengucheck-WB™ 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. Specific Agglutinating sera for Human IgM and Agglutinating sera for Human IgG are immobilized on the nitrocellulose membrane as two individual test bands (IgM and IgG) in the test device at region 'M' and region 'G' respectively. The IgG band is closer to the sample well and the IgM band is close to the control area marked 'C'. As the test sample flows through the membrane assembly within the test device, the colored–Dengue specific antigen-colloidal gold conjugate complexes with specific antibodies (IgM and IgG) 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 a colored band which confirms a positive test result. Absence of these colored bands in the test region indicates a negative test result. 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 anti-Dengue virus antibodies in the specimen and serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED

Dengucheck-WB™ kit contains:

A. Individual pouches, each containing:
   1. DEVICE : Membrane assembly pre-dispensed with Dengue virus specific antigen colloidal gold conjugate, streptavidin gold conjugate, Agglutinating sera for Human IgM at test region 'M', Agglutinating sera for Human IgG at test region 'G' and Biotinylated BSA at control region 'C'.
   2. Desiccant pouch.

B. BUF : Sample running buffer in a dropper bottle.

C. Package Insert

REF	502010010	502010025
Σ	10	25

OPTIONAL 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. After first opening of the sample running buffer bottle, it can be stored between 4ºC to 30ºC for the remaining duration of its shelf life.

Colour	C	M	Y	K
Black	0	0	0	100
Green	100	20	100	10

Size : 137 x 218 mm

NOTES

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. Do not inter mix the reagent or devices from different lots.
6. 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.
7. Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
8. 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.

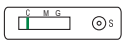
SPECIMEN COLLECTION AND PREPARATION

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 2ºC to 8ºC for upto 24 hours, in case of delay in testing.
3. Whole blood samples collected with a suitable anticoagulant such as EDTA or Heparin or Oxalate can also be used.
4. Do not use turbid, lipaemic, icteric and haemolysed specimen.
5. Repeated freezing, thawing of the specimen should be avoided.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.

TEST PROCEDURE

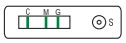
1. Bring the **Dengucheck-WB**™ kit components to room temperature before testing.
2. Open the foil pouch by tearing along the "notch".
3. Retrieve the device and desiccant pouch. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard that test device and use another device.
4. ***Once opened, the device must be used immediately.***
5. Tighten the cap of the sample running buffer bottle provided with the kit in clockwise direction to pierce the dropper bottle nozzle.
6. Label the test devices with patient's identity.
7. Place the device on a flat horizontal surface.
8. By using precision micropipette carefully add 5 µl serum/ plasma/whole blood specimen into the specimen port (S).
9. Add **two drops** of sample running buffer into the same specimen port (S) and immediately start the stopwatch.
10. Read the final result at the end of **15 minutes**.

INTERPRETATION OF RESULT



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 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 Result: The test result is invalid if no band appears at the Control Region (C). In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

1) In an in-house evaluation, 42 known positive and 165 known negative specimens were tested with **Dengucheck-WB**™ and compared with a licensed commercially available ELISA test. The results obtained were as follows:

Specimen Type	No. of Specimens Tested	Licensed Test	Dengucheck-WB™
Negative for Ab. to Dengue	165	165	165
Positive for Ab. to Dengue	42	42	42

Based on the above study, the specificity and sensitivity of **Dengucheck-WB**™ is 100%

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2) 25 samples were evaluated in an external study comprising of primary, secondary and negative Dengue sera, along with Japanese Encephalitis sera (JE) in parallel with Dengue IgM/ IgG ELISA and JE ELISA. **Dengucheck-WB**™ gave concordant results with all the samples with no cross reactivity with JE positive sera.

LIMITATIONS OF THE TEST

1. **Dengucheck-WB**™ test detects the presence or absence of IgM and /or IgG antibodies to dengue virus in the human serum/plasma/whole blood 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-WB**™ 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 have reported interference due to presence of heterophile antibodies in patient's sample. **Dengucheck-WB**™ uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
7. 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 Theng Sang, Lim Siew Hoon, Andrea Cuzzubbo, Peter Devine.
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 Gomber, V.G. Ramachandran, Satish Kumar, K.N. Agarwal, P. Gupta, Piyush Gupta and D.K. Dewan. Indian Pediatrics 2001:38: 477-481.
6. Data on file: Zephyr Biomedicals.

Size : 137 x 218 mm

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 Dilution Buffer
Use by	REF — Catalogue Number	DEVICE — Device	LOT — Batch Number / Lot Number
Σ — Contains sufficient for <n> tests		EC REP — Authorised Representative in the European Community	

Manufactured by:

Zephyr Biomedicals

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EC REP

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

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