

Performance of the Dengue NS1 Rapid Test in Detecting Dengue Virus Infection: A Comprehensive Study

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Abstract: Dengue virus infection presents a substantial global health challenge, impacting millions of individuals worldwide. It manifests in various forms including dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The objective of this study was to assess the diagnostic accuracy of the Dengue NS1 Rapid Test, which is crucial for effective management and prevention of dengue virus infection progression. To assess the sensitivity, specificity and accuracy of the Dengue NS1 Rapid Test, we conducted a comprehensive analysis of 351 specimens. The evaluation showed an accuracy of 96.0%, coupled with a sensitivity of 95.8% and a specificity of 96.1%. The test's specificity was confirmed through cross-reactivity assessments, as it yielded negative results for various other viral strains. Developed by Hangzhou AllTest Biotech Co., Ltd, the Dengue NS1 Rapid Test provides rapid and reliable results, demonstrating a high level of accuracy. As a result, it serves as a valuable diagnostic tool in clinical scenarios, especially in environments with limited resources. The early detection capabilities of this test enable timely interventions, improving the management of dengue virus infections. This leads to better results for patients and a lower rate of virus transmission.

1. Introduction

Dengue is an infection caused by the dengue virus. It's mainly transmitted to humans via bites from infected mosquitoes, specifically the *Aedes aegypti* species.^[1] In recent years, the geographical spread of dengue fever has been continuously expanding due to global climate change, rapid urbanization and increased population mobility.^[2] This expansion poses a severe threat to public health security, with dengue outbreaks becoming increasingly critical. According to a 2023 report, there were over 5 million reported cases of dengue fever worldwide, resulting in approximately 5,000 deaths. The Americas accounted for around 80% of these cases, followed by Southeast Asia and the Western Pacific region.^[3] Majority of cases were reported in developing countries, imposing a significant healthcare burden. Dengue fever lacks a specifically effective treatment, with the main approach to management being supportive care. Therefore, it is vital to diagnose dengue fever infection at an early stage, as this aids in patient management, disease control and prevention and the

execution of etiological studies.^[4]

Dengue fever presents clinically in three stages: the febrile, critical and recovery phase.^[5] The febrile phase typically lasts for 2-7 days, during which patients experience symptoms such as high fever, headache, muscle and joint pain, nausea and rash.^[6] In the clinical remission phase, the symptoms temporarily subside. However, during the recurrence phase, severe complications may arise, including severe bleeding, thrombocytopenia and plasma leakage, leading to dengue shock syndrome, which is the primary cause of mortality. Dengue's symptoms are comparable to those of numerous other illnesses, such as malaria, influenza and Zika.^[3]

Several laboratory methods are available for diagnosing dengue fever infection, including virus isolation, RNA detection and antigen & antibody assays. Diagnosing dengue in its early stages based solely on clinical signs can be challenging. This is due to symptom overlap with other febrile illnesses, making it difficult to ascertain disease severity.^[7] Scientists have devised diagnostic methods that integrate virology and immunology. During the initial phase of the infection, RT-PCR can be used to identify dengue RNA, while ELISA or RDTs can detect the NS1 antigen. Subsequently, ELISA or RDTs can be employed to detect dengue-specific IgM and IgG antibodies. According to the latest guidelines from the WHO, CDC and PAHO, it is recommended to conduct RT-PCR and/or NS1 antigen tests within the first 5 to 7 days of illness to confirm dengue infection.^[8] However, both virus isolation and identification of viral RNA through RT-PCR are time-consuming processes that require specialized laboratories, expensive techniques and trained personnel, making them difficult to implement in resource-limited areas.^[9] Serological tests, predominantly administered through ELISA, are used to detect IgM and IgG antibodies in the majority of instances. When in the acute phase, the detection of IgM antibodies signals an initial infection, typically emerging after the end of viremia or the abatement of fever symptoms. During secondary infections, IgG antibodies tend to increase within the first week following infection and then decrease over a period of 3 to 6 months.^[10] NS1 is a highly conserved glycoprotein applicable to all serotypes and produced in membrane-associated and secretory forms. NS1 antigen can be detected in the blood from day 1 to day 9 after the onset of fever, even in the presence of IgM antibodies and when RT-PCR testing for viral RNA is negative. Therefore, NS1 detection is widely utilized for early diagnosis of dengue fever.

2. Materials and Methods

2.1 Study Design

The purpose of this research was to assess the diagnostic accuracy and utility of the Dengue NS1 Rapid Test, a product from Hangzhou AllTest Biotech Co., Ltd, utilized for identifying dengue virus infection. For the dependability of the research findings, 351 specimens were collected from individuals exhibiting symptoms (0-5 days post-fever onset). The determination of the specimen size was based on statistical requisites and aimed to achieve an adequate level of precision required to assess the diagnostic accuracy.

2.2 Specimen Collection

To evaluate the efficacy of the Dengue NS1 Rapid Test, designed for identifying the Dengue virus NS1 antigen in whole blood, serum or plasma specimens, a comprehensive evaluation was conducted following a stringent standardized protocol. The assay cassette underwent examination using a seroconversion panel, which involved analyzing a sequence of serum specimens collected from individuals across various stages of Dengue infection. This panel included specimens from the acute phase, characterized by the presence of IgM antibodies, progressing to the convalescent phase, where IgG antibodies appear and IgM titers diminish.

2.3 Screening Test

(1) RDT

Prior to conducting the test, it is essential to equilibrate the test cassette, specimens, buffer and control devices to ambient conditions (15-30°C). Following the manufacturer's instructions, researchers should transfer three drops of serum or plasma (approximately 75 µL) into the specimen well of the test cassette for serum or plasma specimens. In the case of venous blood specimens, three drops of whole blood (approximately 75 µL) should be transferred into the sample well, followed by the addition of one drop of buffer (about 40 µL). For fingerstick whole blood specimens, researchers should transfer approximately 75 µL of fingerstick whole blood into the sample well of the test cassette, followed by the addition of one drop of buffer (about 40 µL). Researchers should read and interpret the results after a 10-minute interval.

The Dengue NS1 Rapid Test Cassette, designed for qualitative identification of the Dengue NS1 antigen in whole blood, serum or plasma, is a chromatographic immunoassay based on a membrane. During the assay, the specimen interacts with the Dengue antibody-conjugate. The conjugate carrying a gold-label then binds with the Dengue antigen present within the specimen. Subsequently, this complex connects with the membrane-coated Anti-Dengue NS1. As the reagent traverses the membrane, the Dengue NS1 antibody on the membrane captures the antibody-antigen complex, resulting in the formation of a colored line in the test line region of the test membrane. The presence of a colored line in the test region is indicative of a positive result.

(2) ELISA

In the study, the presence of Dengue NS1 antigen was detected using an Enzyme-Linked Immunosorbent Assay (ELISA) method. Polystyrene microtiter plates were coated with monoclonal capture antibodies specific to the Dengue NS1 protein. Following blocking with bovine serum albumin (BSA) to prevent non-specific binding, serum, plasma or urine specimens from suspected Dengue patients were added to the coated plate. After incubation and washing to remove unbound materials, a secondary antibody conjugated to horseradish peroxidase (HRP) was added to bind to a different epitope on the NS1 protein. Following another round of incubation and washing, tetramethylbenzidine (TMB) was added as a substrate for HRP, resulting in a color change. The intensity of the color was proportional to the amount of NS1 present in the specimen and was quantified by comparing the absorbance to a standard curve generated using known concentrations of NS1 protein. This ELISA method provided a sensitive and specific identification of Dengue NS1 antigen, suitable for high-throughput screening in laboratory settings.

2.4 Cross-reactivity and Interference

To evaluate the specificity of the Dengue NS1 Rapid Test Cassette, a thorough cross-reactivity evaluation was performed using a collection of specimens positive for diverse antibodies and antigens. These included anti-H. pylori IgG, HBsAg, anti-HIV, HBsAb, anti-CMV IgM, anti-MONO IgM, anti-HCV IgG, anti-Rubella IgM, HBeAb, anti-Syphilis IgG, HBcAb, anti-RF IgG, anti-HAMA IgG, anti-TOXO IgM, HBeAg, anti-CMV IgG, anti-TOXO IgG and anti-Rubella IgG. The outcomes of these tests revealed no cross-reactivity with any of these substances, thereby confirming the high specificity of the NS1 Rapid Test for the detection of Dengue NS1 antigen.

The potential interference of various substances commonly found in clinical specimens was also assessed. Dengue-negative and positive specimens were spiked with specified concentrations of acetaminophen, caffeine, acetylsalicylic acid, gentisic acid, ascorbic acid, albumin, creatinine, hemoglobin, bilirubin and oxalic acid. The assay's performance was evaluated in the presence of these substances and none of them, at the tested concentrations, were found to interfere with the assay results. This highlights the robustness of the NS1 Rapid Test Cassette, ensuring reliable results even

in the presence of potentially interfering substances commonly encountered in clinical specimens.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and Specificity

In the process of conducting a seroconversion study, the Rapid Test Cassette for Dengue NS1 (Whole Blood/Serum/Plasma) was compared with the leading Dengue Ag ELISA tests that used clinical specimens. Table 1 showed that this Rapid Test Cassette demonstrated a relative sensitivity rate of 95.8% and a relative specificity rate of 96.1%.

Table 1: Performance Characteristics.

Method		Dengue Ag ELISA Test		Total Results
Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	137	8	145
	Negative	6	200	206
Total Results		143	208	351

Relative sensitivity: $137/143 * 100\% = 95.8\%$ (95%CI*: 91.1%~98.4%);

Relative specificity: $200/208 * 100\% = 96.1\%$ (95%CI*: 92.6%~98.4%);

Accuracy: $(137+200)/(137+6+8+200) * 100\% = 96.0\%$ (95%CI*: 93.4%~97.8%)

*: Confidence Intervals

3.1.2 Precision

To evaluate the intra-assay precision of the Dengue NS1 Rapid Test Cassette, 15 replicates of four distinct specimens were analyzed: one negative, one with low positive NS1 antigen concentration, one with a middle positive concentration and one with a high positive concentration. The assay's intra-assay precision was affirmed as the specimens were accurately pinpointed with a success rate that exceeded 99%, showcasing high uniformity and dependability within one single run.

The Dengue NS1 Rapid Test Cassette's inter-assay precision was evaluated by carrying out 15 separate assays on the same four specimens which included a range from negative to low positive, middle positive, and high positive NS1 antigen concentrations. These assays were performed using three distinct batches of the test cassette. The results showed a correct identification rate of greater than 99% for all specimens across the different lots, indicating excellent agreement and precision between runs. This high level of inter-assay precision validates the consistency and reproducibility of the NS1 Rapid Test Cassette, regardless of the lot used, ensuring reliable results in clinical testing.

3.2 Discussion

Dengue virus infection is predominantly widespread in tropical and subtropical regions globally. Recent years have witnessed an increasing trend in dengue infection outbreaks. Timely detection is paramount in initiating prompt administration of appropriate therapeutic measures, resulting in a significant improvement in disease prognosis and effective management of dengue outbreaks from a public health perspective.^[11] The Dengue NS1 Rapid Test, developed by Hangzhou AllTest Biotech Co., Ltd, has demonstrated an accuracy of 96.0%, a sensitivity of 95.8% and a specificity of 96.1% when evaluated using 351 specimens collected within 0-5 days post-fever onset. These impressive performance metrics suggest that the test can be confidently utilized for the early detection of dengue virus infection. Its reliable performance enables timely intervention and effective disease

management.

It is important to acknowledge the limitations of this study. Firstly, the Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma) is designed for the qualitative detection of dengue antigen, and it can't interpret the concentration of the dengue NS1 antigen from the intensity of the colored line. Moreover, a negative test outcome doesn't completely exclude the chance of exposure to or infection with dengue viruses. It's possible for a negative result to occur if the amount of the dengue antigen present in the specimen falls below the detection thresholds of the assay, or if the specific dengue antigens being detected aren't present at the stage of the disease when the specimen is collected. Therefore, if symptoms persist, it is recommended to retest the patient after a few days or to employ an alternative testing approach, such as PCR or ELISA. Thirdly, it should be noted that certain specimens containing abnormally high levels of heterophile antibodies or rheumatoid factor may affect the expected results. Consequently, the results obtained from this test should be in conjunction with other diagnostic procedures and clinical findings. Furthermore, accurate results are dependent on the hematocrit level of the whole blood specimen falling within the range of 25% to 65%.

4. Conclusion

To conclude, the Dengue NS1 Rapid Test, which was developed by Hangzhou AllTest Biotech Co., Ltd, demonstrates a range of advantages that render it highly appropriate for employment in clinical settings, particularly those with limited resources. The test has shown exceptional effectiveness, characterized by its remarkable precision and consistent results. Consequently, healthcare professionals can promptly identify individuals afflicted with Dengue virus infections, enabling early intervention and suitable strategies for management. The test's rapid diagnostic abilities play a pivotal role in enhancing patient outcomes and mitigating the risk of viral transmission. These features effectively highlight the substantial value that the Dengue NS1 Rapid Test brings to clinical practice.

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