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Performance Evaluation of Dengue NS1 Rapid Test Cassette (WB/S/P) via Clinical Validation

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Abstract: This research undertakes an exhaustive clinical verification of the CITEST Dengue NS1 Rapid Test Cassette-an immunochromatographic device engineered for the qualitative identification of Dengue virus NS1 antigen in human whole blood, serum, and plasma samples; a commercially supplied Dengue Antigen Enzyme-Linked Immunosorbent Assay (ELISA) was employed as the gold standard reference method to assess the efficacy of the rapid diagnostic test, utilizing a total of 383 clinical specimens collected from both symptomatic and asymptomatic patients, and the rapid test exhibited a relative sensitivity of 95.8% (with a 95% confidence interval of 91.1% to 98.4%) and a relative specificity of 98.3% (95% CI: 95.8%–99.5%), which translated to an overall diagnostic precision of 97.4% (95% CI: 95.3%–98.7%), with consistent and high levels of concordance with the reference ELISA assay observed throughout the validation process; precision analyses revealed superior intra-assay and inter-assay reproducibility, with correct identification rates exceeding 99%, and furthermore, no cross-reactivity was found when the test was exposed to a panel of potentially interfering substances and antibodies, such as heterophile antibodies and rheumatoid factor, leading to the conclusion that the Dengue NS1 Rapid Test Cassette is a reliable, precise, and user-friendly point-of-care diagnostic tool, capable of facilitating the timely detection of dengue infections, which in turn supports prompt clinical intervention and effective public health responses in regions where dengue is endemic.

1. Introduction

Dengue virus (DENV), a flavivirus transmitted by mosquitoes, ranks among the primary contributors to illness and death in tropical and subtropical areas across the globe, presenting a formidable challenge to global public health [1]. As reported by the World Health Organization (WHO), there are roughly 390 million dengue infections each year, with approximately 96 million cases showing clinical manifestations [2]. Clinically, dengue can present as a self-resolving febrile condition (dengue fever) or progress to severe, life-threatening forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [3]. Prompt and precise diagnosis is crucial for

proper patient care, including timely fluid replacement therapy and surveillance for warning signs of severe disease, and for implementing effective public health interventions like vector control [4].

Given the non-specific nature of early dengue symptoms, which frequently overlap with those of other febrile illnesses (e.g., chikungunya, Zika virus infection, and malaria), laboratory confirmation of dengue infection is indispensable [5]. Conventional diagnostic approaches include virus isolation, a time-intensive method that demands specialized biosafety infrastructure, and reverse transcription-polymerase chain reaction (RT-PCR). While RT-PCR boasts high sensitivity for early detection, it relies on advanced equipment and professional technical skills, restricting its application in resource-limited or point-of-care environments [6]. Serological assays, particularly Enzyme-Linked Immunosorbent Assay (ELISA) for detecting IgM/IgG antibodies, are widely utilized but usually produce positive results only after the acute febrile phase, making them less effective for early diagnosis [7].

The detection of Dengue non-structural protein 1 (NS1) antigen has become a valuable diagnostic indicator during the acute phase of infection (1-7 days after symptom onset), often occurring prior to seroconversion [8]. Rapid diagnostic tests (RDTs) based on immunochromatographic technology for NS1 detection offer notable advantages, such as simplicity of operation, minimal training requirements, quick result turnaround (generally within 15-30 minutes), and adaptability to decentralized healthcare settings [9].

The Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma) developed by CITEST is intended for the qualitative detection of DENV NS1 antigen. This study seeks to conduct a comprehensive assessment of its clinical performance, encompassing sensitivity, specificity, accuracy, and precision, using a commercial Dengue Ag ELISA as the reference standard. The ultimate goal is to validate its effectiveness as a dependable rapid diagnostic tool.

2. Experimental Procedures

2.1 Source of Clinical Samples

A total of 383 archived clinical specimens were utilized for this evaluation. The specimens comprised human whole blood, serum, and plasma collected from a population of individuals presenting with symptoms suggestive of dengue infection as well as asymptomatic individuals, representing a realistic clinical spectrum. Specimen collection and handling followed standard clinical laboratory protocols. Whole blood samples were collected in EDTA tubes. Serum and plasma were separated by centrifugation at recommended speeds and durations. All specimens were stored at -20 ℃ or below prior to testing to preserve antigen integrity. Before analysis, frozen specimens were completely thawed and acclimated to ambient temperature (15-30 ℃). Specimens were mixed thoroughly to ensure homogeneity.

2.2 Test Kits and Procedures

The evaluation employed the Dengue NS1 Rapid Test Cassette (IDES-402, from Citest Diagnostics Inc.) as the investigational device, with a leading commercial Dengue NS1 Antigen Capture ELISA kit serving as the reference method. The study adopted a parallel-testing design where each specimen was tested simultaneously with both the rapid test and the ELISA.

The rapid diagnostic test was conducted under ambient temperature conditions (ranging from 15 to 30 ℃) following the equilibration of all test components—including the cassette and specimens—to room temperature. For serum or plasma samples, 50 microliters (μL) of the specimen was directly dispensed into the sample well (marked as S) of the test cassette using the accompanying dropper. In the case of whole blood samples, 50 μL of the blood was added to the

sample well, and this was immediately followed by the addition of one drop (equivalent to 40 µL) of the provided assay buffer. A timer was activated as soon as the sample was applied, and test results were read precisely at the 15-minute mark; any readings taken after 20 minutes were deemed invalid. The criteria for interpreting the test results are outlined below:

Positive Result: Two separate colored lines were observed—one in the control region (denoted as C) and another in the test region (denoted as T). Any detectable shade of color in the test region (T) was classified as a positive result.

Negative Result: Only a single colored line appeared in the control region (C), with no visible red or pink line present in the test region (T).

Invalid Result: The control line (C) did not appear. If this occurred, the test was repeated using a new test cassette.

3. Performance Analysis

3.1 Analysis of Performance Characteristics

3.1.1 Key Diagnostic Metrics

The comparative results between the Dengue NS1 Rapid Test Cassette and the reference Dengue Ag ELISA for all 383 specimens are summarized in Table 1.

Table 1: The comparative results between the Dengue NS1 Rapid Test Cassette and ELISA Result

Method		ELISA Result		Total
Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	137	4	141
	Negative	6	236	242
Total Results		143	240	383

Sensitivity Agreement: 95.8%

Specificity Agreement: 98.3%

Overall Accuracy: 97.4%

The high sensitivity indicates the test's effectiveness in correctly identifying individuals with acute dengue infection (low false-negative rate). The high specificity confirms its ability to correctly identify individuals without dengue infection (low false-positive rate), minimizing misdiagnosis.

3.1.2 Precision

Precision was evaluated through intra-assay and inter-assay studies.

Intra-run reproducibility was evaluated through the analysis of 15 duplicate tests for each of four distinct specimens, which included one negative sample, one low-positive sample, one medium-positive sample, and one high-positive sample. Every single duplicate test yielded accurate identification results, achieving a 100% correct classification rate, which confirms the presence of superior repeatability of the assay.

Inter-Assay Precision: Between-run precision was assessed by performing 15 independent assays on the same four panels of samples (negative, low, medium, high positive) using three different manufacturing lots of the test cassette. The results showed correct identification greater than 99% of the time across all lots, indicating high reproducibility and consistent performance between different production batches.

3.2 Discussion

3.2.1 Performance Characteristics

The CITEST Dengue NS1 Rapid Test Cassette demonstrated excellent clinical performance, with sensitivity and specificity exceeding 95% and 98%, respectively, and an overall accuracy of 97.4%. These metrics align with or surpass the performance of other commercially available dengue NS1 RDTs, which typically report sensitivities ranging from 70% to 95% and specificities from 95% to 100%.[10] The high sensitivity is crucial for an acute-phase test to avoid missing true cases, which is vital for clinical management and outbreak control. The high specificity is equally important in dengue-endemic areas where co-circulation of other flaviviruses and febrile illnesses is common, preventing unnecessary anxiety, further testing, and clinical interventions.

The precision studies confirm the test's robustness and reliability. The high intra-assay and inter-assay precision indicates that the test produces consistent results both within the same run and across different manufacturing lots, which is essential for quality assurance in clinical use. The lack of cross-reactivity with common interfering antibodies and substances further validates the assay's specificity and reduces the likelihood of false-positive results in patients with conditions like rheumatoid arthritis or other viral infections.

3.2.2 Limitations

As a qualitative immunochromatographic test, it has certain inherent limitations. The test is designed for the qualitative detection of NS1 antigen and does not provide quantitative viral load information. The intensity of the test line does not linearly correlate with antigen concentration. False-negative results can occur, particularly if the specimen is collected outside the optimal viremic window (typically the first 5-7 days of illness) or if the viral load/antigen level is below the test's detection limit. As indicated in the product information, a negative result does not rule out dengue infection, and clinical correlation with symptoms and epidemiological context is essential. For patients with persistent symptoms and an initial negative rapid test result, re-testing after a few days or confirmation with molecular methods (e.g., RT-PCR) is recommended. Proper specimen collection and adherence to the testing procedure are critical to avoid invalid results.

3.2.3 Comparison with Other Diagnostic Methods

Compared to the reference ELISA, the rapid test offers the significant advantage of speed, providing a result in 15 minutes versus several hours for a standard ELISA run. It also requires minimal laboratory infrastructure and technical training, making it ideal for primary healthcare centers, clinics, and field settings. While ELISA remains a robust laboratory-based method with high throughput, the rapid test serves as an excellent point-of-care screening tool. Compared to RT-PCR, the rapid test is less sensitive but far more accessible and faster, making it a practical first-line diagnostic tool during outbreaks and in resource-limited settings.

4. Conclusion

The CITEST Dengue NS1 Rapid Test Cassette (Whole Blood/Serum/Plasma) demonstrates high sensitivity (95.8%), specificity (98.3%), and overall diagnostic accuracy (97.4%) for the detection of dengue NS1 antigen when compared to a reference ELISA method. Its performance is supported by excellent precision and a demonstrated lack of cross-reactivity with common interfering substances. The test's ability to use whole blood, serum, or plasma provides flexibility for different clinical and field settings.

This rapid diagnostic cassette serves as a dependable, sturdy, and easy-to-operate tool for the early identification of acute dengue infections. The application of this device can greatly improve patient care by enabling timely clinical decision-making, supporting appropriate supportive treatment, and initiating swift public health measures for vector control. When integrated into a comprehensive diagnostic approach that takes into account clinical manifestations, epidemiological information, and confirmatory testing when required, this rapid diagnostic tool can considerably optimize the management of dengue fever. In doing so, it contributes to enhanced patient outcomes and more effective use of healthcare resources in areas where dengue is prevalent.

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