SBio Dengue Combo Test

Rapid test for Dengue NS-1 antigen and IgG/IgM antibodies to Dengue Virus

REF	90241015
Σ	15 T

CE

Temperature Limitation	Manufacturer	PIPETTE — Disposable Plastic Sample Applicator	EC REP — Authorised Representative in the European Community	NS-1 Ag / IgG/IgM — Rapid test for Dengue NS-1 antigen and IgG/IgM antibodies to Dengue Virus
Use by (Last day of stated month)	Consult Instructions for use	DEVICE — Device	BUF — Assay 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.
Date of Manufacture	REF — Catalogue Number	Σ — Contains sufficient for <n> tests	Do not reuse	
LOT — Batch Number/ Lot Number	IVD — *In vitro* Diagnostic Medical Device	This side up	Do not use if package is damaged	

INTENDED USE

SBio Dengue Combo Test is a rapid one step immunochromatographic assay designed to detect Dengue NS-1 antigen and IgG and IgM antibodies to dengue virus in human serum or plasma. This test is intended for professional use as an aid in early diagnosis of dengue infection and for differential diagnosis of primary and secondary dengue infection.

SUMMARY

Dengue virus is a member of the virus family Flaviviridae and is transmitted to people through the bite of the mosquitoes *Aedes aegypti* and *Aedes albopictus*. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Infection with any of these viruses may be asymptomatic or cause a self-limiting febrile illness known as dengue fever. Subsequent infections by other serotypes increase the risk of developing severe dengue or a life-threatening syndrome called dengue hemorrhagic fever (DHF)/ dengue shock syndrome. The significance associated with dengue infection is due to morbidity and mortality caused by the disease. WHO currently estimates there may be 50–100 million dengue infections worldwide every year.

Dengue NS-1 (nonstructural protein-1) antigen is present at high concentrations in the sera of dengue infected patients during early clinical phase. This antigen marker is considered to be a sensitive diagnostic marker for detection of dengue infection in early stage. Dengue NS-1 antigen has been detected in the serum of infected individuals as early as first day and upto 6 days after onset of symptoms in both primary and secondary infection. Dengue NS-1 antigen levels decline when antibodies start appearing.

In primary infection IgM antibodies become detectable about 4-6 days after onset of illness, followed by IgG antibodies which is detectable at low titer at the end of the first week of illness, which slowly increases. Secondary infection in contrast to primary infection, results in appearance of high levels of anti-dengue IgG antibodies before IgM antibodies. The IgG antibody levels rise quickly reaching to a peak in about 2 weeks and may persists for years.

PRINCIPLE

The test system consists of two devices, NS-1 Ag device for detection of dengue NS-1 antigen and IgG/IgM device for the differential detection of IgG & IgM antibodies to dengue virus in human serum/ plasma specimen. Both the devices are based on the principle of immunochromatography.

In NS-1 Ag device

When the test specimen is added to the specimen port of the NS-1 Ag device, dengue NS-1 antigen if present in the specimen binds to the anti- dengue NS-1 antibody - colloidal gold conjugate present in the membrane and this complex then migrates towards the test region (T) where it reacts with another specific anti-dengue NS-1 antibody at the test region (T) thus leading to the formation of a pink-purple band. If the specimen does not contain dengue NS-1 antigen, then no colored band is formed in the test region (T).

In IgG/IgM device

When the test specimen is added to the specimen port of IgG/IgM device, specific antibodies IgM or IgG or both if present in the specimen binds to the dengue specific antigen-colloidal gold conjugate present in the membrane and this complex migrates towards the test region where it reacts with the specific anti-human IgG antibody at the region marked 'G' and specific anti human IgM antibody at the region marked 'M' leading to the formation of colored bands at the respective region indicating a positive test for either IgM/IgG or both. The appearance of a colored band at control region marked 'C' in both NS-1 Ag device and IgG/IgM device indicates that the test has been performed properly. If no band appears at the Control region 'C' then the test is considered invalid and has to be repeated with new device.

REAGENT AND MATERIAL SUPPLIED

SBio Dengue Combo Test kit contains individually packed seperate pouches containing **NS- 1Ag Device** and **IgG/IgM Device**.

A. Individual **NS- 1Ag Device** Pouch contains

1. A test device: Consisting of a nitrocellulose membrane pre-dispensed with
   - Anti- dengue NS-1 antibody colloidal gold conjugate,
   - Test region (T) coated with monoclonal 'anti-dengue NS-1' antibody
   - Control region (C) coated with anti-mouse globulin.
2. A desiccant pouch.
3. A plastic disposable specimen dispensing dropper.

B. Individual **IgG/IgM Device** Pouch contains:

1. A test device: Consisting of a nitrocellulose membrane pre-dispensed with
   - Dengue virus specific antigen colloidal gold conjugate and Streptavidin gold conjugate
   - Test region coated with ' anti human IgG at region marked 'G' and anti-human IgM at region marked 'M'.
   - Control region (C) coated with Biotinylated BSA.
2. A desiccant pouch.

C. Buffer bottles with sample running buffer for IgG/IgM (Dengue) testing.
D. Package insert.

OPTIONAL MATERIAL REQUIRED

5 µl calibrated micropipette and a stop watch.

STORAGE AND STABILITY

SBio Dengue Combo Test kit can be stored at 4-30ºC till the shelf life mentioned on the pouch / carton. DO NOT FREEZE. The opened bottle of sample running buffer can be stored between 4-30ºC for till its shelf life.

NOTE

1. For *in vitro* diagnostic use and professional use only. NOT FOR MEDICINAL USE. DO NOT RE-USE THE DEVICE.
2. Use within the expiry date mentioned in the carton/pouch.
3. Always read the instruction carefully before performing the test.
4. Do not modify the above procedure or use other reagents as this will invalidate the test procedure.
5. The specimen dispensing dropper provided in the kit is to be used for NS-1 antigen testing only.
6. Do not interchange the buffer or the devices of different lots.
7. Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
8. The IgM/IgG device 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. Fresh serum/plasma may be used for the test, if specimens cannot be tested immediately they may be stored at 2-8 °C for up to 24 hours and then used provided they are not contaminated.
2. The use of turbid, lipaemic, icteric and haemolysed serum or plasma specimen should be avoided as it may lead to erroneous results.
3. Repeated freezing, thawing of the specimen should be avoided.
4. Specimen containing precipitates or particulate matter may yield erroneous results. Such specimens must therefore be centrifuged and the clear supernatant should only 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 SBio Dengue Combo Test system to room temperature before testing.
2. Tear open the individual pouches along the "notch'. Remove the NS-1 antigen device and IgG/IgM testing device from respective pouches along with the desiccant and dropper.
3. Check the color of the desiccant in both pouches. It should be blue. If the color of the desiccant has turned colorless or pink, discard that test device and use another device.
4. *Once opened, the devices should be used immediately.*
5. Label the devices with the patient's name or identification number.
6. Place both the devices on a flat horizontal surface and perform the test as below.

A) Dengue NS-1 Ag test:

1. Holding the specimen dropper vertically, add exactly 3 drops i.e., 75 µl of the serum/plasma specimen into the specimen port (S). Or using a 75 µl precision micropipette, carefully dispense exactly 75 µl of the serum/plasma specimen into the specimen port (S).
2. Start the stopwatch immediately and wait for the specimen to flow through the membrane . At the end of 15 minutes read the result.

B) Dengue IgG/IgM antibody test:

1. With a calibrated micropipette, carefully dispense 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).
3. Start the stopwatch immediately and wait for the specimen to flow through the membrane . At the end of 15 minutes read the result.

RESULT INTERPRETATION

A) Dengue NS-1 Ag test:

fig (1)

Negative Result: As shown in the figure (1). Appearance of only one pink / purple colored band at the Control Region (C) indicates absence of Dengue NS-1 antigen in the specimen.

fig (2)

Positive Result: As shown in the figure (2). Appearance of two pink / purple colored bands one at the Control Region (C) and another at the Test Region (T) indicates that the specimen contains detectable level of Dengue NS-1 antigen.

fig (3)

Invalid Result: As shown in the figure (3) the test result is to be considered invalid if no band appears at the Control Region (C). In such cases, repeat the test with a new device.

IgG/IgM antibody test:

fig (4)

Negative result: As shown in the figure (4). Appearance of only one pink / purple colored band at the Control Region (C) indicates the absence of specific antibodies against Dengue virus or that the amount of antibodies is below the detection limit of the test.

fig (5)

Positive Results:
As shown in the figure (5) Appearance of two pink-purple coloured bands in the test region 'G' and region 'M' respectively along with a band in the control region 'C', indicates the presence of Dengue virus specific IgG and IgM antibodies.

fig (6)

As shown in the figure (6) Appearance of a pink-purple coloured band in the test region 'M', indicates the presence of Dengue virus specific IgM antibodies.

fig (7)

As shown in the figure (7) Appearance of a pink-purple coloured band in the test region 'G', indicates the presence of Dengue virus specific IgG antibodies.

fig (8)

Invalid Result: As shown in the figure (8) the test result is to be considered invalid if no band appears at the Control Region (C). In such cases, repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

SBio Dengue Combo Test was evaluated in-house with 80 known dengue positive specimens (NS-1antigen Positive & IgM, IgG positive) and 60 known negative specimens. The test performance was evaluated and compared with a commercially available Dengue NS-1 antigen & IgG/IgM detection test. Evaluation results showed 100% correlation for NS-1 antigen and 98% correlation in result for IgG/IgM tests.

LIMITATION OF THE TEST

(1) SBio 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 SBio Dengue Combo Test 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 specimen SBio Dengue Combo Test uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference. (7) Do not interpret the test results beyond 30 minutes.

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