Dengue Combo Rapid Test

Catalogue Number: RAPG-DEC-001

Please read this manual carefully before operating to ensure proper use.

TEST KIT DESCRIPTION

The Biopanda Dengue Combo Rapid Test qualitatively detects NS1 antigen, IgG and IgM antibodies of dengue virus in human whole blood, serum or plasma samples. This test applies lateral flow immuno-chromatography and is a tool to assist in the diagnosis of Dengue infections.

PRINCIPLE

The Biopanda Dengue IgG/IgM Rapid Test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in the IgG test line region. During testing, the specimen reacts with Dengue antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in the IgG test line region. If the specimen contains IgG antibodies to Dengue, a coloured line will appear in IgG test line region. In the IgM component, anti-human IgM is coated in the IgM test line region. During testing, the specimen reacts with anti-human IgM. Dengue IgM antibodies, if present in the specimen, reacts with the anti-human IgM and the Dengue antigen-coated particles in the test cassette, and this complex is captured by the anti-human IgM, forming a coloured line in the IgM test line region.

Therefore, if the specimen contains Dengue IgG antibodies, a coloured line will appear in the IgG test line region. If the specimen contains Dengue IgM antibodies, a coloured line will appear in the IgM test line region. If the specimen does not contain Dengue antibodies, no coloured line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, to show the test has been performed properly.

The Biopanda Dengue NS1 Rapid 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 cassette. 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 a coloured 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 any coloured line in the test region should be considered as positive result.

KIT CONTENTS

- 10 x Foil wrapped cassettes and desiccant.
- 10 x Disposable sample droppers each for NS1/IgG IgM test
- 1 x Buffer Tube
- 1 x Product insert

STORAGE AND STABILITY

Store the kit between 2-30^{o}C and ensure the kits are not frozen or stored in direct sunlight. The test is valid until the expiration date printed on the foil wrapping.

PRECAUTIONS

Follow these instructions for the best results:

- This kit is for *in vitro* diagnostic use only and should only be used by trained health professionals.
- Blood samples may be potentially infectious and should be handled with standard biosafety procedures.
- Protective clothing such as laboratory coats, disposable gloves, and eye protection should be worn when working with assays.
- Ensure the test kit is at room temperature before running the test.
- Keep the test inside the foil wrapper until it is needed.
- Ensure each test is used only once.
- Test kits that have reached their expiry date should not be used.
- Only use reagents from this kit when performing the test to ensure quality controlled testing.
- Used tests and unused samples should be discarded according to local standard biosafety procedures.

SAMPLE COLLECTION AND PREPARATION

- The Biopanda Dengue Combo Rapid Test can be performed using whole blood, serum, or plasma.
- To collect Fingerstick **Whole Blood** Samples:
- Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
- Puncture the skin with a sterile lancet. Wipe away the first sign of blood. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site. Use whole blood as a sample.
- Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood samples. Whole blood collected by fingerstick should be tested immediately.
- For **serum/plasma** samples: Separate serum or plasma from blood as soon as possible to avoid haemolysis. Use only clear, non-haemolysed samples.
- Testing should be performed immediately after sample collection. Do not leave the samples at room temperature for prolonged periods. Serum and plasma samples may be stored at 2-8°C for up to 3 days. For long-term storage, samples should be kept below -20°C.
- Bring samples to room temperature prior to testing. Frozen samples must be completely thawed and mixed well prior to testing. Samples should not be frozen and thawed repeatedly.
- If samples are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

TEST PROCEDURE

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 it. Remove the test cassette from the sealed pouch and use it within 1 hour.
2. Place the cassette on a clean and level surface.

For **Serum or Plasma specimen**:

For IgG/IgM:

To use a dropper: Hold the dropper vertically, draw the specimen up to the **Fill Line** (approximately 5µl), and transfer the specimen to the **specimen well** (S) of the test cassette, then add **3 drops of buffer** (approximately 120µl) to the **buffer well** (B) and start the timer. Avoid trapping air bubbles in the specimen well.

To use a micropipette: Pipette and dispense **5 µl of serum or plasma** to the **specimen well** (S) of the test cassette, then add **3 drops of buffer** (approximately 120µl) to the **buffer well** (B) and start the timer.

For NS1:

Hold the dropper vertically and transfer **3 drops of serum or plasma** (approximately 75µl) to the specimen well (S), and start the timer. See illustration below.

For **Whole Blood(Venipuncture/Fingerstick)** specimen:

For IgG/IgM:

To use a dropper: Hold the dropper vertically, draw the specimen up to about 1cm above the fill line, and transfer **1 drop of whole blood** (approximately 10µl) to the **specimen well** (S) of the test cassettes, then add **3 drops of buffer** (approximately 120µl) to the **buffer well** (B) and start the timer. See illustration below.

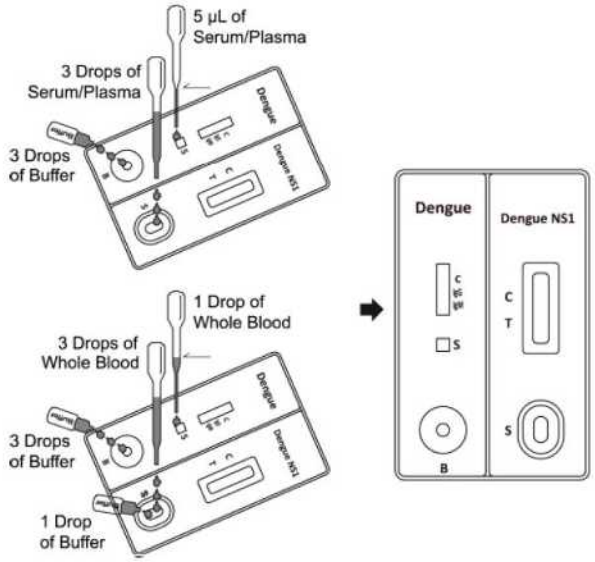
To use a micropipette: Pipette and dispense **10µl of whole blood** to the **specimen well** (s) of the test cassette, then add **3 drops of buffer** (approximately 120µl) to the **buffer well** (B) and start the timer. See illustration below.

For NS1:

To use a dropper: Hold the dropper vertically and transfer **3 drops of whole blood** (approximately 75µL) to the specimen well (s), then add **1 drop of buffer** (approximately 40µL) and start the timer. See illustration below.

To use a capillary tube: Fill the capillary tube and transfer approximately 75µL of fingerstick whole blood specimen to the specimen well(s) of test cassette, then add **1 drop of buffer** (approximately 40µL) and start the timer. See illustration below.

3. **Read the results at 10 minutes**, do not interpret the results after 20 minutes.



INTERPRETATION OF RESULTS

NS1 POSITIVE: * Two distinct coloured lines appear. One coloured line should be in the control region (C) and another coloured line should be in the test region (T).

IgG and IgM POSITIVE:* Three lines appear. One coloured line should be in the control line region (C), and two coloured lines should appear in IgG test line region and IgM test line region. The colour intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and is indicative of secondary Dengue infection.

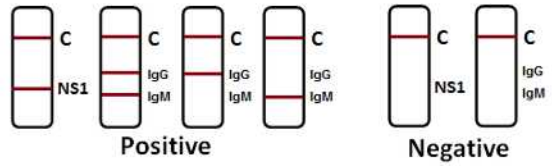
IgG POSITIVE:* Two lines appear. One coloured line should be in the control line region (C), and a coloured line appears in the IgG test line region. The result is positive for Dengue virus specific-IgG and is probably indicative of a secondary Dengue infection.

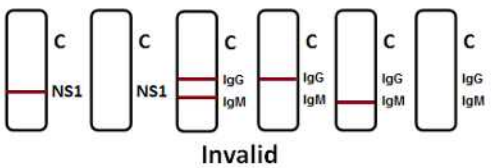
IgM POSITIVE:* Two lines appear. One coloured line should be in the control line region (C), and a coloured line appears in the IgM test line region. The result is positive for Dengue virus specific-IgM antibodies and is indicative of a primary Dengue infection.

***NOTE:** The intensity of the colour in the test line region (NS1 and/or IgG and/or IgM) will vary depending on the concentration of Dengue NS1 antigen and/or IgG and/or IgM present in the sample. Therefore, any shade of red in the test line region should be considered positive.

NEGATIVE: One coloured line appears in the control region (C). No apparent coloured line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient sample 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.





LIMITATIONS

1. The Biopanda Dengue Combo Rapid Test will only indicate the presence of Dengue NS1 antigen and Dengue antibodies in the sample and should not be used as the sole criteria for the diagnosis of Dengue fever.
2. The Assay Procedure and the Assay Result Interpretation must be followed precisely when testing the presence of Dengue Ag in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
3. A negative test result for Dengue NS1 does not preclude the possibility of exposure to or infection with Dengue viruses.
4. A negative result for Dengue NS1 can occur if the quantity of Dengue Ag present in the sample 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 samples containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptoms persist, while the result from the Dengue NS1 Rapid Test is negative or non-reactive, it is recommended to test the patient a few days later or test with an alternative testing method such as PCR or ELISA.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.
8. In the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked immunosorbent assay (ELISA) showed that 80% of the Dengue patients tested exhibited detectable levels of IgM antibody by the fifth day after infection, and 99% of the patients tested IgM positive by day 10. It is recommended that patients be tested within this time. For the secondary infection, a low molar fraction of anti-Dengue IgM and a high molar fraction of IgG that is broadly reactive to flaviviruses characterize the antibodies. The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
9. Serological cross-reactivity across the flavivirus group (Dengue 1, 2, 3 & 4, St. Louis encephalitis, West Nile virus, Japanese encephalitis and yellow fever viruses) is common. Positive results should be confirmed by other means.
10. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
11. Results from immunosuppressed patients should be interpreted with caution.
12. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
13. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Dengue infection.

SENSITIVITY AND SPECIFICITY

The Biopanda Dengue Combo Rapid Test has passed a seroconversion panel and compared with a leading commercial Dengue Ag ELISA test using clinical samples for Dengue NS1 and has been evaluated with samples obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test for IgG and IgM.

The results show that the relative sensitivity of the Dengue NS1 Rapid Test is 95.8%, and the relative specificity is 96.1%. And the overall relative sensitivity for the primary and secondary infection of the Dengue Rapid Test is 95.7%, and the relative specificity is >99.9%,and the relative accuracy is 99.3%.

REFERENCES

1. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue. Rev. Infect. Dis. 1984; 6:251-264.
2. Halstead SB, Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239:476-481.

Thank you for purchasing Biopanda's Dengue Combo Rapid Test kit. Please read this manual carefully before operating to ensure proper use.

Biopanda Reagents Ltd.
Unit 14 Carrowreagh Business Park
Carrowreagh Road
Belfast, BT16 1QQ
United Kingdom
Tel: +44 (0) 28 95438774
E-mail: info@biopanda.co.uk
Website: www.biopanda.co.uk

Revision date: 08/04/2021
PI-RAPG-DEC 001