A Comparative Study on the Performance of Two Commercial Anti-Dengue IgM Assay Kits

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SUMMARY

The performance of a commercial rapid immunochromatographic dengue IgG/IgM assay device was evaluated against an in-place dengue IgM-capture ELISA in the National Public Health laboratory. Of the 239 serum samples from patients with clinical diagnosis of acute dengue illness, 140 and 99 samples were tested positive and negative respectively for anti-dengue IgM by the in-placed ELISA. Comparatively, 72 and 76 samples were tested positive and negative respectively, and 91 samples gave equivocal results by the rapid dengue test device. The rapid immunochromatographic assay device gave a relative sensitivity of 49.3% and a relative specificity of 62.6%. 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.

KEY WORDS:
Dengue diagnostics, ELISA, Chromatography

INTRODUCTION

Dengue viruses belong to the genus *Flavivirus* under the family *Flaviviridae*. The four dengue virus serotypes (dengue virus types 1 to 4) 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].

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 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 virus isolation and identification, detection of viral genomic sequence by a nucleic acid amplification technology assay (RT-PCR, real-time PCR, etc.), and detection of dengue virus-specific IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or rapid dengue immunochromatographic test (DIT)[6-8]. Assay of anti-dengue specific IgM depends on the time taken for an infected person 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 necessarily provide early diagnosis of acute dengue infection, as in most cases the first detectable IgM appears only on days 4 to 5 of 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 pair second serum sample. Virus isolation and characterization is considered as the gold standard of laboratory diagnosis of acute dengue virus infection. However, it is expensive and at least 6 to 10 days are required for the virus to replicate in tissue cell culture or laboratory mosquitoes. Reverse transcriptase-polymerase chain reaction (RT-PCR) is also an expensive method and is not widely available in most hospital diagnostic laboratories.

Due to reasons mentioned above for the later two methods, the most common laboratory test widely performed in hospitals' and health centres' laboratories in Malaysia to support clinical diagnosis of acute dengue virus infection is still based on the assay of anti-dengue specific IgM. We carried out a comparison study to evaluate the performance of a commercial rapid dengue immunochromatographic test device (*Acon* Laboratories Inc., USA) for the serological assay of anti-dengue IgM with reference to an IgM-capture enzyme-linked immunosorbent assay (Panbio, Australia) performed routinely in the National Public Health Laboratory (NPHL) of Malaysia.

Laboratory tests based on the two methods were performed concurrently on daily serum samples received by NPHL from patients with clinical diagnosis of acute dengue infection. The process of performing the tests was strictly adhered to the assay procedures of the respective commercial kits. The interpretation of test result for the Panbio Dengue IgM Capture ELISA was based on the absorbance value of the sample with respect to the cut-off absorbance values of standards included in the test kit. As for the *Acon* Dengue Rapid Test Device, the result of each test was independently read by two scientific officers within the specified time for reading. However, if there was disagreement in the interpretation of reading between the two officers, a third officer would adjudicate the reading. The result was

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Table I: The number of serum samples from patients with clinical diagnosis of acute dengue that were tested positive by Panbio Dengue IgM Capture ELISA and *Acon* Rapid Dengue IgG/IgM Test Device.

***Acon* Dengue Rapid IgG/IgM Test Device**	**Panbio Dengue-IgM Capture ELISA**		
	Positive	**Negative**	**Total**
Positive	69	3	72
Borderline	57	34	91
Negative	14	62	76
Total	140	99	239

interpreted as positive detection of anti-dengue IgM if a coloured line was visible at the expected position on the test device, borderline for equivocal finding, and negative for clear absence of the coloured line.

In the months of February, March and April 2006, 239 serum samples from patients with clinical history of acute dengue illness were received by NPHL for laboratory assay of anti-dengue IgM. Anti-dengue IgM was detected in 137 samples with 12 samples in the equivocal range. Subsequent independent repeat assay confirmed three samples were tested positive for anti-dengue IgM and nine samples were tested negative. A summary finding of the test results on the 239 serum samples by the two methods is shown in Table I.

The *Acon* Dengue Rapid IgG/IgM Test Device is a chromatographic immunoassay for qualitative detection of IgG and IgM antibodies to dengue in human whole blood, serum or plasma. The evaluation results presented in the kit insert shows that the overall relative sensitivity of the device was 73.6% with 82.4% and 70.9% respectively for primary and secondary dengue infection. The relative specificity is >99.0%. In this small comparative evaluation, the performance of the *Acon* Dengue Rapid Test Device was rather disappointing in comparison to the Panbio Dengue Igm Capture ELISA used routinely in NPHL. Equivocal result, which both readers were unable to decide whether the read-off coloured line was present or absent, was relatively high (38.1%, 91/239). The Acon Dengue Rapid Test Device gave a relative sensitivity of 49.3% (69/140) and a relative specificity of 62.6% (62/99). However, if the borderline results were accepted as positive value, 34 serum samples (14.2%, 34/239) would be included as false positive besides the actual three false positive samples. On the reverse, if the borderline results were accepted as negative value, 57 samples (23.8%, 57/239) would be included as false negative besides the actual 14 false negative samples. Despite the poor relative sensitivity and specificity, the positive predictive value for the Acon device was high (95%, 69/72) with moderate negative predictive value (81.6%, 62/76) which was probably due to high prevalence of dengue in the community from which the samples originated. Thus, though the *Acon* Dengue Rapid Test 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 result of the test.

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