Dengue NS1 Ag Rapid Test
(Serum/Plasma/Whole Blood)

For Rapid qualitative detection of NS1 Antigen to dengue virus In human serum, plasma or whole blood.
Only *for In Vitro diagnostic use*.

CLINICAL SIGNIFICANCE

Dengue virus, a virus belonging to the flavivirus group of viruses, is one of the most significant mosquito-borne diseases in the world in terms of morbidity and mortality. Transmitted principally by the mosquito types Aedes aegypti and Aedes albopictus, the virus is found commonly throughout the tropic and subtropic regions of the world. There are four known serotypes of dengue. Symptoms of dengue fever include high fever, headache, muscle pain and skin rash. The complications often associated with this infection are dengue hemorrhagic fever or dengue shock syndrome.
Dengue NS1 Antigen Test provides an excellent methodology for specifically detecting Dengue NS1 antigen within up to 1 day of infection.

PRINCIPLE

The Dengue NS1 Antigen Test Device (Serum/ Plasma/Whole Blood) is a qualitative test for the detection of NS1 antigen to dengue virus in human serum, plasma or whole blood. Only serum, plasma, or whole blood samples may be used with this test. First a specimen is dispensed buffer; 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 AND MATEREIALS PROVIDED

Dengue Ns1 Test cards contain the following:
- Test Device with activated silica gel
- Plastic Dropper.
- Assay Buffer Bottle
- Package Insert (Instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED
- Positive Control
- Negative Control

MATERIALS REQUIRED BUT NOT PROVIDE
- Timer
- Digital Clock
- Specimen collection container Tube

WARNINGS AND PRECAUTIONS:
For in Vitro Diagnostic Use
- This package insert must be read completely before performing the test.
- The test device should remain in the sealed pouch until use
- Do not use expired devices.
- Do not use the kit if the cassette package is damaged or the seal is broken.
- Bring all reagent to room temperature (15-30°C) before use.
- Do not use hemolyzed blood specimens for testing.
- Wear personal protective equipment,such as gloves and lab coats when handling kit reagents.wash hands thoroughly after performing the test.
- Do not smoke,drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and material used to perform the test as bio-hazardous waste.
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REAGENT PREPARATION AND STORAGE

INSTRUCTIONS: All reagents are ready to use as supplied. Store unused test device unopened at 2-30 °C, ensure that the test device brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperature 30°C.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

1. Plasma
- **Step 1:** Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
- **Step 2:** Separate the plasma by centrifugation.
- **Step 3:** Carefully withdraw the plasma into a new per-labeled tube.

2. Serum
- **Step 1:** Collect blood specimen into a red top collection tube (containing no anti coagulants in Vacutainer®) by venipuncture.
- **Step 2:** Allow the blood to clot.
- **Step 3:** Separate the serum by centrifugation.
- **Step 4:** Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store Specimens at 2-8°C if not tested immediately, for up to 5 days. The specimens should be frozen at -20°C for longer storage.

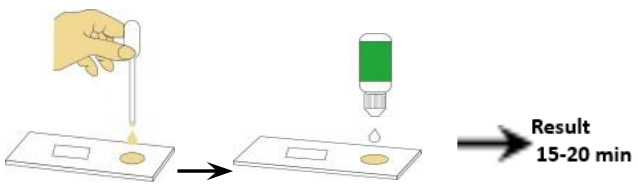
.3.Whole Blood
- Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). Do not use hemolyzed blood for testing.
- Whole blood specimens should be store in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

DIRECTIONS FOR USE

Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening it.
2. Remove the test cassette from the sealed pouch and place it on flat dry surface.
3. **For Serum or Plasma Specimen**: Hold the dropper vertically and transfer **3 drop** of serum or plasma (**approximately 75µl**) to the specimen area, then add **1 drop** of buffer **(approximately 40µl),** and start the timer, see illustration below.

   .For Whole Blood Specimen: Hold the dropper vertically and transfer **3 drop** of whole blood (**approximately 75µl**) to the specimen area, then add **1 drops** of buffer (**approximately 40µl**), and start the timer. See illustration below.

4. Wait for the colored line(s) to appear. The test result should be read at 15-20 minutes.
   Note. ***Do not interpret the result after 20 minutes*.**



3 Drops of Serum/Plasma **1 Drop of Buffer**

3 Drops of Whole Blood **1 Drop of Buffer**

INTERPRETATION OF RESULTS

Please refer to the illustration above.

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 (T).

**NOTE: The intensity of the color in the test line region (T) will vary depending on the concentration of Dengue Ns1 Anitegen present in the specimen. Therefore, any shade of red in the test region should be considered positive.*

NEGATIVE : One color line appears in the control region (C). No apparent red or pink line appears in the test region (T).

INVALID : Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

region (C) is an internal positive procedural control. It confirms sufficient specimen volume. 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 OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of dengue Ag in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Dengue NS1 Rapid Test 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 Test of the specimen.
3. A negative test result does not preclude the possibility of exposure to or infection with dengue viruses.
4. A negative result can occur if the quantity of dengue Ag present in the specimen is below the detection limits of the assay, or the dengue Ag that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptom persists, while the result from Dengue NS1 Rapid Test 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.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma) has passed a seroconversion panel and compared with a leading commercial Dengue Ag EIA test using clinical specimens.

Method		**Dengue Ag EIA Test**		Total Result
Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	143	2	145
	Negative	0	200	200
Total Result		143	202	345

Relative Sensitivity: 98.6%
Relative Specificity: 100.0%
Accuracy : 99.0%

LIMITED EXPRESS WARRANTY DISCLAIMER

Bioline Diagnostics LLP. Products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Bioline Diagnostics LLP. For which liability is limited to either replacement of the product and in no case liable to for claim of any kind for ap amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. Bioline Diagnostics LLP. Disclaims any and I responsibility for any injury or damage or legal implications which may be caused by the fault of the user or buyer in accordance with the limitations and specifications here in. Due to continuous development, the manufacturer reserves the right to improve / change any specification/components without prior information/notice to the buyer.

REFERENCES

1. CDC/NIH Guidelines. Bio-safety in Microbiological and Biomedical Laboratories. 2nd Edition, 1988.
2. Lam, SK. Dengue haemorrhagic fever. Rev. Med. Micro. (1995), 6:39-48.
3. Siti-Strong. Diagnosis, prevention, and treatment of tropical disease, 7th ed., Philadelphia, the Ablakiston Company.
4. Halstead SB, Selective primary health care: strategies for control of disease in thedeveloping world: XI, Dengue. Rev. Infect. Dis. 1984; 6:251-264.
5. Halstead SB, Pathogenesis of dengue: challenges to molecular biology. Science 1988;239:476-481.

Dengue IgG/IgM Rapid Test
(Serum/Plasma/Whole Blood)

One Step kits for the differential detection of IgG and IgM against Dengue virus Type I, II, III and IV using Human Serum/Plasma/Whole Blood.

INTENDED USE
First View Dengue Fever Rapid IgG/IgM is an immunochromatography assay designed for the qualitative detection and differentiation of specific IgM and IgG antibodies to dengue virus in human serum or plasma. It is intended to be used as in vitro diagnostic of dengue fever. The test provides a differential detection of anti-dengue IgM and anti-dengue-IgG antibodies and can be used for the presumptive distinction between a primary and secondary dengue infection.The results obtained should not be the sole determinant for clinical decision.

INTRODUCTION
Dengue is a flavivirus, transmitted by Aedesaegypti and Aedesalbopictus mosquitoes.[1] It is widely distributed throughout the tropical and subtropical areas of the world,[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. Primary Dengue infection causes IgM antibodies to increase to a detectable level in 3 to 5 days after the onset of fever. IgM antibodies generally persist for 30 to 90 days.[3] Most Dengue patients in endemic regions have secondary infections,[4] resulting in high levels of specific IgG antibodies prior to or simultaneous with IgM response.[5] Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.
The Dengue IgM/IgG Rapid Test Device (Whole Blood/Serum/Plasma) is a rapid test that utilizes a combination of Dengue antigen coated colored particles for the detection of IgG and IgM Dengue antibodies in human whole blood, serum, or plasma.

PRINCIPLE OF TEST
The Dengue IgM/IgG Rapid Test Device (Whole Blood/Serum/Plasma) is a qualitative membrane-based immunoassay for the detection of Dengue antibodies in whole blood, serum, or plasma. This test consists of two components, an IgM component and an IgG component. In the IgM component, anti-human IgM is coated in test line region 1 (IgM) of the test. During testing, if the Dengue IgM antibodies present in the specimen, reacts with the Dengue antigen-coated particles in the test strip, and this complex is captured by the anti-human IgM, forming a colored line in test line region 1(IgM). In the IgG component, anti-human IgG is coated in test line region 2(IgG) of the test. During testing, the specimen reacts with Dengue antigen-coated particles in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in test line region 2(IgG). If the specimen contains IgG antibodies to Dengue, a colored line will appear in test line region 2(IgG).

Therefore, if the specimen contains Dengue IgM antibodies, a colored line will appear in test line region I(IgM). If the specimen contains Dengue IgG antibodies, a colored line will appear in test line region 2(IgG). If the specimen does not contain Dengue antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always change from red to blue in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

ACTIVE REAGENTS AND MATEREIALS PROVIDED
Dengue IgG/IgM Test cards contain the following:
- Test Device with activated silica gel
- Plastic Dropper.
- Assay Buffer Bottle
- Package Insert (Instruction for use)

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED
- Positive Control
- Negative Control

MATERIALS REQUIRED BUT NOT PROVIDE
- Timer
- Digital Clock
- Specimen collection container Tube

WARNINGS AND PRECAUTIONS:
For in Vitro Diagnostic Use
- This package insert must be read completely before performing the test.
- The test device should remain in the sealed pouch until use
- Do not use expired devices.
- Do not use the kit if the cassette package is damaged or the seal is broken.
- Bring all reagent to room tempreature (15-30°C) before use.
- Do not use hemolyzed blood specimens for testing.
- Wear personal protective equipment,such as gloves and lab coats when handling kit reagents.wash hands thoroughly after performing the test.
- Do not smoke,drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and material used to performe the test as bio-hazardous waste.

REAGENT PREPARATION AND STORAGE
INSTRUCTIONS: All reagents are ready to use as supplied. Store unused test device unopened at 2-30 °C, ensure that the test device brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperature 30°C.

SPECIMEN COLLECTION, STORAGE AND HANDLING
Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

1. Plasma
- **Step 1:** Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
- **Step 2:** Separate the plasma by centrifugation.
- **Step 3:** Carefully withdraw the plasma into a new pre-labeled tube.

2. Serum
- **Step 1:** Collect blood specimen into a red top collection tube (containing no anti coagulants in Vacutainer®) by venipuncture.
- **Step 2:** Allow the blood to clot.
- **Step 3:** Separate the serum by centrifugation.
- **Step 4:** Carefully withdraw the serum into a new pre-labeled tube.

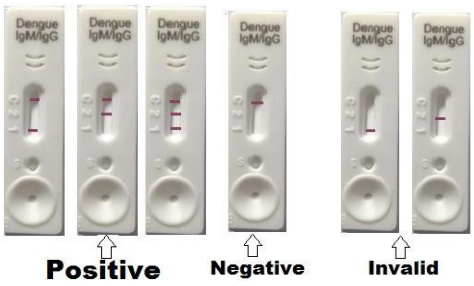
Test specimens as soon as possible after collecting. Store Specimens at 2-8°C if not tested immediately, for up to 5 days. The specimens should be frozen at -20°C for longer storage.

.3.Whole Blood
- Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). Do not use hemolyzed blood for testing.
- Whole blood specimens should be stored in refrigeration (2-8°C),if not tested immediately. The specimens must be tested within 24 hours of collection.

DIRECTIONS FOR USE
Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening it.
2. Remove the test cassette from the sealed pouch and place it on flat dry surface.
3. **For Serum/Plasma OR Whole Blood Specimen**: Hold the dropper vertically and transfer **1 Drop** of Serum/Plasma or Whole Blood (**approximately 5µl**) to the specimen into the sample well (S), then add **2 Drops** of buffer **(approximately 80µl**) into the buffer well (B), and start the timer, see illustration below.
4. Wait for the colored line(s) to appear. The test result should be read at 15 minutes.

Note. ***Do not interpret the result after 15 minutes.***

INTERPRETATION OF ASSAY RESULT



Negative: The presence of one Red band ("C" control line) within the result window indicates a negative result for IgG and IgM antibodies. Retest in 3-5 days if dengue infection is still suspected.

IgM Positive: The control line (C) and IgM line (M) (1) are visible on the test device. This is positive for IgM antibodies to Dengue virus. This is indicative of a primary dengue infection.

IgG Positive: The control line (C) and IgG line (G) (2) are visible on the test device. This is positive for IgG antibodies. This is indicative of secondary or past dengue infection

IgG and IgM Positive: The control line, IgM (M) (1) and IgG line (G) (2) are visible on the test device. This is positive for both IgM and IgG antibodies. This is indicative of a late primary or early secondary dengue infection.

Invalid: If the control band fails to appear within the result window, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be retested.

PERFORMANCE CHARACTERISTICS

First View Dengue IgM/IgG Rapid Test Device (Whole Blood/Serum/Plasma) has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA.

Dengue Infection	Result	IgM	IgG
Primary Infection	Positive	14	0
	Negative	3	17
	Total	17	17
	Relative Sensitivity	82.4%	0%
Secondary Infection	Positive	39	55
	Negative	16	0
	Total	55	55
	Relative Sensitivity	70.9%	>99.0%
Non-Dengue Infection	Positive	0	0
	Negative	378	378
	Total	378	378
	Relative Specificity	>99.0%	>99.0%

For the primary and secondary infection, the overall
Sensitivity: 95.8%,
Specificity: 99.0%
Accuracy : 99.3%.

LIMITATIONS AND INTERFERENCS

- This product is designed for use with human serum, plasma or whole blood only.
- This test detects the presence of antibodies to dengue in the specimen and should not be used as the sole criterion for the diagnosis of a dengue viral infection.
- The test is a qualitative assay and is not for quantitative determination of antibodies concentration levels. The intensity of the band does not have linear correlation with the antibody titer of the specimen.
- The results obtained should only be interpreted in conjunction with other diagnostic results and clinical information.

LIMITED EXPRESS WARRANTY DISCLAIMER

Bioline Diagnostics LLP. products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Bioline Diagnostics LLP. for which liability is limited to either replacement of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in which damages are likely to be claimed. Bioline Diagnostics LLP. disclaims any and all responsibility for any injury or damage or legal implications which may be caused by the fault of the user or buyer in accordance with the limitations and specifications here in. Due to continuous development, the manufacturer reserves the right to improve /change any specification /components without prior information /notice to the buyer.

REFERENCES

1. Sabin, AB and Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice: Science (1945), 101:640
2. Lam, SK. Dengue haemorrhagic fever. Rev. Med. Micro. (1995), 6:39-48.
3. Innis, BL, Nisalak, A., et.al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trap. Med. Hygiene (1989), 40:418-427.
4. CDC/NIH Guidelines. Biosafety in Microbiological and Biomedical
5. Siti-Strong. Diagnosis, prevention, and treatment of tropical disease, 7th ed., Philadelphia, TheAblakiston Company.