Accu-Sreen Dengue IgG/IgM and NS1 Test Device

Package Insert

For qualitative detection of Dengue IgG and/or IgM antibodies and NS1 antigen in Human Serum, Plasma or Whole Blood.
For professional in vitro diagnostic use only.

INTENDED USE

Dengue IgG/IgM and NS1 Rapid Test Device is a solid phase immunochromatographic assay for the qualitative and differential detection of IgG and IgM antibodies to dengue virus and NS1 antigen of Dengue virus in human serum,plasma or whole blood.This test is intended for professional use as an aid in the presumptive diagnosis between primary and secondary dengue infection.This test provides only a preliminary test result.Therefore,a more specific diagnosis method must be used in order to obtain a confirmation of dengue virus infection.

SUMMARY

Dengue viruses,transmitted by Aedes aegypti and Aedes albopictus mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world. There are four known distinct serotypes (dengue virus 1,2,3,and 4).In children,infection is often sub-clinical or causes a self-limited febrile disease. However, if the patient is injected a second time with a different serotype, a more severe disease, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur.Dengue is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality associated with it. Rapid and reliable tests for primary and secondary infections of dengue are essential for patient management. Primary dengue infection is associated with mild to high fever,headache, muscle pain,and skin rash.Immune response includes IgM antibodies produced by the 3rd to 5th day of symptoms and persist for 30-60 days. IgG appear the 14th day and persist for life. Secondary infections often result in high fever and in many cases with haemorrhagic events and circulatory failure.Secondary infections show that IgG rise within 1-2 days after the onset of symptoms and induce IgM response after 20 days of infection.
Dengue is a flavivirus,[1] and causes up to 100 million infections annually.[2] Classic Dengue infection is characterized by a sudden onset of fever,intense headache,myalgia,arthralgia and rash.NS1 is one of 7 Dengue Virus non-structural proteins which are thought to be involved in viral replication.NS1 exists as a monomer in its immature form but is rapidly processed in the endoplasmic reticulum to form a stable dimer.A small amount of NS1 remains associated with intracellular organelles where it is thought to be involved in viral replication.The rest of NS1 is found either associated with the plasma membrane or secreted as a soluble hexadimer. NS1 is essential for viral viability but its precise biological function is unknown.Antibodies raised in response to NS1 in viral infection can cross react with cell surface antigens on epithelial cells and platelets and this has been implicated in the development of Dengue Hemorrhagic fever.
The Dengue NS1 is a rapid test that utilizes a combination of Dengue antibodies coated colored particles for the detection of Dengue NS1 antigen in human whole blood,serum,or plasma.

PRINCIPLE

Dengue IgG/IgM Test is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood.This test can also detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins.
The Dengue NS1 Test is a qualitative membrane-based immunoassay for the detection of Dengue NS1 antigen in whole blood,serum,or plasma.During testing,the specimen reacts with Dengue antibody-conjugate in the test Device.The Gold antibody conjugate will bind to Dengue antigen in the specimen sample which in turn will bind with Anti-Dengue NS1 coated on the membrane.As the reagent moves across the membrane,the Dengue NS1 antibody on the membrane will bind the antibody-antigen complex causing pale or dark pink line to form at the test line region of the test membrane. The intensity of the lines will vary depending upon the amount of antigen present in the sample.The appearance of pink line in the test region should be considered as positive result.

REAGENTS

The test contains Dengue virus envelope protein particles and anti-human IgG,anti-human IgM antibody anti-Dengue NS1 antibody conjugated gold particles and anti-Dengue NS1 antibody coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test must remain in the sealed pouch until use.
- Do not eat,drink or smoke in the area where the specimens or kits are handled.
- Do not use test if pouch is damaged.
- Handle all specimens as if they contain infectious agents.Observe established precautions against microbiological hazards throughout the testing and follow standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats,disposable gloves and eye protection when specimens are being tested.
- Standard personal hygiene measures should be taken in the case of ingestion or direct eye contact with the buffer.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C).The test is stable through the expiration date printed on the sealed pouch.The test must remain in the sealed pouch until use.**DO NOT FREEZE.**Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Dengue IgG/IgM and NS1 Rapid Test Device can be performed using whole blood (from venipuncture),serum or plasma.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis.Use only clear non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods.Serum and plasma specimens may be stored at 2-8°C for up to 3 days.For long term storage,specimens should be kept below -20°C.Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 1 day of collection.Do not freeze whole blood specimens.
- Bring specimens to room temperature prior to testing.Frozen specimens must be completely thawed and mixed well prior to testing.Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped,they should be packed in compliance with local regulations covering the transportation of etiologic agents.

MATERIALS

Materials provided

- Test Devices
- Droppers
- Buffer
- Package insert

Materials required but not provided

- Specimen collection containers
- Micropipette
- Lancets (for fingerstick whole blood only)
- Centrifuge (for plasma only)
- Timer

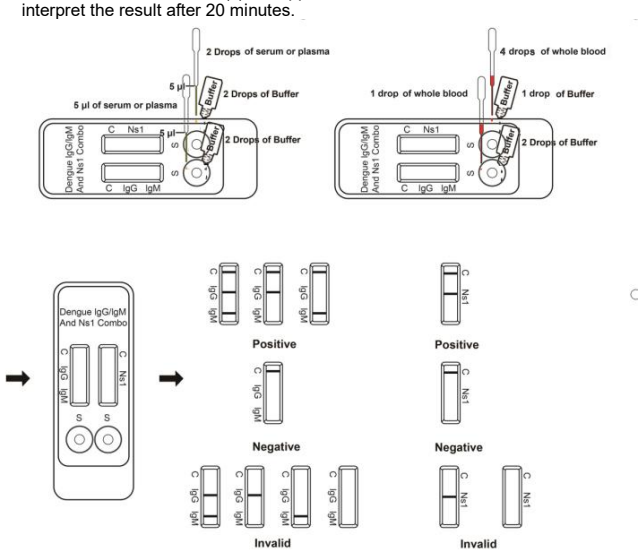
DIRECTIONS FOR USE

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening.Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface.

- For **Serum or Plasma Specimens:**Using the provided dropper,draw the specimen up to the Fill Line,and **transfer 5μL of serum/plasma (approximately 5μL)**,to the left specimen well for the item of IgG&IgM of the test device, and transfer 2 drops **serum/plasma (approximately 20μL)**,to the right specimen well for the item of NS1 of the test device. then **add 2 drops of buffer (approximately 80μL)** into each specimen well and start the timer.See illustration below.
- For **Venipuncture Whole Blood specimen:**Using the provided dropper and **transfer 1 drop (about 10μL) of whole blood** to the left specimen well for the item of IgG&IgM of the test device and transfer 4 drops **whole blood (approximately 40μL)**,to the right specimen well for the item of NS1 of the test device.,then **add 2 drops of buffer (approximately 80μL)**,and start the timer.See illustration below.
- For **Finger stick Whole Blood specimen:**To use a capillary tube: Fill the capillary tube and **transfer 10μL(1 drop) of fingerstick whole blood specimen** to the left specimen well for the item of IgG&IgM of the test device and transfer 40ul(4 drops) of **fingerstick whole blood specimen** to the right specimen well,then **add 2 drops of buffer (approximately 80μL)** and start the timer.

3. Wait for the colored line(s) to appear.**Read results at 10 minutes.**Do not interpret the result after 20 minutes.



INTERPREATION OF RESULTS

(Please refer to the illustration above)

For Dengue IgG/IgM Test

IgG POSITIVE:* Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the test region IgG.The result is positive for Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.
IgM POSITIVE: Two distinct colored lines appear.** One color line should be in the control region (C) and another color line should be in the test region IgM.The result is positive for Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.
IgG AND IgM POSITIVE:*One color line should be in the control region (C), and two colored lines should appear in test line regions IgG and IgM.The color intensities of the lines do not have to match.The result is positive for IgG & IgM antibodies and is indicative of secondary Dengue infection.
***NOTE:**The intensity of the color in the test line region(s) (IgG and/or IgM) will vary depending on the concentration of Dengue antibodies in the specimen.Therefore,any shade of color in the test line region(s) (IgG and/or IgM) should be considered positive.
NEGATIVE: The colored line in the control line region (C). No line appears in test line regions IgG or IgM (IgG or IgM).
INVALID:Control line fails to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure.Review the procedure and repeat the procedure with a new test device. If the problem persists,discontinue using the test kit immediately and contact your local distributor.
For Dengue NS1 Test
POSITIVE: Two colored bands appear on the membrane.One band appears in the control region (C) and another band appears in the test region (NS1).
NEGATIVE: Only one colored band appears,in the control region(C).No apparent colored band appears in the test region (NS1).
INVALID: Control band fails to appear.Results from any test which has not produced a control band at the specified read time must be discarded.Please review the procedure and repeat with a new test.If the problem persists,discontinue using the kit immediately and contact your local distributor.
NOTE:
1.A negative result can occur if the quantity of Dengue virus NS1 antigen present in the specimen is below the detection limits of the assay,or the antigens that are detected are not present during the stage of disease in which a sample is collected.
2.A negative test result cannot exclude a recent infection.
The presence of detectable Dengue virus NS1 Ag may mean positive for early Dengue infection.As with all diagnostic tests,all results must be considered with other clinical information available to the physician.

QUALITY CONTROL

The control line is used for procedural control.Control lines should always appear if the test procedure is performed properly and the test reagents of the control line are working.It confirms sufficient specimen volume and correct procedural technique.A clear background is also required.
Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal positive procedural control.
Control standards are not supplied with this kit;however,it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. This test detects the presence of antibodies to dengue in the specimen and should not be used as the sole criterion of Dengue virus infection.
2. In early infections and some secondary infections,detectable levels of IgM antibodies may be low.Some patients may not produce detectable levels of antibody within the first 7-10 days after infection.If clinical symptoms persist,patients should be retested in 3-4 days.
3. Serological cross-reactivity across the Flavivirus group (Dengue virus,St.Louis encephalitis, Japanese encephalitis,West Nile and yellow fever virus) is common.
4. As with all diagnostic tests,all results must be considered with other clinical information available to the physician.
5. If the test result is negative and clinical symptoms persist,additional follow-up testing using other clinical methods is recommended.A negative result does not preclude the possibility of an early infection of dengue virus.
6. The Dengue IgG/IgM and NS1 Rapid Test Device is limited to the qualitative detection of dengue Ag in human whole blood,serum or plasma.The intensity of the test band does not linear correlate with dengue Ag titer of the specimen.
7. If the symptom persists,while the result from Dengue NS1 Rapid Test device is negative or non-reactive result,it is recommended to re-sample the patient few days late or test with an alternative test device such as PCR,ELISA.
8. The test procedure,precautions and interpretation of results for this test must be followed strictly when testing.

EXPECTED VALUE

Primary dengue is characterized by the presence of detectable IgM 3-5 days after the onset of infection.Secondary dengue is characterized by the elevation of specific IgG 1-2 days after the onset of infection and in the majority of cases this is generally accompanied by an elevation of IgM. The Dengue IgG/IgM and NS1 Rapid Test Device has been compared with another leading commercial rapid test.The correlation between these two systems is 98%.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Dengue IgG/IgM and NS1 Rapid Test Device has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals.Results were confirmed by a leading commercial Dengue ELISA test.The results show that the overall relative sensitivity for the primary and secondary infection of the Dengue IgG/IgM Rapid Test Device is 95.7%,and the

relative specificity is >99.9%,and the relative accuracy is 99.3%,and for the Dengue NS1 Rapid Test Device is 95.8%,and the relative specificity is 96.2%,and the accuracy is 96.1%.

Dengue Primary infection for IgG/IgM test results

Method			ELISA		Negative
Dengue IgG/IgM Rapid Test Device	**Results**		**Positive**	**Negative**	
			IgM	IgG	
	Positive	IgM	15	0	0
		IgG	3	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%	/	/

Dengue Secondary infection for IgG/IgM test results

Method			ELISA		Negative
Dengue IgG/IgM Rapid Test Device	**Results**		**Positive**	**Negative**	
			IgM	IgG	
	Positive	IgM	37	0	0
		IgG	15	52	0
	Negative		0	0	0
Relative Sensitivity			71.2%	>99.9%	/

Non-Dengue infection for IgG/IgM test results

Method			ELISA		Negative
Dengue IgG/IgM Rapid Test Device	**Results**		**Positive**	**Negative**	
			IgM	IgG	
	Positive	IgM	0	0	0
		IgG	0	0	0
	Negative		0	0	338
Relative Specificity			/	/	>99.9%

Relative sensitivity: (15+52)/(18+52)=95.7%(95%CI*:88.0%-99.1%);
Relative specificity:338/338>99.9%(95%CI*:99.1%-100.0%);
Accuracy: (15+52+338)/(18+52+338)*100%=99.3%(95%CI*:97.9%~99.8%);
*Confidence Intervals

Method		ELISA		Total Results
Dengue NSI Rapid Test Device	**Results**	**Positive**	**Negative**	
	Positive	69	4	73
	Negative	3	102	105
Total Results		72	106	178

Relative sensitivity: 69/72*100%=95.8%(95%CI*:88.3%-99.1%);
Relative specificity:102/106*100%=96.2%(95%CI*:90.6%-99.0%);
Accuracy: (69+102)/(69+3+4+102)*100%=96.1%(95%CI*:92.1%~98.4%);
*Confidence Intervals

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SYMBOLS

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer	EC REP	Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse		Consult instruction foe use
LOT	Batch code	CE	Meet the requirements of EC Directive 98/79/EC

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