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Original Research Article

Evaluation of a novel immuno-magnetic assay technology for rapid detection of dengue NS1 antigen

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ABSTRACT

Background: Serological diagnosis of dengue fever is based on the detection of NS1 antigen and further confirmed by the assay of dengue IgM and IgG. Rapid Diagnostic Tests (RDTs) are no longer recommended for use by the Government of India. However, there is a requirement of rapid results without compromising on the test quality. Accordingly, we undertook an evaluation of a novel cartridge-based system employing a unique table-top diagnostic device for dengue NS1 antigen by BluSense Diagnostics incorporating a patented Immuno-Magnetic Assay (IMA).
Methods: A total of 309 samples were tested on unlinked anonymous basis. Each sample was tested using NS1 Ag ELISA (Microlisa, J. Mitra), ViroTrack and NS1 RDT (SD Alere) and results were recorded. Discordant samples were further tested by dengue NS1 AG ELISA (Panbio) and dengue IgG/IgM Maclisa (J. Mitra).
Results: When compared with approved ELISA kits used globally the Virotrack test returned a sensitivity of 94.74%; specificity of 97.44%; PPV of 97.30%; NPV of 95% and accuracy of 96.10%. Hourly throughput is 5-6 samples.
Conclusions: The Virotrack system is highly suitable as a POCT module in HCFs with low to moderate workload that employs novel technology, is rapid, user friendly and comparable to the ELISA in sensitivity and specificity. The equipment is user friendly and can work both on plasma/serum and whole blood. Model with multiple modules (3-4) will improve the throughput and turnaround time. Evaluation of this novel technology has been done in India for the first time.

Keywords: BluBox, Dengue NS1 Ag, Immuno-magnetic assay, Point of care testing, Virotrack

INTRODUCTION

Dengue fever is caused by the dengue virus belonging to genus Flavivirus; family Flaviviridae (serovar DEN 1-4) and transmitted by Aedes aegypti mosquito. In India, the distribution is seasonal and most commonly seen in urban and semi urban regions. For the last few years, several outbreaks of dengue fever have been reported worldwide. More than 100 countries have been affected and nearly 35-40 percent of the global population are now at risk.[1] The fever is often self-limiting but has potential to cause life threatening complications namely Dengue Haemorrhagic Fever (DHF) and Dengue Septic Shock (DSS). Usually, the diagnosis of DHF is made on clinical ground and ancillary investigations. The clinical picture is often complex due to comorbidities and overlapping viral infections. Early and rapid diagnosis is therefore mandatory to monitor the treatment and occurrence of any complications.[2]

Early serological diagnosis is based on the detection of NS1 antigen and further confirmed by the assay of dengue IgM and IgG. These tests are recommended to be performed on ELISA platform. Although accurate and precise, the ELISA tests are labour intensive, time consuming and the samples need to be run in batches. Rapid Diagnostic Tests (RDTs) are no longer recommended for use by the Government of India.

Hence, there is a great requirement of a Point of Care Testing (POCT) module that employs novel technology, is rapid, user friendly and comparable to the ELISA in sensitivity and specificity.[3]

A number of novel technologies are being tested worldwide and some of them are already approved by CE certification.

We undertook the evaluation of a novel cartridge based system employing a unique diagnostic device for infectious diseases by BluSense Diagnostics based on a new, simple and scalable blood testing technology incorporating a patented Immuno-Magnetic Assay (IMA) developed by DTU Nanotech, Denmark and CIC NanoGUNE, Spain for assay of dengue NS1 and comparing with ELISA based tests as well as Rapid Diagnostic Tests (RDTs). The tests were conducted in the innovative product block called the BluBox supplied by BluSense for the first time in India.[4-6]

The evaluation was carried out at Oncquest Laboratories Ltd, New Delhi between August to October 2018.

Primary objective of the study is to evaluate the efficacy of the ViroTrack platform in detection of NS1 antigen in suspected cases of Dengue fever in comparison to the existing NS1 AG ELISA and NS1 rapid card test (RDT) and secondary objective is to ascertain the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of the ViroTrack, NS1 AG ELISA and NS1 rapid card test.

METHODS

BluSense Diagnostics ApS, Symbion Bioscience Park, Fruebjergvej 3, 2100 Copenhagen, Denmark, a provider of assay technologies and various diagnostic test kits approached Oncquest Laboratories Ltd, 3 Factory Road, New Delhi - 110029, India**,** a CAP and NABL accredited diagnostic centre, to conduct performance evaluation for the BluBox equipment and ViroTrack Dengue NS1 diagnostic test assay kit for the purposes of product registration in India in future. Accordingly, an "Evaluation Agreement" between the two parties was signed delineating the procedural, financial and timeline details. Two ViroTrack machines (BluBox) and 325 Dengue NS1 Virotrack cartridges were placed by BluSense at Oncquest. An initial training was conducted for the staff. (Figure 1, Figure 2). The tests were performed in the department of microbiology, Oncquest laboratories, New Delhi over a period of approximately three months (August to October 2018).

Both in-patient and OPD subjects with clinical suspicion of dengue fever were included in the study. 3 ml whole blood was collected aseptically in vacutainers with clot activator gel after obtaining informed consent. Samples for other tests were also collected in appropriate vacutainers and rapidly transported to the main lab under ice and after registration serum was separated by centrifugation and tested on the same day without delay. The residual sample was stored at minus 200C. Samples that were turbid, lipemic or grossly homolysed were rejected and a repeat sample was requested. Samples received and tested within 48 hours was considered fresh. Samples stored between 2-7 days and more than 7 days were randomly selected and tested as per the established lab protocol.

Figure 1: Blubox.

Figure 2: ViroTrack cartridge.

A total of 309 samples were tested on unlinked anonymous basis that were routinely received at the lab for Dengue NS1 assay by ELISA. Informed consent of patients was obtained and there were no invasive interventions.

Testing algorithm

Initially, each sample was tested using Dengue NS1 AG ELISA (J. Mitra), ViroTrack and Dengue NS1 RDT (SD Alere) and results were recorded. All samples showing positive by NS1 AG ELISA (J. Mitra) and ViroTrack were considered 'True positive'. Similarly, samples found non-reactive by NS1 AG ELISA (J. Mitra) and ViroTrack were considered 'True negative'. Any discordance between the ELISA and the ViroTrack was designated as 'discordant'

and further tested using a separate algorithm as under. (Figure 3)

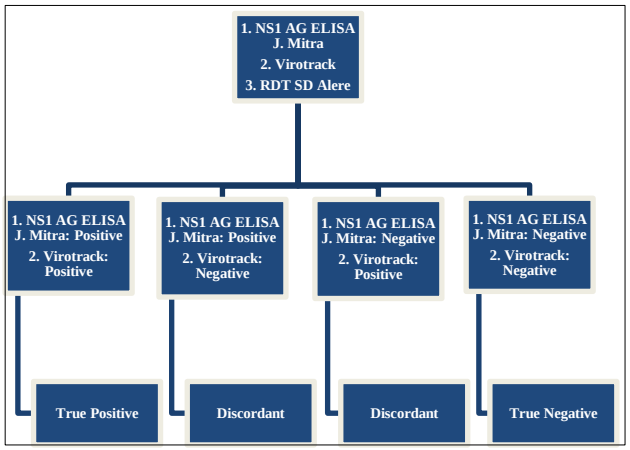

Figure 3: Testing algorithm (all samples).

Testing algorithm (discordant samples)

Samples that showed discordance in results by the two testing systems were designated 'discordant' and were further tested by Dengue NS1 AG ELISA (Panbio) and Dengue IgG/IgM Maclisa (J. Mitra). (Figure 4, Table 2) The assay results were interpreted as under:

- Discordant samples that showed positives with NS1 Ag ELISA (Panbio) were considered true positive.
- IgM positivity was considered 'true positive'.
- IgG positivity alone was reconciled with clinical findings like fever, myalgia, joint pains, rashes, hypotension, reducing platelet counts etc for considering 'true positive'.

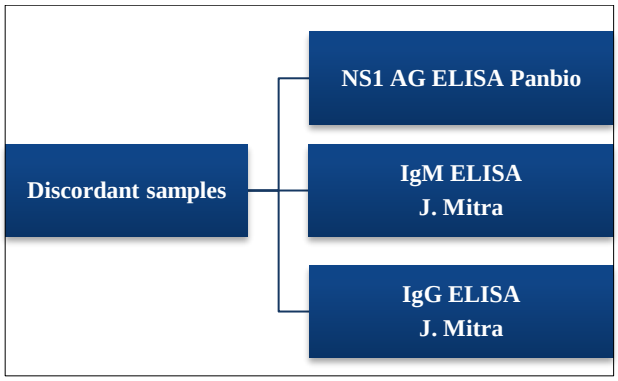

Figure 4: Testing algorithm (discordant samples).

Accordingly, final results were noted as true positive (Positive); true negative (Negative); ViroTrack, false positive, false negative and equivocal; J Mitra ELISA false positive; Panbio ELISA false negative; RDT SD false negative and false positive (Table 3 and 4).

The entire dataset was recorded in Excel format and analysed independently by the Oncquest and BluSense researchers. The preliminary reports were generated by Oncquest and comments were sought from BluSense. Three rounds of discussions were held and final reconciliation on discordant samples were arrived at.

NS1 Ag ELISA, Panbio

At which 44 discordant samples were tested by NS1 AG ELISA Panbio. 39 tests were run on randomly selected samples.

One sample (equivocal in Virotrack) was removed from the test results.

Quality parameters of each test kit and platform have been analysed under sensitivity, specificity, PPV, NPV and accuracy (Table 4).

The following procedure was adopted for the test. After plugging the BluBox to a power source, the machine was switched on. Initial self-check and calibration completed and step by step instructions were displayed on the LCD panel. Sample and patient data were fed and saved. The ViroTrack cartridge was removed from the cover, marked and 30 microlitre of serum (whole blood can also be used) was loaded into the designated well. The cartridge was closed and inserted into a test slot in the BluBox and fixed with a click. The test was set to run. The BluBox conducted a self-test and after ensuring the correctness of the cartridge placement, the test was allowed to continue by the onboard software.

The display panel continued to show the progress of the run and after 8-10 minutes, the run ended with semiquantitative report namely negative (green display), equivocal and positive (red display). A few cartridges did not complete the run and exhibited 'cartridge failure'. The failed cartridges had to be discarded. The machine has memory to archive reports and can be interfaced with the Lab Information System (LIS). The data can be retrieved directly using a standard pen drive.

RESULTS

Out of a total of 309 subjects 176 were males and 133 were females. 41 subjects were in the pediatric age group; rest of the subjects were adults. 241 samples out of 309 were tested within 48 hours of collection. 152 samples were tested "final true positive" (including all false negatives) and 157 samples were tested "final true negative" (including all false positives). 44 samples were found to be "discordant". 14 Virotrack cartridges exhibited errors (Table 1).

Among 44 discordant samples were analysed by the Oncquest lab and the results were reviewed by the BluSense team and the final 'truth' was arrived at. The final truth for 37 samples were designated with unanimity. In 7 cases, a second round of analysis was done, and final truth was arrived in 6 cases. One sample (Virotrack-equivocal) was removed from the calculations (Table 2 and 3).

Table 1: General data.

Total samples		309
Sex distribution	Males	176
	Females	133
Age distribution	<12 Years	41
	12- 27 Years	93
	27-42 Years	89
	42-56 Years	36
	> 57 Years	40
Sample age	Fresh (48 Hours)	241
	< 7 Days	49
	> 7 Days	19
Final True positives		152 (includes all false negatives)
Final True negatives		157 (includes all false positives)
Discordant results		44
Additional tests	Maclisa J. Mitra IgM/IgG	44
	NS1 AG ELISA Panbio	83
ViroTrack cartridge data	Valid runs	309
	Cartridge errors	14
	Repeat tests	02

Table 2: Discordance details.

Total Discordance	False positive ViroTrack	False negative ViroTrack	False positive ELISA J. Mitra	False negative RDT SD Alere	False positive RDT SD Alere
44	4	8	31	34	1

Table 3: Final analytical process: discordant samples.

Virotrack BluSense	NS1 Ag ELISA J. Mitra	NS1 Ag ELISA Panbio	IgM Elisa J. Mitra	IgG Elisa J. Mitra	Remarks
-	+	-	-	-	False positive NS1 Ag ELISA J. Mitra
+	-	-	-	-	False Positive ViroTrack
-	+	+/-	+/-	+/-	False Negative ViroTrack

Table 4: Test results of individual kits.

	Samples	True Positive	True Negative	False Positive	False Negative	Equivocal
Virotrack Blusense	309	144	152	4	8	1
NS1 AG ELISA J. Mitra	309	152	126	31	0	-
RDT NS1 SD Alere	309	118	156	1	34	-
NS1 AG ELISA Panbio	83	24	54	0	5	-

Table 5: Quality parameters of individual kits.

Parameter	NS1 J. Mitra ELISA (n=309)	NS1 Panbio ELISA (n=83)	RDT NS1 SD Alere (n=309)	ViroTrack (n=308)
Sensitivity (In %)	100.00	82.76	77.63	94.74
Specificity (In %)	80.25	100.00	99.36	97.44
PPV (In %)	83.06	100.00	99.16	97.30
NPV (In %)	100.00	91.53	82.11	95.00
Accuracy (In %)	89.97	93.98	88.67	96.10

Significantly, NS1 AG ELISA J. Mitra was found to have 31 false positives and RDT NS1 SD Alere showed 34 false negative results. Virotrack showed 4 false positives and 8 false negative results (Table 4).

NS1 J. Mitra ELISA, and showed sensitivity, specificity, PPV, NPV and accuracy of 100, 80.25, 83.06, 83.06, 100 and 89.97; NS1 Panbio ELISA 82.76, 100, 100, 91.53 and 88.76; RDT NS1 SD Alere 77.63, 99.36, 99.16, 82.11 and 88.67; Virotrack 94.74, 97.44, 97.3, 95 and 96.10 respectively (Table 5).

DISCUSSION

Dengue fever an important arthropod borne viral disease that has taken epidemic proportion in many parts of India. Although most cases of dengue subside without any residual complications, DHF and DSS are being reported with increased frequency with significant mortality. Furthermore, during the season wherein dengue is reported, other febrile illnesses namely, malaria, typhoid fever, scrub typhus and chikungunya are also reported concurrently. This confuses the disease spectrum as early and precise diagnosis of the infectious organism causing the febrile episode becomes that much important for a clinician. This is of importance as early therapeutic intervention is necessary for bacteria and protozoal infections.[1-3,7,8] As of now, detection of NS1 antigen as a screening test for dengue has proved to be invaluable. However, the ELISA protocol commonly used is time consuming, laborious and needs to be run in batches. Dengue IgM Maclisa assay is considered confirmatory. In view of the above we studied the efficacy of the ViroTrack platform in detection of NS1 antigen in suspected cases of Dengue fever in comparison to the existing NS1 AG ELISA and NS1 rapid card test (RDT). Additionally, we tried to ascertain the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of the ViroTrack, NS1 AG ELISA and NS1 rapid card test. Concurrently, we compared the above assay characteristics for the three tests mentioned.

The characteristics of the hardware, consumable and the novel technology is discussed as under:

Dengue NS1 Ag Virotrack cartridges

BluBox is run in combination with single-use disposable cartridges (ViroTrack cartridges), each of which run tests for one patient. The ViroTrack cartridge requires only 30 µL of blood (one drop) and is simple to use, as all blood processing operations are automatically run without need of any additional tool or equipment. In fact, the ViroTrack cartridge is a small plastic device embedding nanoparticles and microfluidics structures, which enable the sample preparation directly on the cartridge. The process functions via centrifugal microfluidics, allowing blood plasma separation, dilution, mixing, and resuspension of nanoparticles without the need of pumps or any user sample-preparation. The design allows splitting the sample into multiple measurement chambers. As a result, it is only necessary, first, to load the drop of blood on the cartridge and, second, to insert the cartridge in the BluBox. All the subsequent processes are completely automated, which leads to a sample-to-answer time below 15 min. The cartridges were thin and packed satisfactorily. The sample loading was easy and biosafe. Loading the cartridge inside the BluBox was easy and the start process was without delay. 14/325(4.3%) cartridges exhibited cartridge failure. No leakages were observed, and disposal was easy.

Technology: Immuno-magnetic assay (IMA)

The patented immuno-magnetic assay is the core of the ViroTrack cartridge and its diagnostics operations. The technological innovation is based on the opto-magnetic readout which is part of BluSense's core IP. This technology has been developed by the founders between DTU Nanotech, Denmark and CIC NanoGUNE, Spain, by combining expertise in nanomagnetism and microfluidics from collaborating research groups.[9-13]

Commercial superparamagnetic nanoparticles (MNP)-size below 200 nm - embedded and dried in the ViroTrack cartridge, are coated with coupled antibody or antigens capable to form agglutination in presence of the target analyte. The nanoparticles, when mixed with the sample and incubated in high magnetic field, agglutinate and form anisotropic nanoclusters. An AC magnetic field is then used to force nano-clusters rotation, which causes a temporal scattering cross-section variation which is optically measured using a low-cost/high-tech Blu-ray unit, and a photodetector, all part of BluBox. The novelty is the intrinsic simplicity and scalability of the technology, which does not require any special sensor or sophisticated optics but just off-the shelf components, both in the cartridge and the reader. As unique edge compared to RDTs, the same specificity and sensitivity of ELISA test can be achieved with the immuno-magnetic assay format which requires minimal sample/buffer exchange, temperature control steps and incubation time.

The compatibility of the same readout method with both antigen and antibodies assays on the same microfluidic support is unique, and no similar microfluidic device embedding dilutions and multiplexing have been developed so far, opening compelling opportunity in different diagnostics areas as well. In fact, this technology allows the detection of virtually any biomarker in blood. BluSense has spent the past 3.5 years optimizing the technology and applying it to the three dengue biomarkers and is currently working towards the development of cartridges for the diagnosis of Zika.

Performance of BluBox: Hardware

BluBox is a portable (<3 kg) optical reader based on Blu-ray technology components. It can be connected via Lan port, Wi-Fi and USB and does not need any calibration as it can perform multiple quality controls upon each cartridge run. The software can be quickly updated through internet connection. It gives access to data analysis which can provide guidance to analyze the biomarkers pattern and translate the result into the

severity of the infections. As advanced features, the BluBox embedded software can analyze the values of the biomarkers and visualize them on a scale of risk. It can also perform automated reporting of a positive case to the healthcare authority, syncing BluBox communication protocols to the databases and servers of the local Healthcare authorities.

- Both the BluBox performed satisfactorily throughout the testing process as a table-top model. Except a minor problem with the power cord in one machine, there were no breakdowns observed.
- The programming was satisfactory and user friendly.
- Average run time per test was around 10 minutes with serum/plasma.
- The results were flashed as positive/ negative/ equivocal with value ranging from 1-100 in different colour codes.

Overall performance

When compared with different other approved ELISA kits used globally the Virotrack test returned a Sensitivity of 94.74%; Specificity of 97.44%; PPV of 97.30%; NPV of 95% and Accuracy of 96.10%. The results are comparable to the existing standards. Both sensitivity and specificity are acceptable. Repeat tests in two cases returned identical results. Some of the problem areas was low throughput in labs/HCFs with higher workload; single test parameter in a cartridge and a cartridge failure rate of 3-5%.

CONCLUSION

The system appears to be highly suitable as a POCT module in HCFs with low to moderate workload. Hourly output is 5-6 samples. Model with multiple modules (3-4) will improve the throughput and turnaround time. Considering electricity failures, battery operations is recommended for 6 hours. In addition to NS1, other parameters singly or in combination (2 per cartridge) e.g. malaria (HRP, pan LDH and LDH for vivax), typhoid, scrub typhus, Leptospira, IgM Dengue, HCV, HBV and HIV can be introduced to fulfil the necessity of a fever panel in India. In future parameters like CRP, PCT, Troponin, CKMB; urinary parameters like sugar, ketone and leucocyte esterase and future parameters: tuberculosis, tumour markers, HPV can be incorporated.

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