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Comparison of the Diagnostic Efficacy of Rapid Immunochromatographic Test with Microwell ELISA in the detection of Dengue NS1 Antigen - A Retrospective Study

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A B S T R A C T

Dengue fever is a *Flavirus* disease transmitted by *Aedes* mosquito. Primary dengue virus infection is characterized by elevations in specific NS1 antigen levels 0 to 9 days after the onset of symptoms. This retrospective study was conducted to assess performance of rapid ICTs in acute dengue diagnosis in comparison to ELISA test. It was conducted on 2234 serum samples received at The Department of Microbiology, Apollo Diagnostics, Regional Reference Laboratory, Chennai from September 2017 to November 2017 by ICT and ELISA to detect NS1 antigen. Total NS1 positive samples were 763(34.1%) by Rapid ICT and 815(36.5%) by ELISA. Five hundred and thirteen (513-63%), 234(28.7%) and 214(26.3%) of the positive samples belonged to male, female and children respectively. Monthly analysis of the data during the study period showed that 318(39.2%), 383(46.9%) and 114(13.9%) of the dengue NS1 antigen were detected during the months of September, October and November 2017 respectively. The sensitivity of Dengue NS1 Rapid ICT when compared with ELISA was found to be 93.6% and specificity was found to be 100%. ELISA has a minimal edge over Rapid ICT in the detection of Dengue NS1 antigen. Hence ICT in combination with ELISA could help in early and accurate diagnosis of acute dengue infection.

Introduction

Dengue fever which is caused by DENV and belongs to the *Flavivirus* genus of the *Flaviviridae* family[1]. It is an arthropod-borne viral infection transmitted to humans by mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus* and is endemic to both tropical and sub-tropical regions of the world[2]. The disease has become global public health concern because the infection incidence rates have increased 30-fold in the past 50 years. WHO has placed India under Category A as Dengue is endemic in our country[3].

DENV is a positive-sense, single-stranded RNA virus. The genome encodes three structural proteins – the capsid (C), membrane (M) and envelope (E) glycoproteins – as well as seven non-structural proteins (NS1, NS2A,

NS2B, NS3, NS4A, NS4B and NS5). There are four distinct DENV serotypes (DENV-1 - 4), all of which are now circulating in Asia, Africa and the Americas[4].

After an incubation period of 4 – 8 days, infection can range from mild dengue fever (DF) to severe forms of DENV infection, referred to as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[5]. In 2009, the WHO introduced a new classification system for DENV, replacing the traditional DF and DHF/DSS with DENV infection with and without warning signs and severe DENV infection. Asymptomatic dengue cases are mostly seen in children and also in adults with primary infection. Symptoms of dengue are fever, headache, fatigue, body pains, rash, petechiae and bleeding through nose, gums and gastrointestinal tract. Though most patients recover after a self-limiting illness, about 5 – 10% of patients progress to hypovolemic shock with respiratory distress, pleural effusion, pericardial effusion, rising haematocrit values, thrombocytopenia etc. If untreated, the mortality of patients with acute DENV infection can be as high as 20%, although appropriate case management can reduce mortality to less than 1%[6].

Accurate diagnosis of dengue is an important component of public health surveillance since clinical diagnosis does not differentiate dengue from other diseases that present with dengue-like signs and symptoms (e.g., malaria, leptospirosis, measles, influenza, Japanese encephalitis, West Nile fever, yellow fever)[7]. Hence, there is the global need for accurate dengue diagnostics. There are three main laboratory methods to diagnose dengue infection: viral isolation in culture, detection of viral RNA, NS1 antigen and specific IgM/IgG antibodies in paired sera. The gold standard is usually a combination of these methods[8]. However, methods such as virus isolation and genomic RNA detection (PCR), need a specialized laboratory, well trained laboratory personnel and costly, which are not widely available in resource limited settings. Primary dengue virus infection is characterized by elevations in specific NS1 antigen levels up to 50µg/ml 0 to 9 days after the onset of symptoms; this generally persist upto 15 days[9]. As per Indian national guidelines, a patient is labelled as a "probable" if he satisfies the clinical criteria during dengue outbreak or positive non ELISA based immuno-chromatography tests (ICT) such as NS1 antigen(Ag) ICT "confirmed" when NS1 Ag is positive by ELISA[10].

There were many studies conducted to calculate the sensitivity and specificity of the rapid diagnostic tests that are used to detect NS1 Ag and there was a considerable variation in the percentages reported. Hence this retrospective analysis of the available data was conducted to assess performance of rapid ICTs in acute dengue diagnosis in comparison to Microwell ELISA test in terms of sensitivity and specificity.

Materials and Methods

This retrospective study was conducted on 2234 serum samples received at The Department of Microbiology in Apollo Diagnostics, Regional Reference Laboratory, Chennai from September 2017 to November 2017. All samples were tested using a commercially available immuno-chromatographic test (ICT) followed by ELISA for NS1 antigen detection.

Detection of NS1 antigen by Immuno-chromatographic Test (ICT)

After bringing the kit to room temperature, place the device on a flat surface and add 100µl of the serum sample (2 drops) in the sample window. Read the results in 20 minutes.

Interpretation of results

1. NEGATIVE: If only one band (Control band) appears in the result area, Interpret the result as negative. This shows that the specimen does not contain Dengue Ns1 antigen.

2. POSITIVE: If two bands (Control & Test) appear in the result area the specimen is reactive for Dengue NS1 antigen

3. INVALID: If no band appears after the test is complete, interpret the result as invalid. This shows that the test has been performed incorrectly or there was some procedural error. Please check the procedure and retest using a new device.

Detection of NS1 antigen by Microwell ELISA

Fit the strip holder with the required number of Anti-Dengue NS1 antibody coated strips. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon reader software. Add 50 µl Diluent in all the wells. Add 50 µl Negative Control in A-1well, 50 µl Calibrator in B-1, C-1 and D-1 well, 50 µl Positive Control in E-1 well and 50 µl sample in F-1 well onwards. Add 100 µl of working Conjugate Solution in each well. Ensure thorough mixing of controls, samples to be tested and working conjugate to get reproducible results. Apply cover seal. Incubate at 37ºC + 1ºC for 90 min. + 1min. While the samples and working Conjugate are incubating, prepare working Wash Solution as specified in preparation of reagents. Take out the plate from the incubator after the incubation time is over and, wash the wells 6 times with working Wash Solution. Add 150 µl of working substrate solution in each well. Incubate at room temperature (20-30ºC) for 30 min. in dark. Add 100 µl of stop solution. Read absorbance at 450 nm. within 30 minutes in ELISA Reader. Calculate the Dengue NS1 antigen units by multiplying the sample O.D. ratio by 10.

Interpretation of results

a. If the Dengue NS1 Ag Units is < 9 then interpret the sample as Negative for Dengue NS1 Antigen.

b. If the Dengue NS1 Ag Units is between 9 - 11 then interpret the sample as Equivocal for Dengue NS1 Antigen.

c. If the Dengue NS1 Ag Units is > 11 then interpret the sample as Positive for Dengue NS1 Antigen.

Results and Discussion

Of the 2234(100%) samples tested for NS1 antigen by Rapid Immuno-chromatographic test (ICT), 763(34.1%) showed positive for the presence of NS1 antigen and 1471(65.9%) were found to be negative. When the samples tested for NS1 antigen by ELISA, 815(36.5%) showed positive for the presence of NS1 antigen whereas 1419(63.5%) were found to be negative. The sensitivity of Dengue NS1 Rapid ICT when compared with ELISA was found to be 93.6% and specificity was found to be 100% (Table 1: Study Results and Comparison of Rapid ICT with ELISA).

Demographic analysis of the NS1 antigen positives showed that 513(63%), 234(28.7%) and 214(26.3%) of the positive samples belonged to male, female and children respectively. Our study showed a male preponderance in concordance with the studies conducted by Gupta *et al*[11]., 2006; Chakravarti *et al*[12]., 2005; Sarkar *et al*[13]., 2012 and a greater prevalence among the adult population. Ankita Nisarta *et al*[14] reported that females were more commonly affected than males with male to female ratio of 1:1.35 in contrast to our study (Figure 1: Demographic analysis of the positive samples).

Table.1 Study results and comparison of rapid ICT with ELISA

	Detection of NS1 Antigen by Rapid ICT	Detection of NS1 Antigen by ELISA		
NS1 Antigen Positive	763(34.1%)	815(36.5%)	**Sensitivity**	93.6%
NS1 Antigen Negative	1471(65.9%)	1419(63.5%)	**Specificity**	100%
Total	2234(100%)	2234(100%)		

Figure.1 Demographic analysis of the positive samples

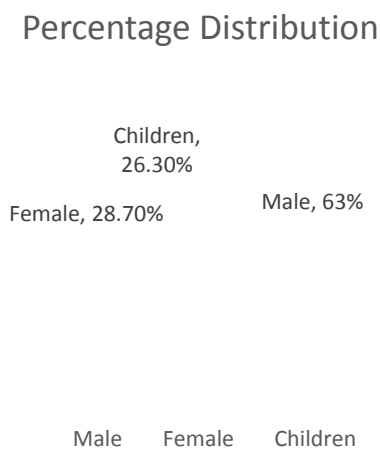
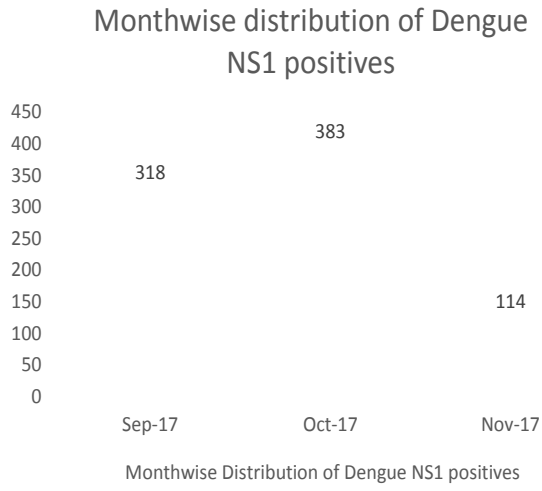


Figure.2 Month wise distribution of dengue NS1 antigen positives during the study period



The prevalence of NS1 antigen was found to be 36.5% from our study which is similar to the studies conducted by J.V. Sathish *et al*[15] (36.34%) in 2017 and Priyadharshini Shanmugam *et al*[16] (38.4%) in 2016. Paradesi Naidu *et al*[17] in 2015 reported a lower prevalence of 12.5% when compared to our study.

From the monthly analysis during the study period we observed that 318(39.2%), 383(46.9%) and 114(13.9%) of the dengue NS1 antigen were detected during the months of September, October and November 2017 respectively. This showed that there was an increase in the incidence of dengue during the month of September 2017 and attained a peak during October and significant decline was seen in November.

Similar observation was also made by Ilamani *et al*[18] (2014) and Priyadharshini Shanmugam *et al*[16] (2016). This was related to the onset of monsoon in South India when the vectors are found in large numbers (Figure 2: Monthwise Distribution of Dengue NS1 antigen positives during the study period).

The sensitivity of Rapid ICT when compared to ELISA was found to be 93.6% and specificity of 100% which corroborates with the results of Mahesh Reddy *et al*[19] in 2016 (90.1% sensitivity and 98.45% specificity). Similar results were also observed by Groen *et al*[20]., (2000) and Shih *et al*[21] (2016). Whereas Subhamoy Pal *et al*[22] (2014) reported that sensitivity ranges from 71.9% - 79.1%. This shows that ELISA is more sensitive and specific in detecting NS1 antigen. NS1 antigen detection assay has an advantage of detecting infection very early (from DAY 1), however it disappears early and is of little use in the early convalescence phase when antibody detection becomes useful[23].

To conclude, ELISA has a minimal edge over Rapid ICT in the detection of Dengue NS1 antigen. Hence NS1-antigen detection through ICT in combination with ELISA could help in early and accurate diagnosis of dengue infection in the laboratories where facilities and trained staff are available. Immunochromatographic test being cost effective, and needs less technical expertise can be used in peripheral health care centres for mass screening activities or in laboratories that have limited resources for the detection of dengue fever. But ELISA is recommended for further confirmation because of its high sensitivity and specificity and specificity.

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