Comparative Analysis of Locally Manufactured Dengue NS1 Antigen Card Test and ELISA with Reference to Commercially Available Bio-Rad Dengue NS1 ELISA Kit

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Abstract

Dengue NS1 is a protein that can be detectable in patients' blood during early infection and aids in swift diagnosis through locally manufactured rapid ICT and ELISA kits, ensuring cost-effectiveness and timely treatment and reducing economic burden. Bio-Rad ELISA tested 122 clinically suspected dengue cases and proceeded with locally manufactured Immunochromatography assay method ICT (R-test) Dengue NS1 and ELISA kit (Beyond ELISA) for comparative analysis. Of 122 suspected cases, 95 samples were confirmed positive by BeYond ELISA kit. Bio-Rad ELISA found 94 positive cases, and 90 were detected as positive by ICT methods. One weak positive sample was detected by Beyond ELISA and missed by Bio-Rad; similarly, 5 ELISA positive samples were missed by R-test ICT. Locally manufactured ELISA were compatible with commercially available Bio-Rad ELISA kit. The sensitivity and specificity of the locally manufactured ELISA kit (98.96% and 100%, respectively) is equal to the commercially available Bio-Rad ELISA kit. Immunochromatographic R-test and locally manufactured ELISA are useful and economical tools for poor resource-setting institutes and labs for detecting Dengue NS1 infection. Furthermore, locally manufactured medical kits can be more readily available in emergencies, where time is of the essence.

Keywords: immuno-chromatographic technique, ELISA, Dengue NS1, Bio-Rad ELISA, R-test

1. Introduction:

Dengue fever is a mosquito-borne viral infection common in tropical and subtropical regions. The disease is caused by four serotypes of the dengue virus (DENV1-4), which belong to the Flaviviridae family. Dengue NS1 (nonstructural protein 1) is a protein produced by the dengue virus. It is an early biomarker for the diagnosis of dengue fever and is used in combination with other laboratory tests to confirm the diagnosis of dengue virus infection.

NS1 is a glycoprotein that is secreted by infected cells during the early stages of infection Dinkar and Singh [1]. It can be detected in the serum of infected individuals as early as day one after the onset of symptoms, making it a valuable tool for early diagnosis. NS1 is a glycoprotein that plays a crucial role in DENV replication and immune evasion. It is secreted by infected cells and circulates in the blood, making it a useful marker for the early diagnosis of dengue fever. NS1 detection tests are highly sensitive and specific and are commonly used in clinical settings to diagnose dengue fever Zahoor *e al* [2].

In addition to its diagnostic value, NS1 has been shown to play a role in the pathogenesis of dengue fever. It has been implicated in the development of severe disease and has been found to modulate the host immune response da Silva *et al*. [3]. Overall, the detection of NS1 is an important tool for the early diagnosis of dengue fever and for monitoring the progression of the disease.

Dengue NS1 ELISA (Enzyme-linked immunosorbent assay) is a laboratory test that detects the presence of the NS1 antigen in the blood of individuals suspected of having dengue fever. The test works by using specific antibodies that bind to NS1 antigen, which is then detected using an enzyme-linked colorimetric reaction Chen *et al*. [4]. The Dengue NS1 ELISA test is a highly sensitive and specific method for diagnosing dengue fever, especially during the early stages of the disease when NS1 is most abundant in the bloodstream. The test can detect NS1 antigen in the blood within 24-48 hours of the onset of symptoms, making it a valuable tool for early diagnosis and effective management of dengue fever. The Dengue NS1 ELISA test is widely used in clinical settings, and it is often used in combination with other diagnostic tests such as PCR (polymerase chain reaction) and IgM/IgG antibody tests to confirm dengue infection Lebeau *et al*. [5].

It is important to note that false-positive and false-negative results can occur with the Dengue NS1 ELISA test, and it should be interpreted in conjunction with clinical symptoms, travel history, and other laboratory test results Kassim *et al*. [6]. The detection of Dengue NS1 antigen can be performed using two methods: the enzyme-linked immunosorbent assay (ELISA) and the rapid immunochromatographic test (ICT). Both methods are designed to detect the presence of NS1 antigen in the blood of individuals infected with dengue virus, but they differ in their speed, sensitivity, and cost.

The Dengue NS1 ELISA method is a laboratory-based test that involves several steps, including coating a

microplate with NS1-specific antibodies, adding the patient's blood sample, and then detecting the presence of NS1 antigen using a secondary antibody conjugated to an enzyme. The results are usually available within a few hours, and the test is highly sensitive and specific.

On the other hand, the Rapid ICT method is a point-of-care test that can be performed in the field or at the bedside Chong *et al*. [7]. This test involves the use of a strip or a cassette that is pre-coated with NS1-specific antibodies. A drop of blood is added to the strip, and the test result is obtained within 15-30 minutes. The Rapid ICT method is less sensitive than the ELISA method but is more convenient, faster, and less expensive.

Overall, both methods have their advantages and disadvantages, and the choice of method depends on the specific needs of the clinical setting. The ELISA method is preferred in settings where high sensitivity and specificity are required, whereas the Rapid ICT method is preferred in resource-limited settings where rapid and low-cost testing is needed.

2. Material and Methods

The study was carried out by randomly collecting 122 blood samples of dengue suspected patients from tertiary care hospital in Rawalpindi. Serum was separated by centrifugation and tested for dengue serologically by locally manufactured Rapid (ICT Method) lateral flow assay kit and locally manufactured ELISA kit.

All the serum were further tested by using commercially available Bio-Rad ELISA kit for detection of Dengue NS1 antigen. All the procedure were carried out as per instruction of the manufacturer.

3. Results:

A total 122 suspected cases of dengue were tested for the detection of NS1 antigen by using locally manufactured R-test (lateral flow assay method) and ELISA. The same sample were subjected to the detection of NS1 antigen by commercially available Bio-Rad ELISA kit. Overall, 95 cases were detected as positive by ELISA and Rapid test. Total 90 detected as positive by R-test and 95 detected as positive by locally manufacturered ELISA kit. 94 samples were positive by Bio-Rad.

Table 1: Total serologically positive Dengue tests.

Table 1: Serologically positive cases diagnosed			
	T. Positive	T. Negative	Total
R-test Dengue NS1 Rapid Test	90	32	122
Elisa (Bio-Red)	94	28	122
Locally manufactured Elisa kit	95	27	122

Table 2: Total no. of serologically positive dengue NS1 test by different kits.

Rapid test	ELISA	ELISA Bio-Rad	ELISA + Rapid	Rapid + Bio-Rad ELISA
90	95	94	90	89

P value < 0.05, is significant)

Table 3: Comparison of locally manufactured ELIS and Rapid test

Rapid test	Beyond ELISA
90	95
73%	77.80%

P value < 0.05, is significant)

Table 4: Comparison between locally manufactured ELISA and BIO-Rad ELISA kit for detection of Dengue NS1.

ELISA Bio-Rad	Beyond ELISA
94	95
77%	78%

P value < 0.05, is significant)

A total 78% positive dengue NS1 cases detected by using three different confirmatory tests.

Table 5: Specificity and sensitivity of Beyond ELISA Dengue NS1 kit.

Statistic	Value	95% CI
Sensitivity	98.96%	94.33% to 99.97%
Specificity	100.00%	85.18% to 100.00%

4. Discussion:

A total 122 sample were subjected to three different kit or by two different methods.95 (77%) were positive for the dengue NS 1 assay. Results of the current study shows high prevalence of cases with the results (23.3%) of Datta *et al*. [8], (30%) of Kulkarni *et al*. [9] and (16%) of Srivastava *et al*. [10]. As Rapid chromatographic lateral flow assay technique is seeming to be very effective due to rapid detection of particular infection. Five sample were observed or detected false positive with comparison to locally manufactured ELISA and four Positive cases were detected as false positive with comparison to commercially available Bio-Rad Dengue NS 1 ELISA. In this study a weak positive sample detected by locally manufactured ELISA kit and also detected by R-test but Bio-Rad ELISA kit fails to the detect the weak positive sample. Rapid R-test were missed only four positive cases which were confirmed by Bio-Rad ELISA. As the literature suggest that the ELISA is more sensitive then Rapid as in this study the rapid test detection is 73% whereas by ELISA is 77% which similar with the other study Hora *et al*. [11]. Locally manufactured ELISA were compatible with commercially available Bio-Rad ELISA kit. Efficiency and Specificity of locally manufactured ELISA kit is equals to commercially available Bio-Rad ELISA kit.

5. Conclusion:

Locally manufacturing medical kits have several advantages over commercially available kits, including cost-effectiveness and accessibility. There is often less reliance on expensive imported materials and technology, which can result in lower costs. Furthermore, locally manufactured medical kits can be more readily available in emergency situations, where time is of the essence. However, it is important to note that the quality and safety of locally manufactured medical kits must be carefully regulated and monitored to ensure that they meet the necessary standards for effectiveness and safety. In some cases, it may be more appropriate to use commercially available medical kits that have been

thoroughly tested and approved by regulatory agencies.

6. Reference:

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