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Full Length Research Paper

Comparison of five serological diagnostic assays for the detection of IgM and IgG antibodies to dengue virus

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Difficulties in the management of dengue infections include the lack of rapid diagnostic methods and symptoms of dengue are often confused with those of other diseases. Five commercial kits were evaluated for the detection of dengue-specific IgM and IgG antibodies using sera obtained from patients with primary and secondary dengue infections, as well as other febrile illnesses. All kits were compared to the in-house IgM ELISA and HI assays. The rapid test kits took either 30 to 45 min (PanBio Dengue Duo Cassette, Accusens Dengue Virus Rapid Strip Test, and Unitest Dengue IgM and IgG Combo Rapid Test) or between 2 to 4 h (PanBio Dengue Duo Capture ELISA, and Antivirus IgM Detecion Kit 96 [Pentax Corporation]). Most kits were able to detect IgM in more than 90% of the secondary convalescent sera, while IgG detection was generally high (80 to 100%). All five kits showed high specificity when tested against sera from other febrile patients, and have been shown to be extremely useful in the diagnosis of dengue infections.

Key words: Dengue virus, IgM and IgG, diagnosis, ELISA, PCR.

INTRODUCTION

Arthropod-borne viruses commonly cause infections in the tropical and subtropical regions of the world. Dengue virus, a virus belonging to the Flavivirus group of viruses, is one of the most significant mosquito-borne diseases in the world in terms of morbidity and mortality (Gubler and Clark, 1995). The virus is transmitted principally by the mosquito species Aedes aegypti and Aedes albopictus, which are found commonly throughout the tropic and subtropical regions of the world. The infection caused by this virus can be asymptomatic, a self-limiting febrile illness or, in some cases, can result in severe haemorrhagic manifestations with plasma leakage that could lead to shock if not treated promptly and appropriately (Lee et al., 2006). Symptoms of dengue fever include high fever, headache, muscle pain and skin rash. The complications often associated with this infection are dengue hemorrhagic fever or dengue shock syndrome.

In countries where dengue is endemic, the majority of patients will have secondary infection. Detection of antibodies to dengue is a valuable procedure, particularly in second and subsequent infections where the occurrence of complications is high. Rapid and reliable tests for primary and secondary infections of dengue are essential for patient management. Primary dengue infection is associated with mild to high fever, headache, muscle pain and skin rash. Dengue viremia occurs prior to onset of fever and symptoms and peaks 2 - 3 days after onset of symptoms, which is 2 - 3 days before defervescence. Immune responses include production of IgM antibodies produced by the 5th day of symptoms and persist for 30 - 60 days. IgG antibodies appear by the 14th day and persists for life. Secondary infections often result in high fever and, in many cases, with haemorrhagic events and circulatory failure. Secondary infections show that IgG antibodies rise within 1 - 2 days after the onset of symptoms, simultaneously with IgM antibodies (Gubler, 1996; Innis, 1997). Therefore, patients with secondary infections will have a positive IgG result, usually, but not always with a positive IgM result.

Serology has been the main method employed in the diagnosis of dengue infections (Gubler, 1996). However, to confirm the infection, a four-fold rise in antibody titre needs to be done with paired samples collected at least 3 to 7 days apart. The classic haemagglutination inhibition assay (HI) and virus neutralization tests are still being used, despite their tedious and time-consuming nature (Clarke and Cassals, 1958; Russell and Nisalak, 1967). Currently, diagnosis depends on the detection of virus-

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Table 1. List of samples used in the evaluation.

Category	Sub-category	Number
Primary Dengue *	Acute sample	30
Primary Dengue †	Convalescent Sample	30
Secondary Dengue ‡	Acute sample	30
Secondary Dengue §	Convalescent Sample	30
Other febrile illnesses	Malaria	10
	Leptospirosis	7
	Typhoid fever	10
	Scrub typhus	2
	Measles	10
	Herpes	8
	Rubella	10

* Samples as determined by gold standard HI : 80
† Samples as determined by gold standard HI : 4-fold higher
‡ Samples as determined by gold standard HI : 640
§Samples as determined by gold standard HI : 640

specific IgM which can only be detected after 3 days of fever. Hence, many laboratories have developed various assays that enable early and specific detection. Several commercial kits such as the PanBio Dengue Duo IgM and IgG (developed in 1999), PanBio Rapid Immuno-chromatographic Test (developed in 1999), IgM capture (MAC) ELISA (developed by MRL Diagnostics, 1999), the Dengue Dot ELISA dipstick (INDX, USA), Genelabs IgG Blot and the Progen IgG IFA have been evaluated by several laboratories worldwide (Cuzubbo et al., 1999; Lam and Devine, 1998; Palmer et al., 1999; Sang et al., 1998; Vaughn et al., 1998; Lam et al., 1996). Each kit varies in its sensitivity and specificity depending on the assay format, the type of antigen employed and the detection system used.

The objective of this study was to evaluate five different systems for the detection of dengue specific antibodies using the same panel of sera taken from patients with primary and secondary dengue infections, as well as sera from patients with other febrile illnesses.

MATERIALS AND METHODS

Serum samples

A total of 177 serum samples (97 males and 80 females between the ages of 25 and 40 years) were used to evaluate the kits. These sera were from patients admitted to the University Malaya Medical Center for acute viral infection and consisted of 30 pairs of confirmed primary dengue infections, 30 confirmed pairs of secondary dengue infection, and 57 single samples from patients with confirmed viral infections other than dengue (Table 1).

In-house IgM capture ELISA

This is an in-house IgM capture ELISA developed by Lam et al. (1987). Briefly, 100 l of a 1:100 dilution of sample was added to human anti-IgM coated 96-well flat bottomed plates and incubated for 1 h at 37^{0}C. After washing thrice with PBS-Tween 20 (0.05%), 100 l of a 1:100 dilution of dengue antigen was added and the plates incubated for 1 h at 37^{0}C. The antigen used in this assay was obtained from the National Institute of Health, Thailand. The antigen concentration was given as an HA titer and was in different concentrations for each serotype, that is, Dengue 1 – HA 5120; Dengue 2 – HA 2560; Dengue 3 – HA 2560; Dengue 4 – HA 1280. Each antigen was reconstituted in 0.5 ml distilled water and stored at -20^{0}C until further use. The plates were then washed three times with PBS-Tween 20 (0.05%) and 100 l of a 1:5 000 dilution of mouse monoclonal antibody was added prior to incubation at 37^{0}C for 1 h. The plates weree washed again with PBS-Tween 20 (0.05%) and 100 l of a 1: 50 000 dilution of goat anti-mouse IgG conjugated with horse radish peroxidase (HRP) was added prior to incubation for 1 h at 37^{0}C. After a further three washes, 100 l of O-Phenylenediamine.2HCl (OPD) was added to all wells and incubated in the dark at room temperature. The reaction was stopped with 50 l 4 N sulphuric acid and the absorbance (OD) of each well was read at a wavelength of 490 nm with a reference filter of 630 nm using an ELISA plate reader. The positive control/sample OD was divided by the mean of the negative OD to obtain a positive:negative ratio (P/N). A P/N ratio of greater or equal to 2.0 is considered positive. A result with a P/N ratio of less than 2.0 is reported as a negative if the sample was collected two weeks after disease onset.

Differentiation of primary and secondary dengue infections

Primary and secondary dengue infections are generally differentiated based on the haemagglutination inhibition (HI) titers. Patients were classified as having secondary dengue virus infections when the HI test titer in their sera was greater than or equal to 1:2 560, and were classified as having primary dengue virus infection if the HI test titer was less than 1:2 560 (WHO, 1997)

PanBio Dengue Duo IgM and IgG Rapid Cassette

This assay is used for the qualitative presumptive detection of IgG and IgM antibodies to dengue virus in human serum and whole blood. This rapid immunchomatographic assay captures dengue-specific IgM or IgG antibodies in two lines across the test cassette. Colloidal gold complexes containing dengue 1-4 antigens are captured by the bound patient's IgM or IgG to give visible pink line(s). A procedural control is included to indicate that the assay has been performed correctly. Briefly, 10 µl of whole blood, plasma or serum was added to the circular well using a micropipette or MicroSafe pipette provided. The sample was then allowed to be absorbed entirely into the specimen pad within the circular well. Two drops of buffer provided was added vertically and 1 cm above the square well at the base of the cassette. The result was then read 15 min after adding the buffer to the cassette. Any trace of a pink line in the test area is indicative of a positive result.

PanBio Dengue Duo IgM and IgG Capture ELISA

In the PanBio Dengue Duo Capture ELISA, IgM and IgG are determined in separate wells of the assay plate, following the manufacturer's instructions. Briefly, two microtiter plates are supplied, one containing stabilized dengue-1 through dengue-4 (antigen plate) and the other containing either anti-human IgM or anti-human IgG bound to separate wells (assay plate) . Peroxidase-labeled monoclonal antibodies to dengue virus (125 µl/well) were added to the antigen plate to solubilize the antigens and form antibody-antigen complexes. An aliquot (100 µl) of patient serum (diluted 1:100 in the diluent provided) was added to each well of the assay plate containing either bound anti-human IgM or bound anti-

human IgG, and human IgM or IgG in the patient's serum was captured. The plates were incubated for 1 h at room temperature (antigen plate) or 37^{0}C (assay plate). The assay plate was washed and 100 µl of antibody-antigen complexes per well was transferred from the antigen plate to the assay plate. These complexes were captured by dengue virus-specific IgM or IgG during incubation for 1 h at 37^{0}C. The plate was washed, and bound complexes were visualized through the addition of 100 µl of tetramethylbenzidine substrate per well. After 10 min, the reaction was stopped by the addition of 100 µl of 1 M phosphoric acid in each well. The strips were read at 450 nm with a microtiter plate reader. The test was interpreted according to manufacturer's instructions.

Accusens Dengue Virus Rapid Strip Test

Accusens Dengue IgG/IgM Rapid Test is a solid phase immuno-chromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. The test is designed to simulta-neously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood. This test also can detect all four Dengue serotypes by using a mixture of recombinant Dengue envelope proteins. Accusense Dengue IgG/IgM test strips have three pre-coated lines "G" (Dengue IgG test Line), "M" (Dengue IgM test Line) and "C" (Control line) on the surface of the strip. The three lines are not visible before applying any samples. The control line is used as a procedural control. The control line should always appear if the test procedure is performed properly and the test reagents are working. Purple "G" and "M" lines will be visible in the result window if there are enough IgG and/or IgM antibodies to dengue virus in the sample. If IgG and/or IgM antibodies to dengue virus are not present in the sample, no lines appear. When a specimen is added to the test, anti-dengue IgGs and IgMs in the specimen sample react with recombinant dengue virus envelope proteins of colloidal gold conjugates and form a complex of antibodies and colloidal gold conjugates. As this mixture migrates along the length of the test strip by capillary action, the anti-dengue IgG or IgM complex is captured by the relevant anti-human IgG and/or anti-human IgM immobilized in two lines across the test strip and generate a colored line. This assay was carried out according to the manufacturers' instructions. Briefly, 1 µl of specimen was added to a disposable tube containing 100 µl of assay buffer provided. To each tube a test strip was added and the result was read after 15 - 30 min. The test was interpreted accord-ing to manufacturers' instructions.

Unitest Dengue IgM and IgG Combo Rapid Test

The UniTest Dengue IgM and IgG Combo Rapid Test is a quail-tative test for the detection of IgM and IgG antibodies to dengue virus in human serum, plasma or whole blood. When a specimen is added to the test, IgG and IgM antibodies in the specimen sample react with blue particles coated with dengue envelope proteins. As this specimen/particle mixture migrates along the length of the test strip, the anti-dengue IgG or IgM antibody particle complex is captured by the relevant IgG and/or IgM test bands located on the test strip, causing a pale to dark blue band to form at the IgG or IgM region of the test strip. The intensity of the bands will vary de-pending upon the amount of antibody present in the sample. The appearance of any color in a specific test region (IgG or IgM) should be considered as positive for that particular antibody type (IgG or IgM). A red procedural control line should always develop on the test strip to indicate that the test has been performed test tube containing four drops (100 l) of dengue wash buffer provided. The strip was then inserted into the tube and read after properly. The test was carried out according to manufacturer's instructions. Briefly, a 1 µl sample was added to a 15 - 30 min. The strips were then read according to the instructions provided with the kit.

Anti-Dengue Virus IgM Detection PA Kit 96 (Pentax Corporation)

This kit is a particle agglutination test kit for dengue virus-specific IgM in human serum samples. The test kit essentially consists of two parts, anti-human IgM antibody- coated microplate (strip) and purified dengue virus antigen-coated Ha-Ny (hydroxypatite-coated nylon) beads. The microplate captures human IgM antibodies from the serum samples. The surface of Ha-Ny beads are coated with four serological types of dengue virus antigens. The beads can bind to anti-dengue virus -specific IgM molecules, which are captured on the microplate, and adhere to the face of the wells. Dengue virus antigen-coated Ha-Ny beads adhere to the face of the wells when a tested sample is anti-dengue virus IgM positive. When the Ha-Ny beads form a button pattern at the bottom of the well, the sample is considered negative in Ha-Ny bead agglutination assays. This assay was carried out according to manufacturers' instructions. Briefly, the coated human IgM capture plate was washed three times with distilled water and tapped dry. Two drops of sample diluent was then added followed by a loop of serum from either a serum sample, positive control or a negative control (equivalent to 1 µl). The plate was then incubated at room temperature for 30 min. After washing three times with distilled water, two drops of bead suspension was added to all the wells. The plates were left undisturbed for 1 h at room temperature and the bead patterns were then read. A button in the well is indicative of a negative reaction, while a mattered agglutination pattern indicates presence of specific IgM antibodies.

Calculations of sensitivity, specificity and efficiency

The sensitivity, specificity and efficiency of the assays were calculated as follow:

Percent Sensitivity = a/(a+c) X 100%
Percent Specificity = b/(b+d) X 100%
Efficiency = (a+b)/(a+b+c+d) X 100%

Where a = number of samples positive by the assay; b = number of positive samples negative by the assay; c = number of negative samples that were positive by the assay; d = number of negatives samples that were negative by the assay.

RESULTS

Serological tests

The results of the serological tests done with the kits are shown in Tables 2 and 3. As seen in Table 2, of the 30 acute primary dengue cases, 22 (73.3%) were detected using the PanBio ELISA IgM, 21 by the PanBio Cassette IgM (70%) and 20 (67%) by the in-house IgM ELISA. In the primary dengue convalescent sera, the in-house IgM assay, the PanBio ELISA IgM and the Pentax IgM test detected IgM in all samples, while 29 (96.7%) samples were detected by the Accusens IgM and the Unitest IgM kits. For the secondary dengue cases, IgM was detected in 27 samples (90%) by both the in-house IgM and PanBio cassette IgM, while the Pentax IgM, and PanBio ELISA detected IgM in 28 samples. Most kits were able to detect IgM in 90 to 100% of the samples in secondary

Table 2. Comparison of detection of IgM and IgG antibodies by commercial serological kits with the in-house assays.

Kits	Category				
	Primary Acute[n=30]	Primary Convalescent[n=30]	Secondary Acute[n=30]	Secondary Convalescent[n=30]	Other Febrile Illnesses[n=57]
In-house IgM	20	30	27	30	0
In-house HI	0	27	30	30	0
PB C IgG	21	30	27	27	4
PB C IgG	0	14	24	27	1
PB ELISA IgM	22	30	28	29	2
PB ELISA IgG	0	21	28	30	0
Pentax IgM	17	30	28	30	0
AccuS IgM	18	29	3	2	0
AccuS IgG	10	29	30	30	15
UniTestIgM	14	29	16	13	13
UniTest IgG	2	24	29	30	13

PB C IgM = PanBio Dengue Duo IgM Rapid Cassette PanBio IgM; PB C IgG = PanBio Dengue Duo IgG Rapid Cassette; PB ELISA IgM = PanBio Dengue Duo IgM Capture ELISA; PB ELISA IgG = PanBio Dengue Duo IgG Capture ELISA; Pentax IgM = Anti-Dengue Virus IgM Detection PA Kit 96 (Pentax Corporation); AccuS IgM = Accusens Dengue Virus IgM Rapid Strip Test; AccuS IgG = Accusens Dengue Virus IgG Rapid Strip Test

Table 2. Comparison of detection of IgM and IgG antibodies by commercial serological kits with the in-house assays.

Category	N	In house IgM	In-house HI	PB C IgM	PB C IgG	PB ELISA IgM	PB ELISA IgG	Pentax IgM	AccuS IgM	AccuS IgG	UniTest IgM	UniTest IgG
Primary Acute	30	20	0	21	0	22	0	17	18	10	14	2
Primary Conv	30	30	27	30	14	30	21	30	29	29	29	24
Secondary Acute	30	27	30	27	24	28	28	28	3	30	16	29
Secondary Conv	30	30	30	27	27	29	30	30	2	30	13	30
Other Febrile illness	57	0	0	4	1	2	0	0	0	15	13	13

PB C IgM = PanBio Dengue Duo IgM Rapid Cassette PanBio IgM; PB C IgG = PanBio Dengue Duo IgG Rapid Cassette; PB ELISA IgM = PanBio Dengue Duo IgM Capture ELISA; PB ELISA IgG = PanBio Dengue Duo IgG Capture ELISA; Pentax IgM = Anti-Dengue Virus IgM Detection PA Kit 96 (Pentax Corporation); AccuS IgM = Accusens Dengue Virus IgM Rapid Strip Test; AccuS IgG = Accusens Dengue Virus IgG Rapid Strip Test .

convalescent sera.

Generally, all kits performed well with respect to detection of IgG antibodies (80 to 100%) with the exception of the PanBio Cassette. This could be due to the lower sensitivity of rapid immunochromatographic assays during the acute phase of infections when IgG levels are generally lower especially in primary infections.

The overall specificity of the rapid tests to sera collected from non-flaviviral febrile infections is summa-rized in Table 3, with false positives occurring mainly in sera from patients with leptospirosis, herpes and rubella. All five kits tested showed high specificity with little cross-reactivity with the 57 sera from other febrile infections. However, the highest specificity was seen with the PanBio ELISA IgM, which showed cross-reaction only with sera from two patients with leptospirosis (Table 4c). All 57 sera collected from patients with non-flaviviral febrile infections were negative in the in-house IgM and HI assays (Table 3).

The sensitivity, specificity and efficiency of the kits were compared and are shown in Table 4a, 4b and 4c. For primary dengue infection, a sensitivity of 71.7 - 86.5% was observed for the detection of IgM antibodies, while for IgG antibodies the sensitivity ranged from 46.1 to 100% (Table 4a). Table 4b shows that for secondary infection the sensitivity ranged from 8 - 96% for detection of IgM antibodies and 86.7 to 96.7% for IgG detection (Table 4b). The overall sensitivity, specificity and efficiency are shown in Table 4c.

DISCUSSION

The difficulty faced by physicians in the management of dengue infections is the lack of rapid diagnostic methods available. This problem is compounded by the fact that symptoms of dengue are often confused with those of other diseases such as leptospirosis (WHO, 1997).

Current diagnostic methods are based on serology (de-

Table 3. Specificity of the dengue rapid tests to other non-specific febrile infections.

Rapid tests	No. of negative samples/total no. tested (% specificity)						
	Malaria	Leptospira	Typhoid	Scrub typhus	Measles	Herpes	Rubella
PanBio IgM	12/12 (100)	9/12 (75)	11/12 (92)	4/5 (80)	ND	ND	ND
PanBio ELISA IgM	12/12 (100)	10/12 (83)	12/12 (100)	5/5 (100)	10/10 (100)	10/10 (100)	10/10 (100)
Pentax IgM	10/12 (83)	12/12 (100)	12/12 (100)	5/5 (100)	9/10 (90)	7/8 (87.5)	10/10 (100)
Accusens IgM	12/12 (100)	9/12 (75)	12/12 (100)	5/5 (100)	ND	ND	ND
PanBio IgG	12/12 (100)	10/12 (83.5)	12/12 (100)	4/5 (80)	ND	ND	ND
PanBio ELISA IgG	12/12 (100)	10/12 (83.5)	12/12 (100)	4/5 (80)	ND	ND	ND
Accusens IgG	12/12 (100)	10/12 (83.5)	12/12 (100)	4/5 (80)	10/10 (100)	4/8 (50)	7/10 (70)

Table 4a. Analysis of the sensitivity of the 5 kits evaluated in the diagnosis of primary dengue infection from sera.

Kit/test	Sensitivity of assay (%) *		
	Acute	Convalescent	Overall
In-House IgM	66.7	100	83.4
PanBio ELISA IgM	73	100	86.5
PanBio cassette IgM	70	46.7	58
Pentax PA IgM	56.7	100	78.4
Accusens IgM	60	96.7	78.4
Unitest IgM	46.7	96.7	71.7
In-house HI	-	100	100
PanBio ELISA IgG	-	70	70
PanBio cassette IgG	-	46.7	46.7
Accusens IgG	-	96.7	96.7
Unitest IgG	-	80	80

* Percentage calculated using formula stated in materials and methods

Table 4b. Analyses of the sensitivity of the 5 kits evaluated in the diagnosis of secondary dengue infection from sera.

Kit/Test	Sensitivity of assay (%) *		
	Acute	Convalescent	Overall
In-House IgM	90	100	95
PanBio ELISA IgM	96.7	96.7	96.7
PanBio cassette IgM	90	93.3	91.7
Pentax PA IgM	93.3	100	96.7
Accusens IgM	10	6	8
Unitest IgM	53.3	43.3	48.3
In-house HI	100	100	100
PanBio ELISA IgG	93.3	100	96.7
PanBio cassette IgG	80	93.3	86.7
Accusens IgG	100	100	100
Unitest IgG	96.7	100	98.4

* Percentage calculated using formula stated in materials and methods .

ELISA), particle agglutination (Antivirus IgM Detecion Kit 96 [Pentax Corporation]), and immunochromatographic assays (PanBio Dengue Duo IgM and IgG Rapid Cassette, Unitest Dengue IgM and IgG Combo Rapid Test). All five test kits are extremely rapid methods taking between 30 to 45 min (PanBio Dengue Duo Cassette

Table 4c. Analyses of the percentage sensitivity, specificity and efficiency of the 5 kits evaluated in the diagnosis of dengue infections from sera.

Kit/Assays	Sensitivity (%)*	Specificity (%)*	Efficiency (%)*
In-House IgM	89.2	100	98
PanBio ELISA IgM	91.6	97.6	96.6
PanBio cassette IgM	74.8	93.8	90.3
Pentax PA IgM	87.5	100	97.7
Accusens IgM	43.2	100	89.3
Unitest IgM	60	89.2	83.8
In-house HI	100	100	98.1
PanBio ELISA IgG	83.3	100	93.6
PanBio cassette IgG	70	99.2	90.6
Accusens IgG	98	86.9	86.1
Unitest IgG	89.2	89.2	85.7

* Percentage calculated using formula stated in materials and methods

Accusens Dengue Virus Rapid Strip Test and the Unitest Dengue IgM and IgG Combo Rapid Test) and 2 to 4 h (Anti-dengue Virus IgM Detection PA Kit 96 [Pentax Corporation], in-house IgM ELISA and the PanBio Dengue Duo ELISA).

The PanBio cassette and ELISA tests are able to simultaneously detect both IgM and IgG in a single well and plate, respectively. This reduces the overall cost of the assay. Furthermore, differentiation between IgM and IgG using a single application of diluted serum negates the need of serial dilutions required by the HI assay. The Anti-Dengue Virus IgM Detection PA Kit 96 [Pentax Corporation] showed the highest efficiency among all the five kits that were evaluated, followed by the PanBio Dengue Duo IgM and IgG Capture ELISA, PanBio Dengue Duo IgM and IgG Rapid Cassette, the Accusens Dengue Virus Rapid Test Strip and the Unitest Dengue IgM and IgG Combo Rapid Test (Table 4c).

Most of the kits that were evaluated showed fairly high specificity when tested with sera from non-flavivirus febrile infections (97.6 to 100%). Nonetheless, it is important to include these, and in particular other flavivirus infections (e.g. JE, yellow fever) with similar clinical presentation (scrub typhus, typhoid, leptospirosis and rubella) in the serum panels when evaluating dengue diagnostic assays, since high levels of cross-reactivity have been reported in previous studies evaluating similar dengue kits (Lam et al., 1996; Vaughn et al., 1999).

The choice of an assay for the detection of dengue infections that is to be used in any laboratory is dependent on several factors, including the facilities available and the anticipated workload. The ELISA and cassette kits described in this study have shown to be extremely useful in the diagnosis of dengue infections. In addition to being a rapid method, the ELISA assays have the advantage of being able to diagnose a large number of samples. The only drawback is that specialized, automated equipment is required to carry out this assay. Thus, in well established laboratories that generally handle larger number of samples, the ELISA should be the assay of choice. However, in smaller laboratories where small batches or even single samples are analysed, the Dengue duo cassette or the Unitest and Accusens test strips should be considered. The Dengue duo cassette has the advantage of portability and can be used with a certain degree of accuracy in field testing of suspected dengue cases, or to screen travelers who have returned from countries endemic for dengue.

Although the serological tests are widely used in the diagnosis of dengue, nevertheless, the assays, including the five kits that were analysed, cannot efficiently detect the presence of antibodies in acute phase sera (Day 1 to 3) in primary infections. In addition, these assays are unable to distinguish the serotype of dengue causing the infection. The methods employed for the detection of dengue virus in the early stages of infection include cell culture, polymerase chain reaction (PCR) and NS1 detection (Henchal et al., 1991; Alcon et al., 2002). Currently, dengue virus NS1 antigen detection has become increasingly useful in the diagnosis of dengue virus infections (Dussart et al., 2006; Sekaran et al., 2007). Commercial kits for NS1 antigen detection such as the PLATELIA™ Dengue NS1 antigen assay (BioRad) and the PanBio Dengue NS1 antigen capture ELISA (PanBio, Australia) have been developed and evaluated (Sekaran et al., 2007). PCR is fast becoming the method of choice for the rapid detection of dengue viruses (Lanciotti et al., 1992; Kong et al., 2007). With the recent advances in real-time PCR, several methods have been developed using this versatile tool that enables rapid detection, serotype identification, as well as viral RNA quantitation (Drosten et al., 2002; Yong et al., 2006). .The only drawback of these methods is the need for specialized equipment and the cost incurred using this method. Thus, the choice of an assay that is to be used in the diagnosis of dengue infections depends on several factors, including the labo-

ratory infrastructure, preference and availability of equipment.

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