A COMPARATIVE STUDY ON THE PERFORMANCE OF TWO COMMERCIAL SEROLOGICAL DENGUE KITSSaha Dalal Bibhas¹, Roy Swagnik², Mitra Saurabh³, Dasgupta Rajat⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: The performance of a commercial rapid immunochromatographic dengue NS1, IgG/IgM assay device was evaluated against an in-place dengue IgM-capture ELISA in the KPC Medical College & Hospital, Jadavpur. Of the 932 serum samples from patients with clinical diagnosis of acute dengue illness, 542, 237 and 90 samples were tested positive respectively for NS1 ELISA, anti-dengue IgM (MAC ELISA) and anti-dengue IgG ELISA. Comparatively, 476, 205 and 79 samples were tested positive respectively by the immunochromatographic method, has less sensitivity of 13% and a relative specificity of 12% compared to the ELISA method. Though the rapid immunochromatographic assay device has the advantages of rapid testing which simultaneously detects both IgG and IgM and can also be performed with whole blood, serum or plasma, the user has to exercise extreme caution with the interpretation of the test result.

KEYWORDS: Rapid immunochromatographic dengue NS1, NS1 ELISA, MAC ELISA, Flavivirus, DHF, DSS, PCR.

INTRODUCTION: Dengue viruses are a major public health problem in tropical and subtropical areas and being the cause of one of the most important mosquito-borne viral diseases.⁽¹⁾ Infection with dengue virus can result in a relatively benign, acute febrile illness (Dengue fever) or in severe disease with abnormalities in vascular permeability (Dengue hemorrhagic fever [DHF]) which can sometimes lead to sudden and often fatal hypovolemic shock (Dengue shock syndrome [DSS]).⁽²⁾ Dengue viruses belong to the genus *Flavivirus* under the family *Flaviviridae*. The four dengue virus serotypes (Dengue virus types 3 to 6) are closely related yet antigenically distinct.^{1,2}

In terms of health (Morbidity and mortality) and economic costs, dengue virus infection is the most important mosquito-borne virus disease in the world, with an estimated 50 to 100 million cases of human infections worldwide and resulting in around 24, 000 deaths. Infection with a dengue virus may be clinically inapparent or may be present as a nonspecific febrile illness, classic dengue fever (DF), or dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).⁽³⁻⁵⁾ All four dengue virus serotypes are capable of causing dengue fever, with the induction of an immune response that in most cases leads to lifelong protection against clinical disease arising from infection with the homologous serotype.

Secondary infection with a heterologous serotype, however, may lead to the severe complications of DHF and DSS.^(6,7) Antibody-dependent enhancement of dengue virus growth in cells of the monocyte/macrophage lineage resulting from the presence of preexisting, non-neutralizing dengue virus-specific antibodies has been proposed as the pathogenetic mechanism that underlies DHF and DSS.⁽⁸⁾ However, the link between this enhanced replication and the vascular permeability that characterizes these diseases is still the subject of conjecture.⁽⁹⁾

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MATERIALS AND METHOD: The test kits of X Co.'s Immunochromatography (Dengue NS1 Antigen and differential detection of IgM& IgG antibodies in Human Serum/ Plasma) as well as ELISA (Microwell ELISA Test for the Detection of Dengue NS1 Antigen, IgM Antibodies and IgG Antibodies in Human Serum/Plasma) kits were used.

FOR IMMUNOCHROMATOGRAPHIC METHOD TEST PROCEDURE:

A) For Dengue NS1 antigen device was as follows:

1. 2 drops (70 µl) sample (serum/ Plasma) was added to the specific well.
2. Reaction takes to occur for 20 minutes.
3. Results were read at 20 minutes. Positive results may appear as early as 2-10 minutes. However, negative results must be confirmed after 20 minutes only.

B) Dengue IgM/ IgG device was as follows:

1. 10µl of sample was added using micropipette to the sample well of the antibody device. 2 drops of dengue antibody assay buffer was added to the Buffer well "B" of the device.
2. Reaction takes to occur for 20 minutes.
3. Results were read at 20 minutes. Positive results may appear as early as 2-10 minutes. However, negative results must be confirmed after 20 minutes only.

FOR MICROWELL ELISA TEST FOR THE DETECTION OF DENGUE NS1 ANTIGEN IN HUMAN SERUM/PLASMA TEST PROCEDURE:

1. 50 µl Diluent was added in all the wells.
2. 50 µl Negative Control was added in A-1 & B-1 well.
3. 50 µl Positive Control was added in C-1 well.
4. 50 µl sample was added in D-1 well onwards.
5. 100 µl of working Conjugate Solution was added in each well.
6. Thorough mixing of controls were done, samples to be tested & working conjugate to get reproducible results.
7. Cover seal was applied.
8. Incubated at 37°C + 1°C for 90 min. + 1min.
9. While the samples and working Conjugate were incubating, working Wash Solution was prepared as specified in preparation of reagents.
10. The plate from the incubator was taken out after the incubation time is over and, washed the wells 6 times with working Wash Solution.
11. 50 µl of working substrate solution was added in each well.
12. Incubated at room temperature (20-30°C) for 30 min. in dark.
13. 100 µl of stop solution was added.
14. Absorbance at 450 nm. Was read within 30 minutes in ELISA READER.

MICROWELL ELISA TEST FOR THE DETECTION OF DENGUE IGMANTIBODIES (MAC ELISA) AND DENGUE IGG ANTIBODIES IN HUMAN SERUM/PLASMA TEST PROCEDURE:

1. A-1 well was left as substrate blank.
2. 100 µl Negative Control was added in B-1well.

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3. 100 µl Positive Control was added in C-1 well.
4. 100 µl calibrator was added in D-1 & E-1 wells.
5. 100 µl of sample diluent was added in each well, starting from F-1 followed by addition of 1µl sample.
6. Cover seal was applied.
7. Incubated at 37°C + 1°C for 60 min. + 1min.
8. While the samples are incubating, working Wash Solution was prepared and working Conjugate as specified in preparation of reagents.
9. The plate was taken out from the incubator after the incubation time is over and, washed the wells 5 times with working Wash Solution.
10. 100 µl of working Conjugate Solution was added in each well excluding A-1.
11. Cover seal was applied.
12. Incubated at 37°C + 1°C for 60 min + 1min.
13. Aspirated and washed as described in step no. 9.
14. 100 µl of working substrate solution was added in each well including A-1.
15. Incubated at room temperature (20-30°C) for 30 min. in dark.
16. 50 µl of stop solution was added.
17. Absorbance was read at 450 nm. Within 30 minutes in ELISA READER after blanking A-1 well.

INTERPRETATION OF THE TEST IMMUNOCHROMATOGRAPHY METHOD:

A) Dengue NS1 Ag Device:

REACTIVE: Appearance of pink coloured line, one each in test region “T” and control region “C” indicates that the sample is REACTIVE for Dengue NS1 Ag.

NON-REACTIVE: Appearance of one distinct pink line in the control region “C” only, indicates that the sample is “NONREACTIVE” for Dengue NS1 Ag.

INVALID: When neither control line nor the test line appears on the membrane the test should be treated as invalid.

B) Dengue IgM& IgG Antibodies Device:

IgM& IgG REACTIVE: appearance of red coloured line in the control region ‘C’ and Test region; IgM region ‘M’ and IgG region ‘G’ indicates that the sample is reactive for both IgM & IgG antibodies. This is indicative of a secondary dengue infection.

IgM REACTIVE: (i) appearance of red coloured line in the control region ‘C’ and Test region; IgM region ‘M’ indicates that the sample is reactive for IgM antibodies. This is indicative of a primary dengue infection.

IgG REACTIVE: appearance of red coloured line in the control region ‘C’ and Test region; IgG region ‘G’ indicates that the sample is reactive for IgG antibodies. This is indicative of a secondary dengue infection.

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NON-REACTIVE: appearance of one distinct red coloured line in the control region 'C' only (With no line in the IgM region 'M' & IgG region 'G') indicates that the sample is nonreactive for dengue antibodies.

INVALID: When neither control line nor the IgM/ IgG line appears the test should be treated as Invalid which may be because of following reasons:

CALCULATION OF RESULTS (For NS1 ELISA):

TEST VALIDITY: Ensured that the following is within specified acceptance criteria:

1. NC or NCx O. D. must be < 0.3 . If it is not so, the run is invalid and must be repeated.
2. PCx O. D. must be > 1.0 . If it is not so, the run Cut-off Value.

Cut-off value can be determined by using the following formula:

Cut-off Value = $NCx + 0.30$.

Where NCx is mean absorbance (O.D) of Negative Control.

e.g. $0.052 + 0.30 = 0.352$.

INTERPRETATION OF RESULTS: The absorbance of the unknown sample is compared with the calculated cut-off value:

- a) Test specimens with absorbance value less than cut-off value are non and may be considered as negative for Dengue NS1 Antigen.
- b) Test specimens with absorbance value greater than or equal to cut off reactive for Dengue NS1 Antigen.
- c) Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of Dengue NS1 Antigen and should be retested in duplicate.
- d) Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the Kit criteria. Original specimen should be retested in duplicate.
- e) If both duplicate retest sample absorbance value is less than cutoff value, is considered non-reactive.
- f) If any one of the duplicates retest sample absorbance value is equal the cutoff or both duplicate retest value are equal to or greater than the cutoff, the specimen is considered reactive by the Kit criteria.

TEST VALIDITY: (MAC ELISA, dengue IgG antibody ELISA): Ensured that the following is within specified acceptance criteria:

1. Blank must be < 0.100 in case of differential filter being used. In case differential filter is not available in the reader the blank value may go higher.
2. NC or NCx O.D. must be < 0.3 . If it is not so, the run is invalid and must be repeated.
3. Ratio of PCx O.D. / Calibrator O.D. must be > 1.1 . If it is not so, the run is invalid and must be repeated.
4. Calibrator O.D. should not be < 0.35 . If it is not so, the run is invalid and must be repeated.

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CALCULATION OF RESULTS:

- Cut off value = mean O.D. of calibrator
- Calculation of sample O.D. ratio: Calculate sample O.D. ratio as follows:
 Sample O.D.
 Sample O.D. ratio =
 Cut off Value
- Calculation of Dengue IgM units: Calculate by multiplying the sample O.D. ratio by 10.
 Dengue IgM units = sample O.D. ratio x 10.
 e.g.: sample absorbance (O.D.) = 0.925
 Cut off value = 0.512
 Sample O.D. ratio = $0.925 / 0.512 = 1.806$
 Dengue IgM units = $1.806 \times 10 = 18$

INTERPRETATION OF RESULTS:

- If the Dengue IgM Units is < 9 then interpret the sample as Negative for Dengue IgM antibodies.
- If the Dengue IgM Units is between 9 - 11 then interpret the sample as Equivocal for Dengue IgM antibodies.
- If the Dengue IgM Units is > 11 then interpret the sample as Positive for Dengue IgM antibodies.

RESULTS:

No. of Samples	Status	Dengue NS1 immunochromatography on the 1 st day of fever		
		Positive	Equivocal	Negative
550	Dengue positive	476	0	74
390	Dengue Negative	55	0	335

Table 1

Sensitivity 86.54%, Specificity 85.9%

No. of Samples	status	Dengue NS1microlisa on the 1 st day of fever		
		Positive	Equivocal	Negative
550	Dengue positive	542	0	8
390	Dengue Negative	3	0	387

Table 2

Sensitivity 98.54%, Specificity 99.23%

No. of samples	status	Dengue IgM Immunochromatography after 5 days of fever		
		Positive	Equivocal	Negative
250	Dengue positive	205	0	45
300	Dengue Negative	42	0	258

Table 3

Sensitivity 82.0%, Specificity 86.0%

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No. of Samples	status	Dengue IgM microlisa (MAC ELISA) after 5 days of fever		
		Positive	Equivocal	Negative
250	Dengue positive	237	0	3
300	Dengue Negative	3	0	297

Table 4

Sensitivity 94.8%, Specificity 99%

No. of samples	status	Dengue IgG immunochromatography after 15 days of fever		
		Positive	Equivocal	Negative
100	Dengue positive	79	0	21
450	Dengue Negative	59	0	391

Table 5

Sensitivity 79%, Specificity 86.9%

No. of Samples	status	Dengue IgG microlisa after 15 days of fever		
		Positive	Equivocal	Negative
100	Dengue positive	90	0	10
450	Dengue Negative	3	0	447

Table 6

Sensitivity 90%, Specificity 99.33%

DISCUSSION: The performance of a commercial rapid immunochromatographic dengue NS1, IgG/IgM assay device was found to be poor against an in-place dengue IgM-capture ELISA, NS1 and Dengue IgG antibody ELISA in our study. Results of both the parameters were confirmed by PCR and was found to be similar with parameters obtained by the ELISA method. Of the 932 serum samples from patients with clinical diagnosis of acute dengue illness, 542, 237 and 90 samples were tested positive respectively for NS1 ELISA, anti-dengue IgM (MAC ELISA) and anti-dengue IgG ELISA. Comparatively, 476, 205 and 79 samples were tested positive respectively by the immunochromatographic method, has less sensitivity of 13% and a relative specificity of 12% compared to the ELISA method. Though the rapid immunochromatographic assay device has the advantages of rapid testing which simultaneously detects both IgG and IgM and can also be performed with whole blood, serum or plasma.

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