

Original Research Article

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## Evaluation and Comparison of Currently Available Fluorescence Immunoassay (FIA) Test with Rapid Immuno Chromatographic Test (ICT) Considering NS 1 ELISA as a Reference Test

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### ABSTRACT

Dengue is one of the rapidly emerging diseases globally. Early diagnosis can improve clinical outcome by ensuring close follow up, initiating appropriate supportive treatment and increasing awareness of hemorrhage and shock. Many diagnostic kits are available commercially. Amongst these gold standard test is IgM and IgG ELISA with seroconversion, but we can advice this test only after 5 days when the antibodies will start developing. Nonstructural glycoprotein 1 (NS-1) is considered to be a useful marker for early diagnosis of dengue, as this can be advised early. NS 1 ELISA is carried out only in institutional laboratories where technical expertisation as well as specific machines are available. So, for a routine laboratory with limited resources rapid ICT test is a better choice. In the present study the currently available Fluorescence immunoassay (FIA) test is evaluated and compared with rapid ICT considering NS 1 ELISA as a reference test. To evaluate the currently available fluorescence immunoassay test for detection of Dengue NS 1 antigen. To compare the currently available Fluorescence immunoassay (FIA) test for detection of Dengue NS 1 antigen with rapid ICT test and Microwell ELISA test. Cross sectional observational study. In this study, 200 samples from dengue suspected patients are tested for presence of NS 1 antigen by rapid ICT test. From these 50 positive and 50 negative samples are further retested by NS 1 ELISA and NS 1 by FIA. Results are analyzed and compared with each other. NS 1 ELISA is considered as a reference test and NS 1 Fluorescence immunoassay and rapid ICT as an index tests. Data is analyzed and sensitivity, specificity, positive predictive value and negative predictive value are derived for each test. FIA test is more specific and having positive predictive value of 97.43% which states that it is more reliable than rapid ICT. FIA is less time consuming and easy to perform whereas Microwell ELISA is time consuming and require highly technical expertise. NS 1 Microwell ELISA and FIA results are very much comparable with each other. Whereas false positive less with FIA and more with rapid ICT card test.

#### Keywords

Dengue, FIA, ELISA, ICT, NS 1 Antigen.

#### Article Info

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### Introduction

Dengue is an important arthropod borne viral disease, transmitted by *Aedes aegypti* mosquito,<sup>(1)</sup> affecting tropical and subtropical regions.<sup>(2,3)</sup> It is one of the rapidly emerging disease globally,<sup>(1)</sup> affecting 100 million

people/year, of which 5,00,000 cases presenting with dengue hemorrhagic fever and resulting in around 30,000 deaths mostly among children.<sup>(2)</sup> Early diagnosis can improve clinical outcome by ensuring *close*

follow up, initiating appropriate supportive treatment, and increasing awareness of hemorrhage and shock. Dengue disease is caused by one of four closely related but antigenically distinct virus serotypes DENV 1, DENV 2, DENV 3, and DENV 4 of genus *flavivirus*.<sup>(2,4,5,6)</sup> The dengue viral genome is positive sense, enveloped, single stranded RNA virus, approximately 11 kb in size.<sup>(1,2,5,6,7)</sup> It contains 10 genes of 3 structural proteins (capsid C, membrane M, envelope E) and of seven Non Structural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5.<sup>(1,2,4,6)</sup> NS 1 is glycoprotein released from dengue virus infected cells, involved in virus replication.<sup>(2,3,5,6,7,8)</sup> It is hexameric soluble protein released during infection and accumulate in higher concentrations upto 50 µg/ml, which is detectable in primary as well as secondary infections in early phase.<sup>(2,5)</sup> Clinically fever starts with malaise, chills and headache, soon leads to backache, joint pain, muscular pain and pain behind the eye ball.

Temperature persists for 3 to 5 days or may raises in about 5 to 8 days (saddle back fever). Myalgia is severe with deep bone pain, which characteristic of fever (break bone fever). Dengue manifest in three forms, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).<sup>(9)</sup> Infection with one of four serotypes provide immunity to only that serotype for life. Reinfection with different serotype after primary attack is more dangerous causes DHF. Majority infections are self-limiting, subside on its own.

Death is a rare event.<sup>(10)</sup> Patient may present with decreased blood cells like leucopenia (<4000/cumm) / thrombocytopenia (<one lakh/cumm), petechiae, ecchymosis / purpura, mucosal bleeding / epistaxis / bleeding gums, hematemesis / malena, > 20% rise in HCT for age/sex. Release of cytokines, release of vasoactive mediators, release of procoagulants, may manifest as disseminated

intravascular coagulopathy, with signs of plasma leakage like - Pleural effusion, Ascites, Hypoproteinemia.<sup>(10)</sup>

Currently there are three basic methods for diagnosis in laboratories, these are virus isolation, detection of viral genome by RT-PCR and detection of dengue virus specific antibodies and detection of NS 1 antigen.

### **Diagnostic tests**<sup>(1)</sup>

Serology

Identification – RT PCR

Isolation – Virus culture

### **Disease monitoring tests**

White Blood Count

Haematocrit value

Platelet count

Liver Function Tests

Analysis of paired acute and convalescent rise in sera to show four fold rise in antibody titer by ELISA is the most reliable evidence of an active dengue infection.<sup>(6)</sup>

NS 1 is non-structural protein present in febrile phase from day 0 to day 9, generally persist upto 15 days.<sup>(11)</sup> IgM antibodies are not detectable until 5 to 10 days in primary infection and day 4 to 5 days in secondary infection after onset of illness. IgG antibodies appear after 14 days and persist for life in primary infection and rise within 1 to 2 days after the onset of symptoms in secondary infection. NS 1 is considered to be a useful marker for early diagnosis of dengue. Estimated by Rapid ICT and Microwell ELISA methods. Rapid ICT gives false positives with Ebstein Barr virus, Malaria, Rheumatoid factor, Leptospirosis, Japanese encephalitis, Yellow fever and West Nile virus.<sup>(10,11)</sup> NS 1 is considered to be a useful marker for early diagnosis of dengue

infection. In routine laboratories rapid ICT (immunochromatographic test) is the test of choice, as ELISA (Enzyme Linked Immunosorbant Assay) tests require technical expertisation and special equipments, while PCR (Polymerase Chain Reaction) and viral cultures are expensive and are carried out in reference laboratories where facilities are available. Serological tests may give false positive (cross reactivity) / false negative (related to time of testing) results. PCR is only available in few centers where special equipments and trained personnel are available and it is more expensive. Virus isolation has poor yield, difficult to isolate, time consuming and require special lab equipped with tissue and virus isolation, and it is also more expensive.

The present study was aimed to evaluate the currently available FIA test, which is being made available and is being evaluated at different centers, as per the request of manufacturer, the test have been evaluated by testing samples detecting NS 1 Antigen simultaneously by FIA, Microwell ELISA and Rapid ICT card tests.

### **Aims and objectives**

To evaluate the currently available fluorescence immunoassay test for detection of dengue NS 1 antigen.

To compare the FIA test for detection of NS 1 Antigen by rapid ICT and ELISA tests.

### **Materials and Methods**

Duration of study: 1<sup>st</sup> September 2016 to 20<sup>th</sup> September 2016.

Design: Cross sectional observational study.

Total 200 samples from dengue suspected patients from tertiary care hospital, collected

during 1<sup>st</sup> September 2016 to 20<sup>th</sup> September 2016 (this was the period when many cases of dengue were there) were tested with rapid ICT to detect NS 1 antigen. Out of 200, 59 were positive for either NS 1, IgM and/or IgG and 141 were negative by rapid ICT. From 59 positive samples, 46 were positive for NS 1 Ag only, 4 were for NS1+IgM, 6 were for IgM, 2 were for IgM + IgG and 1 was positive for IgG. Out of 200 samples 50 NS 1 positive and first 50 negative samples were further tested for detection of NS 1 antigen by Microwell ELISA and by fluorescence immunoassay (FIA). Only IgM, Only IgG, and IgG and IgM positive samples were excluded from this study. The kits were supplied by J.mitra and Co. Pvt. Ltd. In this study Microwell ELISA test was considered as a reference test and FIA and ICT were as index tests. Only IgM, Only IgG, and IgM and IgG positive were excluded from this study. In this study Microwell ELISA test was considered as a reference test and FIA and ICT were as index tests.

### **NS 1 by FIA**

It is dengue NS 1 antigen Quanti card. It detects dengue NS 1 antigen qualitatively in human serum/whole blood/plasma. It is sensitive immune-chromatographic test with iQuant Analyser. It is fluorescence immunoassay based on sandwich principle. In this kit test area is coated with specific anti dengue NS 1 antigen antibodies. When sample added, if dengue NS 1 antigen is present, it forms a complex with another specific NS 1 antibodies conjugate to fluorochrome. After addition of assay buffer, complex formed migrates along nitrocellulose membrane to the test region and antibody-antigen-antibody fluorescence immunocomplex is formed. Which is detected by iQuant analyser and results will be displayed on screen. Care should be taken of QR code, anything should not be

stuck/written on it, which leads to erroneous results. It is only operational with iQuant analyser. Advantages are – we can use fresh finger prick blood, only 5 µl sample is required, easy to perform, less time required as compared to ELISA.

### **Procedure**

5 µl of whole blood/serum/plasma is added in well 'S'. Avoid spillage on QR code. Add three drops of assay buffer in well 'B'. Allow to react for 25 mins. Then insert this kit into iQuant analyser with arrow ← towards analyser. Then press 'RUN' icon. Note value on the screen. Do not read after 25 mins.

### **NS 1 by Microwell ELISA**

Here there is in vitro qualitative detection of dengue NS 1 antigen in human serum/plasma. It detects all four serotypes of dengue virus. It is solid phase enzyme linked immunosorbant assay (ELISA) based on 'Direct sandwich' principle. Microwells are coated with anti-dengue NS 1 antibodies with high reactivity for dengue NS 1 antigen. Dengue NS 1 antigen from sample is trapped / sandwiched between antibody and Horse Reddish Peroxidase conjugate antibody. Amount of bound peroxidase gives concentration of dengue NS 1 antigen. Addition of substrate buffer and chromogen develops blue colour. Intensity of developed colour is proportional to the concentration of dengue NS 1 antigen in the sample. After addition of stop solution yellow colour develops which is read at 450 nm spectrophotometrically.<sup>(13)</sup>

### **Rapid ICT**

It is rapid visual test for detection of NS 1 antigen and differential detection of IgM and IgG antibodies in human serum/plasma. It is based on antigen-antibody reaction. The kit contains two devices containing testing for

NS 1 antigen and IgM/IgG antibodies. The tests are run following manufacturer's instructions. If NS 1 antigen present in sample this antigen forms Antigen antibody complex that migrates along the membrane to the test region and forms the visible pink line at T region as antigen antibody gold conjugate complex.<sup>(12)</sup>

Interpretation: Reactive: 1 U and above. Non-Reactive: < 1 U.<sup>(11)</sup>

### **Results and Discussion**

50 negative samples by rapid ICT were also negative by Microwell ELISA and FIA.

The sensitivity, specificity, positive predictive value, and negative predictive value were calculated for Rapid ICT and FIA considering Microwell ELISA as a reference test

As no study was carried out using comparative studies of NS 1 detection by FIA, Rapid ICT and NS 1 ELISA, can't compare with others.

Subhamoy Pal *et al.*, states that rapid ICT is simple, easy and less time will be required, although NS 1 ELISA is superior with better sensitivity requiring trained personnel and special equipments. Considering NS 1 ELISA as a reference test in this study, rapid ICT gives 76 % positive predictive value, stating that false positives more by rapid ICT. Meng Ling Moi *et al.*, (2013) and Seok Mui Wang *et al.*, (2010) states that NS 1 ELISA is useful, sensitive, specific and promising tool for early diagnosis of dengue viral infection. FIA is the test, which requires less time than NS 1 ELISA and its sensitivity is 100 %, specificity is 98.38 %, positive predictive value is 97.43 % and negative predictive value is 100 % which states that NS 1 Microwell ELISA and FIA results are very much comparable.

Tables 1–6 show the following findings.

In present study, 50 (25%) samples were positive for NS1 by rapid ICT, which is comparable with studies of Ronni Mol (27.8%), Pramiladevi *et al.*, (27.27%), Jigna Karia *et al.*, (20.35%) and with slight deviation with Badave *et al.*, (16.51%) and Deora *et al.*, (15.6%)

In present study, 19% samples were positive by NS 1 Microwell ELISA, while Ronni Mol showed 27.8 % positive cases.

Present study showed that majority of cases (i.e. 33 and 69 of dengue suspected cases) belong to age group 11-20 and 21-30 yrs.

This was comparable to other studies of Ronni Mol.

The disease was predominantly seen in males (72%) than females (38%) i.e., 1.8:1 which was corresponding to the other studies of Ronni Mol, males (59%) than females (41%) i.e. 1.4:1 and Jigna Karia *et al.*, males (67%) and females (32%) i.e. 2:1.

**Table.1** Comparison of NS 1 detection by rapid ICT and NS 1 Microwell ELISA

ELISA → Rapid ICT ↓	NS 1 POSITIVE	NS1 NEGATIVE	Chi-square	P-value
NS 1 POSITIVE	38	12	141	<0.0001 S
NS 1 NEGATIVE	00	150		
Total (200)	38	162		

P-value S = significant.

From 50 positive samples by rapid ICT, 38 samples were positive and 12 were negative by Microwell ELISA.

**Table.2** Comparison of NS 1 detection by FIA and NS 1 Microwell ELISA

ELISA → FIA ↓	NS 1 POSITIVE	NS 1 NEGATIVE	Chi-square	P-value
NS 1 POSITIVE	38	01	194	< 0.0001 S
NS 1 NEGATIVE	00	161		
Total (200)	38	162		

P-value S = significant.

From 50 positive samples by rapid ICT, 39 samples were positive and 11 were negative by FIA.

**Table.3** Parameters of interest for Rapid ICT and FIA considering NS 1 ELISA as a reference test.

	Rapid ICT	FIA
Sensitivity	100 %	100%
Specificity	80.64%	98.38%
Positive predictive value	76%	97.43%
Negative predictive value	100%	100%

FIA test is more specific and having positive predictive value of 97.43% which states that it is more reliable than rapid ICT.

**Table.4** Age wise distribution of cases with reference to sex

Age	Male	Female	Total cases
0 to 10	15 (12.93 %)	07 (8.3 %)	22
11 to 20	20 (17.24 %)	13 (15.4 %)	33
21 to 30	36 (31.03 %)	33 (39.28 %)	69
31 to 40	11 (9.48 %)	08 (9.5 %)	19
41 to 50	12 (10.34 %)	13 (15.47 %)	25
>50	22 (18.96 %)	10 (11.90 %)	32
Total	116 (100 %)	84 (100 %)	200

Present study showed that majority of cases (i.e. 33 and 69 of dengue suspected cases) belong to age group 11-20 and 21-30 yrs.

**Table.5** Sex wise distribution of dengue positive and negative cases

Sex	Dengue positive	Dengue negative	Total
Male	36 (72 %)	80 (53.33 %)	116
Female	14 (38 %)	70 (46.66 %)	84
Total	50	150	200

The disease was predominantly seen in males (72%) than females (38%) i.e 1.8:1

**Table.6** Comparison of NS 1 Antigen by rapid ICT and NS 1 Ag ELISA

Test	NS 1 Ag Positive	Percentage
FIA	39 (out of 200)	19.5 %
NS 1 Ag ELISA	38 (out of 200)	19 %
Rapid ICT	50 (out of 200)	25 %

In present study, 39 (19.5%) samples were positive by FIA, which is comparable with NS 1 ELISA 38 (19%) in same study.

Sr. No	Year	Author	Total samples	NS 1 by rapid ICT	NS 1 by ELISA
1	2015	Ronni Mol P	90	25 (27.8%)	25 (27.8%)
2	2015	Pooja S. Deora <i>et al.</i> ,	288	45 (15.6%)	-
3	2015	Gitanjali K. Badave <i>et al.</i> ,	327	54 (16.51%)	-
4	2013	Pramiladevi <i>et al.</i> ,	66	18 (27.27%)	-
5	2011	Jigna Karia <i>et al.</i> ,	280	57 (20.35%)	-
6	2016	This study	200	50 (25%)	38 (19%)

It indicates that male predominance than female and commonest age group affected (11 to 30), disease is more transmitted at work place.

ELISA is as good as gold standard tests but it is time consuming and technical expertisation is required.

ICT is less time consuming, easy to perform but it may give false positive results.

FIA is less time consuming and easy to perform whereas Microwell ELISA is time consuming and require highly technical expertise.

NS 1 Microwell ELISA and FIA results are very much comparable in relation with sensitivity, specificity, positive predictive value, and negative predictive value.

Hence for routine laboratories with limited resources one can use FIA test over Rapid ICT for early diagnosis of acute dengue infection

### **Limitations**

More studies are required to assess the performance of FIA test device and that too, on a bigger sample size. Also this should be correlated with RT PCR/viral isolation / paired analysis of IgM and IgG titers by ELISA.

Study should include day of fever, platelet counts, symptoms of patients, so can correlate with time and conditions of patients.

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