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Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection

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Abstract

A commercial dengue NS1 antigen-capture ELISA was evaluated to demonstrate its potential application for early laboratory diagnosis of acute dengue virus infection. Dengue virus NS1 antigen was detected in 199 of 213 acute serum samples from patients with laboratory confirmation of acute dengue virus infection but none of the 354 healthy blood donors' serum specimens. The dengue NS1 antigen-capture ELISA gave an overall sensitivity of 93.4% (199/213) and a specificity of 100% (354/354). The sensitivity was significantly higher in acute primary dengue (97.3%) than in acute secondary dengue (70.0%). The positive predictive value of the dengue NS1 antigen-capture ELISA was 100% and negative predictive value was 97.3%.

Comparatively, virus isolation gave an overall positive isolation rate of 68.1% with a positive isolation rate of 73.9 and 31.0% for acute primary dengue and acute secondary dengue, respectively. Molecular detection of dengue RNA by RT-PCR gave an overall positive detection rate of 66.7% with a detection rate of 65.2 and 75.9% for acute primary dengue and acute secondary dengue, respectively.

The results indicate that the commercial dengue NS1 antigen-capture ELISA may be superior to virus isolation and RT-PCR for the laboratory diagnosis of acute dengue infection based on a single serum sample.

Keywords: Dengue; Antigen detection; Diagnostic; ELISA

1. 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 the regions (World Health Organization, 2000; Gubler and Meltzer, 1999). 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 approximately 25,000 deaths annually (World Health Organization, 2000; Gubler and Meltzer, 1999). 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 (World Health Organization, 2000; Gubler and Meltzer, 1999; Gibbons and Vaughhn, 2002).

Dengue virus is an enveloped 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, a membrane-associated protein, an envelope protein, and seven nonstructural (NS) protein genes. The polycistronic coding region is flanked by a non-coding region of about 100 nucleotides at its 5′ end and a longer non-coding region at its 3′ end. The gene order of the genome is 5′-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′, as for other flaviviruses (Monath and Heinz, 1990; Henchal and Putnak, 1990). Among the nonstructural proteins, NS1 is a highly conserved glycoprotein which appears essential for virus replication, 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

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cellular secretory pathway to the cell surface (Mason, 1989; Schlesinger et al., 1990; Mackenzie et al., 1996; Westaway et al., 1997). A soluble hexameric form of the flavivirus NS1 proteins were released from infected mammalian cells but not from vector-derived mosquito cells (Crooks et al., 1990, 1994; Pryor and Wright, 1993; Flamand et al., 1999). The hexameric form of dengue virus NS1 protein was also found circulating in the sera of patients during acute phase of the illness (Young et al., 2000). 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 (Alcon et al., 2002).

In this report, an evaluation of a commercially available dengue NS1 antigen-capture ELISA was carried out to demonstrate its potential future application for early laboratory confirmation of acute dengue virus infection based on a single acute serum sample with reference to the existing in-place laboratory diagnostic test methods, such as, dengue virus isolation and molecular detection of dengue genomic RNA by reverse transcriptase-polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Serum specimens

During an outbreak of dengue that occurred in the Klang valley, Peninsular Malaysia from January to March 2005, more than 1000 patients clinically suspected of acute dengue virus infection were admitted to Hospital Tengku Ampuan Rahimah (HTAR), Klang, as a precautionary proactive management. Blood specimens were collected from patients and sent to the National Public Health laboratory for laboratory confirmation of acute dengue. In the laboratory, dengue virus isolation using C6/36 cell-line (ATCC CRL-1660) and molecular detection for dengue virus genome by RT-PCR based on published oligonucleotide primers were carried out on the acute serum samples besides the standard serological assay for dengue specific IgM by IgM-capture ELISA (Panbio, Australia) (Lanciotti et al., 1992; Bundo and Igarashi, 1985; Palmer et al., 1999; Shu and Huang, 2004). Remaining excess acute serum samples were stored in a minus 80 °C freezer for later use.

Only acute serum specimens from patients with laboratory confirmation of acute dengue virus infection by the positive molecular detection of dengue virus RNA and/or positive isolation of dengue virus were used to evaluate the sensitivity of the dengue NS1 antigen-capture ELISA. Serum specimens from healthy blood donors were used to evaluate the specificity of the assay kit.

2.2. Serological assay of dengue NS1 antigen

The test system is based on a one-step sandwich format microplate enzyme immunoassay (PLATELIA^{TM} DENGUE NS1 AG, Bio-Rad, France) to detect dengue virus NS1 antigen in human serum or plasma. The test uses murine monoclonal antibody (MAb) for capture and revelation. If 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 calculation was strictly adhered to the assay procedure of the commercial Platelia Dengue NS1 antigen capture ELISA kit. Briefly, the acute serum specimens were allowed to thaw to laboratory ambient temperature (21–22 °C). Sample diluent (50 μl), respective samples and controls (50 μl each) and 100 μl of diluted conjugate were incubated for 90 min at 37 °C within the respective microplate wells coated with purified mouse anti-NS1 monospecific antibodies. 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 stopped by adding 100 μl 1N H_2SO_4. The optical density (OD) reading was taken with a spectrophotometer at a wavelength of 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.

2.3. Serological assay of anti-dengue IgG

The Panbio Dengue IgG Capture ELISA (Australia) was used to determine the elevated level of serum IgG antibodies to dengue virus (serotypes 1–4) in a patient with acute secondary dengue virus infection. Anti-dengue specific IgG present in the serum sample was assayed according to the manufacturer's instructions. Briefly, 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 diluted with 1000 μl of serum diluent, respectively. One hundred microlitre of diluted patient samples and controls were pipetted into respective microwells and incubated for 60 min at 37 °C. After six times of washing with diluted wash buffer, 100 μl of antigen-monoclonal antibody (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 stopped by adding 100 μl of 1M phosphoric acid. The absorbance reading in each well was taken at a wavelength of 450 nm. The results were interpreted as negative (suggestive of acute primary dengue in the light of positive virus isolation and/or RT-PCR) for Panbio units of 22 or less, and positive (suggestive of acute secondary dengue) for Panbio unit of >22, based on previous evaluations of the commercial kit against the haemagglutination inhibition (HAI) test which had been used as the gold standard for differentiating acute secondary from acute primary dengue infection by a cut-off HAI titre of 2560 or higher dilution. The Panbio unit of greater than 22 was taken as equivalent to a HAI titre of 2560 (package insert) (Sang et al., 1998; Lam and Devine, 1998).

2.4. Data analysis

The derived data were tabulated in Microsoft Excel worksheets and analysed by Chi-square test using the Epi Info