Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection

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ABSTRACT

Introduction: The aim of this report is to establish an accurate diagnosis of acute dengue virus infection early, in order to provide timely information for the management of patients and early public health control of dengue outbreak.

Methods: 224 serum samples from patients with a clinical diagnosis of acute dengue infection, which were subsequently confirmed by laboratory tests, were used to evaluate the performance of a commercially-available dengue NS1 antigen-capture ELISA kit.

Results: The dengue NS1 antigen-capture ELISA gave an overall sensitivity rate of 93.3 percent (209/224). The sensitivity rate was significantly higher in acute primary dengue (97.4 percent) than in acute secondary dengue (68.8 percent). In comparison, the virus isolation gave an overall positive isolation rate of 64.7 percent, with a positive rate of 70.8 percent and 28.1 percent, for acute primary dengue and acute secondary dengue, respectively. Molecular detection of dengue RNA by RT-PCR gave an overall positive detection rate of 63.4 percent, with a positive rate of 62.5 percent and 68.8 percent, for acute primary dengue and acute secondary dengue, respectively. Of the 224 acute serum samples from patients with laboratory-confirmed acute dengue infection, dengue IgM was detected in 88 specimens, comprising 68 acute primary dengue specimens and 20 acute secondary dengue specimens. NS1 antigen-capture ELISA kit gave an overall sensitivity rate of 88.6 percent in the presence of anti-dengue IgM and 96.3 percent in the absence of anti-dengue IgM.

Conclusion: Of the 224 acute serum samples, the sample ages of 166 acute serum samples are known. The positive detection rate of dengue NS1 antigen-capture ELISA, on the whole, was higher than the other three established diagnostic test methods for laboratory diagnosis of acute dengue infection.

Keywords: dengue virus, NS1 antigen-capture ELISA

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INTRODUCTION

Dengue is an endemic arboviral disease affecting more than 100 countries in the tropical and subtropical regions of Africa, the Americas, the eastern Mediterraneans, Southeast Asia, and the Western Pacific. The disease threatens more than 2.5 billion people living in these regions.[1,2] The World Health Organization estimates that there may be 50–100 million cases of dengue virus infections worldwide every year, resulting in 250,000–500,000 cases of dengue haemorrhagic fever (DHF) and 24,000 deaths each year.[1,2] Dengue fever (DF) and its more serious forms, DHF and dengue shock syndrome (DSS), are becoming prominent public health problems predominantly in urban and suburban areas.[1-3]

Dengue virus is an envelope positive-sense RNA virus. The genomic RNA is approximately 11 kb in length, and is composed of three structural protein genes that encode the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E), and seven nonstructural (NS) protein genes. The gene order is 5'-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3', as for other flaviviruses.[4,5] Among the nonstructural proteins, NS1 is a highly-conserved glycoprotein, which appears essential for virus viability, although no precise function has yet been assigned to it. During acute dengue virus infection, NS1 is found associated with intracellular organelles or is transported through the cellular secretory pathway to the cell surface.[6-9] A soluble hexameric form may be released from infected mammalian cells but not

from vector-derived mosquito cells.[10-13] NS1 was also found circulating during the acute phase of the disease in sera from patients.[14] An enzyme-linked immunosorbent assay, specific to dengue virus type 1 nonstructural protein NS1, has been developed for detection of dengue NS1 antigen during the acute phase of disease in patients experiencing primary and secondary infections.[15]

In this report, we evaluated the sensitivity of a commercially-available dengue NS1 antigen-capture ELISA kit to demonstrate its potential future application for early laboratory confirmation of acute dengue infection, in comparison to the existing in-place diagnostic test methods, such as, dengue virus isolation, molecular detection of dengue genomic RNA by RT-PCR and serological assay of dengue specific IgM.[16,17]

METHODS

Blood samples were collected from patients, who were clinically diagnosed to have acute dengue virus infection, at the time of hospital admission and the time of discharge at Hospital Tengku Ampuan Rahimah, Klang from January to December 2005. The blood samples were sent to the National Public Health Laboratory, and the sera were separated from the clots on the day of collection. Dengue virus isolation using C6/36 cell-line, molecular detection for dengue virus genome by RT-PCR and serological assays for dengue specific IgM by IgM-capture ELISA (Panbio Diagnostics, Brisbane, Australia) were carried out on the acute serum samples. The remaining acute serum samples were stored in a -80°C freezer for later use. Only serological assays of dengue specific IgM by IgM-capture ELISA were carried out on the convalescent serum samples. In this study, the serum specimens which met the criteria of laboratory-confirmed acute dengue infection were used to evaluate the Platelia dengue NS1 antigen-capture ELISA kit. The laboratory criteria used to confirm acute dengue infections were as follows: (i) isolation of dengue virus in C6/36 cells (ATCC CRL-1660) (virological definition); and/or (ii) detection of dengue virus RNA by RT-PCR based on published work by Lanciotti et al (molecular definition);[16] and/or (iii) demonstration of seroconversion or ≥ two-fold rise in dengue specific IgM in paired serum samples collected at ≥ 3 days apart, assayed by an IgM-capture ELISA (serological definition). Sample age was defined as the interval in days between the collection of blood sample and the date of fever onset. It was considered as 0 days, if the blood sample was collected on the same day of the date of fever onset.

The test system is based on a one-step sandwich format microplate enzyme immunoassay (PLATELIA™ DENGUE NS1 AG) (Bio-Rad Corporate HQ, Hercules, USA) to detect the dengue virus NS1 antigen in human serum or plasma. The test uses murine monoclonal antibody (MAb) for capture and revelation. If the NS1 antigen is present in the sample, an immune-complex MAb-NS1-MAb/peroxidase will be formed. The process of performing the tests, and subsequent calculations, strictly conformed to the assay procedure of the commercial Platelia Dengue NS1 antigen capture ELISA kit. The acute serum specimens were allowed to thaw to laboratory ambient temperature (21–22°C). Diluent, samples and controls (50 µL each) and 100 µL conjugate were then incubated for 90 min at 37°C within the respective microplate wells sensitised with MAb. After a six-times washing step, 160 µL of substrate was added into each well and incubated for 30 min at room temperature in the dark. The presence of immune-complex was demonstrated by a colour development and the enzymatic reaction was quenched by adding 100 µL 1N H_2SO_4. The optical density (OD) reading was taken with a spectrophotometer at 450/620 nm, and the amount of NS1 antigen present in an individual serum sample was determined by comparing the OD of the sample to the OD of the cut-off control serum.

The Panbio Dengue IgG Capture ELISA (Panbio Diagnostics, Brisbane, Australia) was used to determine the elevated level of IgG antibodies to dengue virus (serotypes 1–4) in a patient's serum with secondary dengue virus infection. Anti-dengue specific IgG present in the serum sample was assayed according to the manufacturer's instructions. The acute serum specimens were allowed to thaw to laboratory ambient temperature (21–22°C). The negative control, reactive control, calibrator and patients' samples (10 µL each) were then diluted with 1,000 µL of serum diluent respectively. 100 µL of diluted patient samples and controls were pipetted into respective microwells and incubated for 60 min at 37°C. After six washings with diluted wash buffer, 100 µL of antigen-MAb tracer was added to each well and incubated for another 60 min at 37°C. After incubation, the microwell plate was washed another six times and 100 µL tetramethylbenzidine (TMB) was subsequently added to each well. The plate was incubated at room temperature for 10 min and the reaction was quenched by adding 100 µL of 1M phosphoric acid. The absorbance reading in each well was taken at 450 nm. The results were interpreted as negative for ≤ 22 Panbio units (primary dengue) and positive for > 22 Panbio units (secondary dengue).

The derived data was tabulated in appropriate worksheets using the Microsoft Excel programme and evaluated by chi-square test using the Epi Info 6 (Center for Disease Control and Prevention, Atlanta) free computer programme for any statistical significant association. A probability (p) value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables.

Table I. The number of serum samples tested positive in acute primary and secondary dengue infections by diagnostic method.

Diagnostic method	Number of serum samples tested positive		
	Primary dengue (Platelia NS1)	Secondary dengue (Platelia NS1)	Combined (Platelia NS1)
Virus isolation alone	64 (62)	7 (6)	71 (68)
RT-PCR alone	48 (45)	20 (12)	68 (57)
Virus isolation + RT-PCR	72 (72)	2 (2)	74 (74)
Serology (Pair sera)	8 (8)	3 (2)	11 (10)
Total	192 (187)	32 (22)	224 (209)

Table II. The number of serum samples tested positive in acute primary and secondary dengue infections by diagnostic method with respect to the positive detection of anti-dengue IgM in the first acute serum specimen.

Diagnostic method	Number of serum samples tested positive		
	Primary dengue (Platelia NS1)	Secondary dengue (Platelia NS1)	Combined (Platelia NS1)
Virus isolation alone	29 (28)	5 (4)	34 (32)
RT-PCR alone	13 (12)	12 (6)	25 (18)
Virus isolation + RT-PCR	18 (18)	0 (0)	18 (18)
Serology (Pair sera)	8 (8)	3 (2)	11 (10)
Total	68 (66)	20 (12)	88 (78)

Table III. The number of serum samples tested positive in acute primary and secondary dengue infections by diagnostic method with respect to the absence of anti-dengue IgM in the first acute serum specimen.

Diagnostic method	Number of serum samples tested positive		
	Primary dengue (Platelia NS1)	Secondary dengue (Platelia NS1)	Combined (Platelia NS1)
Virus isolation alone	35 (34)	2 (2)	37 (36)
RT-PCR alone	35 (33)	8 (6)	43 (39)
Virus isolation + RT-PCR	54 (54)	2 (2)	56 (56)
Serology (Pair sera)	0 (0)	0 (0)	0 (0)
Total	124 (121)	12 (10)	136 (131)

RESULTS

In this study, 224 specimens which fulfilled the set criteria of acute dengue infection were selected to evaluate the sensitivity of the PLATELIA™ DENGUE NS1 AG test kit for early diagnosis of acute dengue virus against established diagnostic test methods; namely, dengue virus isolation, molecular detection of dengue genomic RNA and assay of dengue IgM. Based on the results of serological assays of dengue specific IgG using the Panbio Dengue IgG-Capture ELISA, 192 and 32 serum samples were derived from patients with acute primary and acute secondary dengue virus infections, respectively.

The overall performance of the Platelia NS1 antigen-capture ELISA with respect to virus isolation, molecular detection and serology in each category, is shown in Table I. The PLATELIA™ DENGUE NS1 AG test kit gave an overall sensitivity rate of 93.3% (209/224). The sensitivity rate was higher in acute primary dengue (97.4%; 187/192) than in acute secondary dengue (68.8; 22/32). Statistically, the detection rate of Platelia NS1 antigen-capture ELISA was better in patients with acute primary dengue than acute secondary dengue (Fisher exact test, $p < 0.0000$). In comparison, virus isolation gave an overall positive isolation rate of 64.7% (71+74/224), with a positive rate of 70.8% (64+72/192) and 28.1% (7+2/32), for acute primary dengue and acute secondary dengue, respectively. The isolation of the dengue virus was significantly higher in acute primary dengue virus infections than acute secondary dengue ($\chi^2 = 20.08$, $p < 0.0000$). Molecular detection of dengue RNA by RT-PCR gave an overall positive detection rate of 63.4% (68+74/224), with a positive rate of 62.5% (48+72/192) and 68.8% (20+2/32), for acute primary dengue and acute secondary dengue, respectively. There was no statistically significant difference in the molecular detection rate between acute primary dengue and acute secondary dengue ($\chi^2 = 0.23$, $p = 0.6303$).

Of the 224 acute serum samples from patients with laboratory-confirmed acute dengue infection, dengue IgM was detected in 88 specimens, comprising 68 acute

primary dengue specimens and 20 acute secondary dengue specimens, respectively. Thus, dengue IgM detection gave an overall sensitivity rate of 39.3% (88/224), with sensitivity rates of 35.4% (68/192) and 62.5% (20/32), for acute primary dengue and acute secondary dengue, respectively. There was a significant higher detection rate of dengue specific IgM in acute secondary dengue as compared to that of acute primary dengue (χ^2 = 7.34, p = 0.0068). The performance of the Platelia NS1 antigen-capture ELISA with respect to virus isolation, and molecular detection of dengue in the presence, or absence, of dengue specific IgM, are shown in Tables II and III. Platelia NS1 antigen-capture ELISA kit gave an overall sensitivity rate of 88.6% (78/88) in the presence of anti-dengue IgM, and 96.3% (131/136) in the absence of anti-dengue IgM. There was an inverse correlation in the dengue NS1 antigen positive detection rate with respect to the positive detection of anti-dengue IgM.

Of the 224 acute serum samples, the sample ages of 166 acute serum samples are known. The mean sample age was 4.5 days (range 0–8, SD 1.8), with a median sample age of 4 days. The sensitivity rate of each test method with respect to the sample age is shown in Fig. 1.

DISCUSSION

In order to provide timely information for the management of the patients, and early public health control of dengue outbreaks, it is important to establish a diagnosis of acute dengue virus infection during the first few days after manifestation of clinical symptoms. Early laboratory diagnosis of acute dengue virus infection still remains a problem. At present, the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation and identification, detection of viral genomic sequence by a nucleic acid amplification technology assay (RT-PCR), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test (DIT). Though virus isolation and characterisation are considered as the gold standard of laboratory diagnosis for acute dengue virus infection, it is expensive and it takes at least 6–10 days for the virus to replicate in tissue cell culture or laboratory mosquitoes. Detection of viral genomic sequence by RT-PCR is also an expensive method and is not widely available in most hospital diagnostic laboratories. The third method, assay of anti-dengue specific IgM, depends on the time taken for an infected person's immunological response to produce IgM antibodies against dengue virus antigens. Thus, both DIT (often considered as the rapid test for diagnosis of dengue infection) and MAC-ELISA do not provide early diagnosis of acute dengue infection, as in most cases, the first detectable IgM only appears on Days 4–5 of the illness. Moreover, a single serological detection of IgM is merely indicative of a recent dengue virus infection, and should not be interpreted as a diagnosis of acute infection without a paired second serum sample.

This evaluation clearly shows that the PLATELIA™ DENGUE NS1 AG test kit gives an overall higher sensitivity rate than the current three established diagnostic test methods for laboratory diagnosis of acute dengue infection. Compared to dengue virus isolation and molecular detection of viral RNA, the Platelia NS1 antigen-capture ELISA gave a higher positive detection within the first four days of illness. However, the NS1

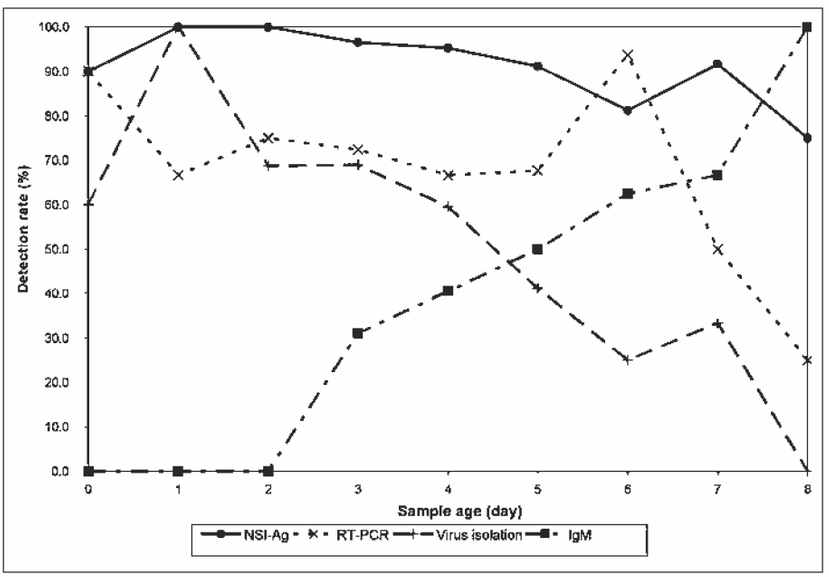

Fig. 1 Positive detection rate of each dengue test method with respect to the sample age.

antigen-capture ELISA has the added advantage of continuing to give good detection rates up to seven days of the illness. In this evaluation, the NS1 antigen-capture ELISA gave a significantly higher detection rate in acute primary dengue than in acute secondary dengue. Despite the lower detection rate for serum samples from patients with acute secondary dengue, the Platelia NS1 antigen-capture ELISA still gave a higher detection rate than the other dengue diagnostic methods used in this laboratory.

Fig. 1 shows the positive detection rate of Platelia NS1 antigen-capture ELISA, which on the whole, gave a higher detection rate than the other test methods at the various sample ages. The sensitivity rate of IgM assay for early diagnosis of dengue was poor in the first three days of the illness, notwithstanding the presence of dengue specific IgM was merely indicative of recent dengue infection, and not confirmative of acute dengue infection. The finding of this evaluation shows that no dengue specific IgM was detected within the first two days of the fever and only 50% of patients had detectable dengue IgM in their sera, even at the fifth post-fever day. Thus, the Platelia NS1 antigen-capture ELISA should be considered as the test of choice for patients suspected of acute dengue illness, especially those with fever lasting five days or less. For those patients with a history of fever for more than six days and are suspected to have acute dengue infection, the test could also be considered concurrently with an assay of dengue specific IgM. The Platelia NS1 antigen-capture ELISA test has the prospect of wide usage for early diagnosis of acute dengue virus infection in dengue endemic countries, since it uses the same instruments as that of the dengue IgM-capture ELISA (MAC-ELISA) test, which is normally carried out in the hospital diagnostic laboratories. This study was limited by the lack of negative controls to evaluate the specificity of the test kit. Further work is ongoing to evaluate the specificity of the Platelia NS1 antigen-capture ELISA kit and the possibility of cross-reactivity with NS1 antigens of other flaviviruses. The possibility of a correlation between a high level of circulating dengue NS1 antigen with the occurrence of dengue haemorrhagic fever, as demonstrated by other studies, is also included in the ongoing evaluation work.[18-20]

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