Development of ASSURE® Dengue IgA Rapid Test for the Detection of Anti-dengue IgA from Dengue Infected Patients

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ABSTRACT

Background: Rapid and early dengue diagnosis is essential for patient management and early disease intervention. MP Diagnostics ASSURE® Dengue IgA Rapid Test (Dengue IgA RT) was developed for the rapid detection of anti-dengue IgA in patients' biological samples. The performance of Dengue IgA RT was examined using multiple categories of well-characterized samples. **Materials and Methods:** Dengue IgA RT was designed and developed. Following characterization of samples by reference ELISAs, the performance of the kit was evaluated. **Results:** The overall sensitivity and specificity of Dengue IgA RT were 86.70% (*n*=233) and 86.05% (*n*=681) respectively; in which Dengue IgA RT detected 77.42% primary and 92.86% secondary cases; compared to 70.97% and 72.14% by IgM-Cap ELISA and 89.25% and 20% by Non-Structural Protein 1 (NS1) Ag ELISA respectively. Using 125 paired samples, Dengue IgA RT showed 84.80% sensitivity at acute phase and 99.20% sensitivity at convalescent phase; with 92% specificity at both phases. Dengue IgA RT also demonstrated a consistent performance (sensitivity: 85.53%, specificity: 80%) with 76 whole blood samples. In detecting all four serotypes of DENV (*n*=162), the performance of Dengue IgA RT was comparable with in-house IgM-Cap ELISA. Kinetics of anti-dengue IgA production was elucidated with 42.86% detection level as early as one-two days after fever onset, which increased to 83.33% between five and seven days after fever onset. **Conclusion:** Dengue IgA RT demonstrated a good performance and is applicable as one of the dengue early diagnostic tools at all levels of health care system.

Key words: Anti-dengue IgA, ASSURE® Dengue IgA Rapid Test, Dengue

INTRODUCTION

Dengue virus (DENV), a member of the genus flavivirus, is mostly found in tropics and subtropics regions. Four serotypes of the Dengue virus (DENV 1-4) are closely related but antigenically distinct and co-circulated in dengue endemic countries.[1,2] The virus is principally transmitted by *Aedes aegypti* and causes infection in humans. In terms of morbidity, mortality and economic costs, dengue is the most important mosquito-borne disease in the world, with an estimated 50 to 100 million infections annually.[3,4]

Clinically, two major types of dengue fever are recognized: primary (first time) and secondary (subsequent) infections. Patients with primary DENV infections typically result in a self-limiting disease. It is characterized by the sudden onset of fever lasting from three to seven days, and a variety of nonspecific signs and symptoms including severe headache with retro-orbital pain, body aches, joint pain and rash.[5-7] Secondary DENV infection is caused by a second exposure by a different DENV serotype. According to Gubler, it is a common form of the disease in many parts of Southeast Asia and South America.[6] This form of the disease is more serious and can result in dengue hemorrhagic fever and dengue shock syndrome. Major clinical features include high fever, hemorrhagic events, circulatory failure, and the fatality rate can be as high as 30%.[6] Early diagnosis of dengue shock syndrome (DSS) is particularly important, as patients may die within 12 to 24 h if appropriate treatment is not administered.[5,8]

Early and rapid diagnostic tool, management and confinement of dengue cases are critical to halt the dengue virus transmission. In recent years, good progress has

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been made in the development of dengue diagnostic tools, resulting in the availability of suitable tests for each stage of the disease. Reverse transcription-polymerase chain reaction (RT-PCR) which detects specific dengue viral ribosomal nucleic acid (RNA) has been widely used to diagnose and serotype dengue viruses in the early phase of the disease.[9-15] These techniques, whilst rapid and effective in providing early and definite dengue diagnosis, is costly and requires trained personnel to perform. Thus, its use is limited to few clinical laboratories. Development of Non-Structural Protein 1 (NS1) antigen detection in the Enzyme-linked immunosorbent assay (ELISA) and rapid lateral flow platform has offered clinical laboratories a very promising tool for early diagnosis during the febrile phase of the disease.[16-18] However, its performance in detecting dengue secondary cases was reported to be not as good as primary infection.[19]

Serological tests have been routinely used for the detection of DENV infection. Typically, basic serologic tests include hemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC ELISA) and indirect immunoglobulin G (IgG) ELISA.[6] In recent years, the detection of anti-dengue IgM based technique has become the most widely used serologic test, due to its simple approach and nearly all patients (93%) developed detectable IgM antibody 6 to 10 days after onset.[6] However, detection at 6-10 days could result in a delayed diagnosis. Moreover, IgM persists in circulation for more than eight months and thus in dengue endemic countries, the detection of IgM in a febrile patient does not necessarily indicate an acute dengue infection.[20-22] Analysis of paired samples at least seven days apart for definitive diagnosis is required, and this further discourages the use of these tests among clinicians and patients. On the other hand, around 30% of dengue secondary patients either do not develop anti-dengue IgM or develop low level of anti-dengue IgM which is undetectable by current diagnostics.[23]

The use of anti-dengue immunoglobulin A (IgA) as a diagnostic marker has previously been explored. The simultaneous increase of dengue-specific IgA and IgM in dengue patients using an anti-dengue virus IgA capture ELISA (AAC-ELISA) and short life span of IgA, compared to IgM has been reported.[24] Another report indicated that anti-dengue IgA typically appeared a day after IgM and disappeared within 45 days following detection.[22]

Recognizing the potential role of IgA in dengue diagnosis, a new rapid test, MP Diagnostics ASSURE® Dengue IgA Rapid Test (Dengue IgA RT) was developed. Dengue IgA RT is an immunochromatographic test device intended for the detection of IgA antibodies to dengue virus in human whole blood, plasma and serum, within 20 min. The rapid detection of dengue infection offers the advantage in making testing feasible and applicable at any level of healthcare system.

In this study, we report on the development and validation of Dengue IgA RT using multiple categories of samples.

MATERIALS AND METHODS

Antigen

Inactivated Dengue 2 antigen (Microbix Biosystems Inc, Ontario, Canada) was immobilized on nitrocellulose membrane and used as an antibody capturing agent in test line. Dengue Type 2 antigen (strain 16681) was propagated in Vero cells, concentrated from tissue culture supernatants by precipitation and ultracentrifugation. The antigen was then purified by sucrose density gradient centrifugation, inactivated by formaldehyde, neutralized by sodium bisulfite and lastly verified the inactivation by cell culture technique.

Recombinant protein

Recombinant Protein L (Thermo Scientific, Illinois, U.S.A) was immobilized on nitrocellulose membrane and used as a control line. Protein L is an immunoglobulin-binding protein that was originally derived from the bacteria *Peptostreptococcus magnus*. It captures human antibodies including IgA through kappa light chain interactions without interfering with an antibody's antigen-binding site.

Gold conjugate

Goat anti-human IgA (Zymed Laboratories Inc / Invitrogen, California, U.S.A) is used to detect anti-dengue IgA bound on Dengue antigen and human IgA captured by Protein L, resulting in pink-purplish color band development. Goat anti-human IgA was conjugated to colloidal gold using in-house standard conjugation protocol.

Test principle of Dengue IgA RT

Dengue IgA RT is a reverse flow technique (US Patent -6316205, WO/2009/139725) immunochromatographic rapid test for the qualitative detection of anti-dengue IgA from patient's biological samples (serum, whole blood or plasma). In this platform, test sample migrates upward from the sample well and eventually form antibody-antigen complexes with dengue virus antigens in the test window. The bound antibody-antigen complexes is subsequently

detected by goat anti-human IgA gold conjugate carried by chase buffer that flows downward giving a pink-purplish color line in the test window. Control line which contains protein L captures human IgA from patient's sample and binds with the anti-human IgA-gold conjugate, resulting in pink-purplish color line in the control window. The appearance of control line indicates the proper addition and migration of serum/plasma/whole blood sample and chase buffer [Figure 1].

Assay procedure of Dengue IgA RT (Serum / plasma samples)

Step 1: 25 μl of specimen was added into the sample;
Step 2: Sample wicked up the membrane and once the sample front reached the blue indicator line, three drops of chase buffer were added into the oval well;
Step 3: "Den IgA" marked tab was pulled out until resistance was felt. One drop of chase buffer was added into the sample well and result was interpreted at 20 mins [Figure 2a].

Assay procedure of Dengue IgA RT (Whole blood samples)

Step 1: 25 μl of specimen was added into the sample well, followed by one drop of chase buffer into the same well;
Step 2: Sample wicked up the membrane and once the sample front reached the blue indicator line, three drops of chase buffer were added into the oval well;
Step 3: "Den IgA" marked tab was pulled out until resistance was felt. One drop of chase buffer was added into the sample well and result was interpreted at 20 mins [Figure 2b].

Test specimens

Test samples used in this study comprised of whole blood, serum and plasma and were categorized into three groups. Serum samples used in in-house validation and clinical trial at International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) were stored sera from archives. The collection of human serum samples in University of Malaya were approved by Medical Ethics Committee, University Malaya Medical Centre, Kuala Lumpur, Malaysia.

Group-1. In-house assay development and test validation

(i) Dengue IgA RT was designed and developed based on MP Biomedicals Asia Pacific standard quality procedure, which compiles and meets requirements defined in international standards (ISO 9001; ISO 13485, *In-vitro* Diagnostics Directive – 98/79/EC; Canadian Medical Device Regulations (MDR P.C. 1998-783, 7 May, 1998; Common Technical Specification (OJ L131). (ii) Assay validation was designed to determine the robustness of the assay procedure by selecting four important parameters of the assay including type of specimens (whole blood, plasma and serum), amount of specimens, amount of chase buffer and the total test time; (iii) Precision assays, (a) Between-run Precision (or Inter-assay precision): 3 production batches using three different lots of raw material were used and three operators ran the assay in triplicates using three production batches with one set of control sera on the same day; (b) Within-run, Within-day and Day to day precision (or Intra-assay Precision): 3 production batches of test kits were used and each production batch was assayed with 1 set of control sera at five days interval for 20 days (day to day). At each time point, each sample was tested twice at different times (within-day), in triplicates (within-run).

Group-2. In-house (Laboratory) performance validation

233 serum samples characterized by dengue reference ELISAs (Platelia Dengue NS1 Ag ELISA, PanBio Dengue IgM capture ELISA and PanBio Dengue IgG Capture ELISA), along with 681 negative serum samples (355 healthy donors and 326 cross-reactive and interfering

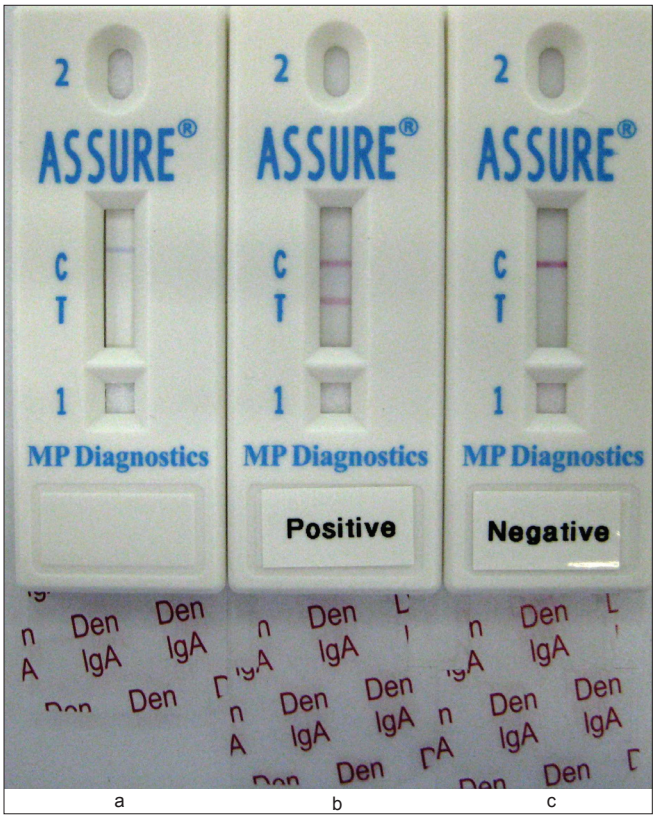

Figure 1: Pictures for Dengue IgA RT devices (a) blank device (b) positive for Dengue IgA antibody with colored band appearing at control line (C) and test line (T) (c) negative for Dengue IgA antibody as test line (T) is absent

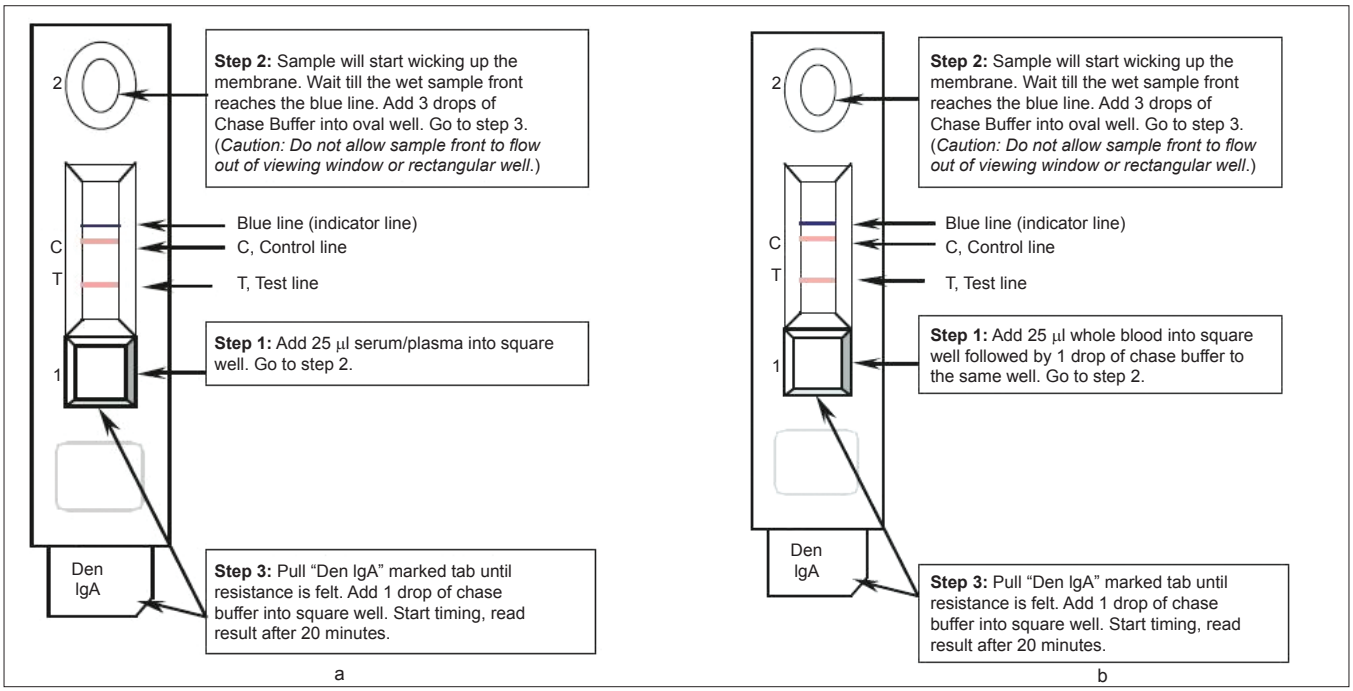

Figure 2: (a) Schematic diagram showing Dengue IgA RT assay procedure for serum or plasma samples; (b) Schematic diagram showing Dengue IgA RT assay procedure for whole blood samples

samples) obtained from MP Biomedicals archives were utilized for the optimization, development and laboratory validation of the rapid test.

Group-3. Clinical validation

(i) 150 paired sera collected from dengue suspected patients collected at 1 - 35 days intervals were used for clinical validation of Dengue IgA RT. The paired samples were obtained from Laboratory Science Department, ICDDR, B. All serum samples were characterized using dengue reference ELISAs and defined accordingly. (ii) 126 dengue suspected whole blood samples obtained from Haematology Laboratory, ICDDR, B were used for the evaluation of Dengue IgA RT. Corresponding plasma collected from whole blood were characterized by dengue reference ELISAs. (iii) 162 RT-PCR based serotype specific dengue sera obtained from the Department of Medical Microbiology, Faculty of Medicine, University of Malaya were used to evaluate the performance of Dengue IgA RT against all four types of dengue viruses. (iv) Dengue sero-converted sera from the Department of Medical Microbiology, Faculty of Medicine, University of Malaya were used to study early detection of infection using Dengue IgA RT. Of the 70 sero-converted sera, 50 samples were negative with in-house IgM-Cap ELISA during acute phase of infection and were sero-converted at convalescent phase. The in-house IgM-Cap ELISA was conducted according to the method described by Lam *et al*., 1987.[25]

Reference dengue assays for characterization

(i) Dengue IgM capture ELISA (IgM-Cap ELISA): A commercial kit (PanBio Diagnostics, Australia) was used for the detection of anti-dengue IgM from serum/plasma samples and carried out according to the manufacturer's instructions. (ii) Dengue IgG capture ELISA (IgG-Cap ELISA): The presence of high level of anti-dengue IgG in dengue suspected samples as an indicator of dengue secondary infection was detected by IgG-Cap ELISA (PanBio Diagnostics, Australia). Tests were performed and results were analyzed according to the manufacturer's instructions. (iii) Platelia Dengue NS1 Ag ELISA (NS1 Ag ELISA): The presence of dengue NS1 in dengue suspected samples was identified using NS1 Ag ELISA (Bio-Rad, USA). All tests were carried out using manufacturer's instructions. (iv) Dengue IgA ELISA (in-house): PAN-dengue monoclonal antibodies (1.15mg/ml; Immunology Consultants Laboratory, USA) were diluted at 1:500 in carbonate-bicarbonate buffer and subsequently coated in 96-well plate (MaxiSorp–NUNC, Denmark). The plate was incubated at 4°C overnight. After blocking of the plate with blocking buffer (5% skim milk containing 0.1% Triton X 100), the plate was washed five times with wash buffer [1 X Phosphate Buffered Saline (PBS) containing 0.5% Triton X 100]. Dengue type 2 antigen was then added at 1:25 dilutions in 1X PBS into each well and the plate was incubated at 37°C for an hour. The plate was again washed

six times using wash buffer. 100 µl of test serum at 1:100 dilution in diluent buffer (same as blocking buffer) was added into each well. One positive and three negative controls were added into each plate. After 1 h incubation at room temperature, the plate was washed again six times, and 100 µl of 1:1000 rabbit anti-human IgA conjugated with horse-radish peroxidase (HRP) was added into each well. The plate was then incubated for 30 mins at room temperature. Following incubation, the plate was then washed six times and 100 µl of tetra-methyl-bencidine (TMB) was added into each well and incubated for 15 mins at room temperature. Further color development was stopped using 100 µl 2M sulfuric acid (H_2SO_4). Optical density (OD) value was measured at 450 nm.

Data analysis

Data analysis was carried out using MedCalc Statistical software, Belgium (Version 10). Sensitivity and specificity of Dengue IgA RT was analyzed using 2×2 tables. Sensitivity, specificity, positive and negative predictive values and 95% confidence intervals (CI) were provided as the estimates of the effectiveness of Dengue IgA RT.

RESULTS

ASSURE® Dengue IgA Rapid Test development and validation

Results of assay validation against different parameters showed insignificant level of deviation from predefined standard (data not shown). Four aspects were studied in the precision validation: within run, between run, within day and day-to-day. Dengue IgA RT devices showed the reproducibility with consistent results across the three production batches for both inter-assay and intra-assay. The observed variations in intensities for the three calibrators were all within the expected range in the test period (data not shown).

Laboratory test validation

In-house validation of Dengue IgA RT was carried out using well-characterized dengue positive sera based on dengue reference ELISAs and the results are presented in Table 1. A combination of both antigen and antibodies ELISAs was used for characterization and reported to be able to detect 90-95% of true dengue positive cases.[17,19]

In-house sensitivity and specificity

The performance validation of Dengue IgA RT was examined using 233 dengue confirmed positive, 355 healthy donors and 326 cross-reactive and interfering serum samples and the results are shown in Table 2. Out of 233 dengue positive sera, Dengue IgA RT detected 202 samples with 86.70% sensitivity, and 95 out of 681 dengue negative samples showed positive reactions with Dengue IgA RT (specificity 86.05%).

Comparative analysis of Dengue IgA RT against dengue reference ELISAs

A comparative performance between the Dengue IgA RT and dengue reference ELISAs was investigated and presented in Table 3. Dengue IgA RT detected more dengue positive cases compared to dengue reference ELISAs covering both primary and secondary dengue infections.

Clinical evaluation of Dengue IgA RT using confirmed dengue positive and negative paired samples

The sensitivity and specificity of Dengue IgA RT was

Table 1: Characterization of 233 dengue positive samples using dengue reference ELISAs

Dengue positive sera*	NS1 Ag ELISA	IgM-Cap ELISA	IgG-Cap ELISA
233	47.64% (111/233)	71.67% (167/233)	60.09% (140/233)

*Dengue serum samples (*n*=233) were considered as positive using either NS1 Ag ELISA, IgM-Cap ELISA, IgG-Cap ELISA or all

Table 2: Analysis of sensitivity and specificity of Dengue IgA RT using 233 dengue positive and 681 negatives samples (healthy donors, cross-reactive and interfering serum samples)

Estimated performances of Dengue IgA RT			
Estimated sensitivity* (*n*=233) (*95% CI*)	Estimated specificity† (*n*=681) (*95% CI*)	Positive predictive value (PPV) (*95% CI*)	Negative predictive value (NPV) (*95% CI*)
86.70% (202/233) (81.65%-90.78%)	86.05% (586/681) (83.22%- 88.56%)	68.01% (62.38%- 73.28%)	94.86% (92.94%- 96.56%)

*Estimated sensitivity is defined as the percentage of positivity of Dengue IgA RT against acute samples positive with Dengue reference ELISAs; †Estimated specificity indicates the percentage of negativity of Dengue IgA RT against total dengue negative samples

Table 3: Comparative performance of Dengue IgA RT against dengue reference ELISAs (IgM-Cap ELISA and NS1 Ag ELISA)

Dengue diagnostics	Sensitivity		
	Primary infection (*n=93)-95% CI*	Secondary infection (*n=140)-95% CI*	Total (*n=233)-95% CI*
NS1 Ag ELISA	89.25% (83/93) (81.11%- 94.71%)	20.00%(28/140) (13.72%- 27.59%)	47.64% (111/233) (41.08%- 54.26%)
IgM-Cap ELISA	70.97% (66/93) (60.64%- 79.92%)	72.14% (101/140) (63.94%- 79.38%)	71.67% (167/233) (65.42% - 77.36%)
Dengue IgA RT	77.42% (72/93) (67.58%- 85.44%)	92.86% (130/140) (87.25%- 96.52%)	86.70% (202/233) (81.65%-90.78%)

Table 4: Sensitivity and specificity of Dengue IgA RT using 125 dengue confirmed positive and 25 dengue negative samples

Phases of dengue infection	Sensitivity (*n*=125) *95% CI*	Specificity (*n*=25) *95% CI*	Positive predictive value *(95% CI)*	Negative predictive value *(95% CI)*
Acute	84.80% (106/125) (77.28%- 90.59%)	92.00% (23/25) (73.93%- 98.78%)	98.15% (93.46%- 99.72%)	54.76% (38.68%- 70.15%)
Convalescence	99.20% (124/125) (95.61%- 99.87%)	92.00% (23/25) (73.93%- 98.78%)	98.41% (94.37%- 99.76%)	94.83% (78.81%- 99.30%)

evaluated using both acute and convalescence dengue confirmed positive (*n*=125) and negative (*n*=25) serum samples and results are presented in Table 4. Dengue IgA RT detected 99.20% of convalescent sera collected between 1 and 35 days interval, whilst 84.80% during acute phase of infection. On the other hand, the specificity in both collections was found to be 92%.

Of 125 dengue positive cases, 55 cases were primary infected patients while remaining 70 patients sera showed very high level of anti-dengue IgG (≥22) at the acute phase of infection and considered as dengue secondary infected cases. In convalescent phase, Dengue IgA RT detected 100% (55/55) (95% CI: 93.45% to 100.00%) of primary and 98.57% (69/70) (95% CI: 92.27% to 99.76%) of secondary dengue infected cases. The sensitivity at acute phase of infection for primary and secondary dengue cases were 72.73% (40/55) (95% CI: 59.04% to 83.85%) and 94.29% (66/70) (95% CI: 86.00% to 98.39%) respectively.

Performance of ASSURE® Dengue IgA Rapid Test in detection of dengue infection using whole blood samples

126 whole blood samples collected during 2008 dengue outbreaks obtained from haematology laboratory, ICDDR,B, were tested blindly by Dengue IgA RT. Following collection of plasma from corresponding whole blood samples, plasma samples were characterized by dengue reference ELISAs (NS1 Ag, IgM and IgG capture ELISAs). Out of 126 plasma samples, 76 samples were found either positive with one or more dengue reference ELISAs and considered as samples from true dengue infected patients while remaining 50 samples were negative against all dengue reference ELISAs. Using whole blood samples, Dengue IgA RT showed 85.53% sensitivity and 80.00% specificity, as presented in Table 5.

Detection of DENV Serotypes by Dengue IgA RT

Dengue IgA RT was used to detect dengue positive samples comprising all four serotypes of dengue viruses (*n*=162) and results are presented in Table 6. Of 162 dengue RT-PCR confirmed samples, Dengue IgA RT detected 101 cases as positive of which 66.34% DENV1 (67/101), 52.38% DENV2 (11/21), 60.87% DENV3 (14/23) and 52.94% DENV4 (9/17).

Table 5: Sensitivity and specificity of Dengue IgA RT against whole blood samples (*n*=126)

	Sensitivity (*n*=76) *95% CI*	Specificity (*n*=50) *95% CI*	Positive predictive value *(95% CI)*	Negative predictive value *(95% CI)*
Dengue IgA RT	85.53% (65/76) (75.57%- 92.54%)	80.00% (40/50) (66.28%- 89.95%)	86.67% (76.84%-93.41%)	78.43% (64.67%- 88.70%)

Table 6: Comparative performances of Dengue IgA RT with in-house IgM-Cap ELISA in detecting dengue virus serotypes (*n*=162)

Dengue diagnostics	Dengue virus serotypes			
	Serotype-1 *(n=101) 95% CI*	Serotype-2 *(n=21) 95% CI*	Serotype-3 *(n=23) 95% CI*	Serotype-4 *(n=17) 95% CI*
Dengue IgA RT	66.34% (67/101) (56.25%-75.44%)	52.38% (11/21) (29.81%-74.25%)	60.87% (14/23) (38.55%-80.26%)	52.94% (9/17) (27.86%- 76.96%)
In-house IgM-Cap ELISA	63.37% (64/101) (53.19%-72.73%)	57.14% (12/21) (34.04%-78.14%)	43.48% (10/23) (23.22%-65.49%)	29.41% (5/17) (10.42%- 55.95%)

Table 7: Comparative performances of Dengue IgA RT with in-house IgM-Cap ELISA and RT-PCR based on duration illness using sero-converted samples (*n*=70)

Days after onset of fever	RT-PCR	In-house IgM-Cap ELISA	Dengue IgA RT
Day 1-2 (*n*=14)	71.43% (10/14)	0.00% (0/14)	42.86% (6/14)
Day 3-4 (*n*=38)	73.68% (28/38)	15.79% (6/38)	63.16% (24/38)
Day 5-7 (*n*=18)	44.44% (8/18)	77.78% (14/18)	83.33% (15/18)
Total (*n*=70)	65.71% (46/70)	28.57% (20/70)	64.28% (45/70)

Use of Dengue IgA RT for the early detection of dengue virus infection

The performance of the rapid test as one of the early dengue diagnostics using acute collection of 70 confirmed acute dengue samples is shown in Table 7. Out of 70 dengue cases, Dengue IgA RT detected 45 dengue cases (64.28%) compared to 20 cases by in-house IgM-Cap ELISA (28.57%) and 46 cases (65.71%) by RT-PCR. In terms of detection of early dengue cases, Dengue IgA RT also showed better performance than by in-house IgM-Cap ELISA. Dengue IgA RT detected 42.86% dengue cases as

early as one-two days after onset of fever, 63.16% by third-forth day and 83.33% by fifth-seventh day of illness; whilst by in-house IgM-Cap ELISA detected 0.00%, 15.79% and 77.78% on first-second day, third-forth day and fifth-seventh day respectively.

DISCUSSION

Based on evaluation studies conducted in MP Biomedicals laboratory (in-house) and various clinical stations, Dengue IgA RT demonstrated its usefulness for the early detection of dengue infection. The overall sensitivity of the assay varied from 84.80% to 86.70% during acute phase of infection and 99.20% during convalescence phase, whilst the specificity varied from 80% to 92%. The performance of Dengue IgA RT is comparable with dengue reference ELISAs during primary infection, whilst it is better in dengue secondary infection.

The kinetics of anti-dengue IgA production was also elucidated, where the anti-dengue IgA appeared earlier than anti-dengue IgM and was detected by Dengue IgA RT. More than 90% of anti-dengue IgA positive cases were detected among secondary infected patients compared to 72.73%-77.42% in primary infection. This could be due to the presence of IgA memory cells from the previous dengue infection which was triggered by the second dengue antigen contact. Similar types of results on Dengue IgA using AAC-ELISA were reported previously.[26-29] However, further study is required to define the kinetics of production of anti-dengue IgA among dengue primary and secondary infected patients using multiple collections of samples.

S. Vazquez and coworkers also utilized AAC-ELISA in their exploration of the kinetics of dengue antibodies production in sera, saliva and urine.[30] They demonstrated low detection levels of anti-dengue IgA compared to IgM in all the body fluids in both primary and secondary infections. In primary dengue-confirmed patients, serum IgA was only detected in about 20% of the samples after four days of fever onset. In contrast, our data showed more than 50% patients produced anti-dengue IgA in serum on third day of illness, which increased to 83.33% between fifth and seventh days. The poor performance of Vazquez studies could be due to the test platform where high levels of non-dengue reactive IgA compete with dengue virus reactive IgA to be captured by anti-human IgA coated plate. Hence it failed to detect low levels of dengue-specific IgA present in suspected serum samples. In contrast, Dengue IgA RT platform eliminated the competition of non-dengue reactive IgA by capturing only dengue reactive IgA using immobilized dengue antigen on the nitrocellulose membrane. This may allow Dengue IgA RT to detect very low level of dengue specific IgA and hence demonstrated a better performance during early stage of dengue infection compared with in-house IgM-Cap ELISA [Table 7]. In addition, the presence of anti-dengue IgA in dengue infected patents at the early stage of infection could be due to the involvement of mucosa- associated lymphoid tissues (MALT). Particularly, DENV multiplies in antigen presenting cells (dendritic cells and macrophages) and stimulate the local immune cells to produce IgA-producing plasma cells, which also enter into the circulation through high endothelial venules (HEV) and produce anti-dengue IgA.

The potential benefits of reverse flow technique of Dengue IgA RT for the detection Dengue IgA using various types of specimens (whole blood, plasma and serum) are numerous. It eliminates the need to prepare serum from whole blood and provide rapid results at all levels of healthcare system. Even though serum IgA in primary infection is not as effective as RT-PCR in detecting cases from day 0 to 2 after fever onset (its detection capability starts on day 3 after fever onset), it is still a useful early diagnostic tool in areas where PCR is not available. In secondary dengue infections, Dengue IgA RT showed better performance compared to NS1 Ag ELISA and IgM-Cap ELISA. In addition, Dengue IgA RT demonstrated sastifactory level of performance in detecting all four serotypes of dengue virues. This technique could thus be very useful in current endemic areas where all four serotypes of dengue viruses are co-circulating and giving more and more dengue secondary infection.

CONCLUSION

Dengue IgA RT has been successfully developed and validated in both laboratory and clinical stations using a wide range of well characterized samples. The assay is easy to perform, cost-effective and results can be interpreted at 20 mins of test run. The use of whole blood specimens further facilitates the rapidness of the assay at all levels of healthcare system. Dengue IgA RT showed comparable level of performance against dengue reference ELISAs and detected both primary and secondary dengue infected patients. The assay also showed satisfactory level of performance against all four serotypes of dengue viruses. Hence, Dengue IgA RT could be equally applied as one of the dengue early diagnostic tools all over the dengue endemic countries.

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