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Evaluation of the MRL Diagnostics Dengue Fever Virus IgM Capture ELISA and the PanBio Rapid Immunochromatographic Test for Diagnosis of Dengue Fever in Jamaica

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We evaluated two new commercial dengue diagnostic tests, the MRL Diagnostics Dengue Fever Virus IgM Capture ELISA and the PanBio Rapid Immunochromatographic Test, on serum samples collected during a dengue epidemic in Jamaica. The MRL ELISA method correctly identified 98% (78 of 80) of the samples as dengue positive, while the PanBio test identified 100% (80 of 80). Both tests were 100% (20 samples of 20) specific.

Dengue fever (DF) is an acute febrile illness caused by a mosquito-borne flavivirus. The more severe form of DF, known as dengue hemorrhagic fever (DHF)-dengue shock syndrome (DSS), can prove fatal, especially among young children, who account for the majority of the 5% annual case-fatality rate in countries where DF is endemic (4). The most challenging problem associated with patient management in dengue infection is rapid diagnosis. Early symptoms of DF mimic other diseases often prevalent in areas where DF is endemic, such as malaria, leptospirosis, and even influenza. Thus, a rapid differential diagnosis is crucial to proper patient care. The traditional diagnosis of dengue infection is performed by using hemagglutination inhibition (HAI) assays or immunoglobulin M (IgM) capture enzyme-linked immunosorbent assays (ELISA) on paired serum samples. Reagents for these techniques have not been commercially available in the past. Mosquito cell cultures are also used but are effective only during the first week of infection while the virus circulates in the blood (5). Additionally, few laboratories in areas where DF is endemic have the ability to maintain mosquito cell lines. Clearly, the need for more rapid and efficient diagnostic tools is evident. This study evaluated two newly introduced commercial tests for the detection of antibodies to dengue virus, the MRL Diagnostics Dengue Fever Virus IgM Capture ELISA (Cypress, Calif.) and the PanBio Rapid Immunochromatographic Test (Brisbane, Australia), on serum samples collected during a dengue epidemic in Jamaica in 1995.

Serum samples were chosen at random from a bank of patient sera collected during the Jamaica dengue outbreak. We selected 50 samples from patients with DF, 30 from those with DHF, and 20 samples from those who were dengue negative. The self-reporting of onset of symptoms by patients showed that the serum samples were obtained an average of 7 to 10 days after DF symptoms had appeared. Sera had been stored at −70°C and were previously diagnosed as dengue positive by using HAI assays (2), IgM ELISA (8), and/or a tissue culture. Dengue cases from this outbreak were attributed to dengue serotype 2, as determined by the Centers for Disease Control and Prevention.

Serum samples were diluted 1:100 and tested in duplicate with the MRL IgM ELISA, a qualitative assay for the detection of IgM antibodies to dengue virus in human serum. The procedure was performed per the manufacturer's instructions and took 4 h to complete. Rapid testing was performed with the PanBio Rapid Immunochromatographic Test. This test detects both dengue-specific IgM and IgG with a test card format. The test required the addition of 30 μl of serum, and results, in the form of the appearance of red lines in the test card viewing window, were read after 5 min. The test format has been previously described by others (1, 9–11).

The MRL test correctly identified 98% (78 of 80) (confidence intervals, 95, 91.3, and 99.7%) of the dengue samples as positive. One hundred percent (30 of 30) of the samples from patients with DHF were positive with the MRL test, while 2 of the 50 DF patient samples were judged negative. The two DF patient samples had been judged positive previously by IgM ELISA in the Jamaican laboratory. Results from the PanBio rapid dengue test revealed 100% (80 samples of 80) agreement with those of the previous Jamaican laboratory diagnosis. The 20 negative control sera were negative with both the MRL and PanBio dengue tests, indicating 100% (20 samples of 20) specificity.

While not representative of the entire infected population during the 1995 Jamaican dengue outbreak (Table 1), the PanBio test results reveal interesting trends. The test detected both dengue-specific IgM and IgG in 84% (42 of 50) of the samples from patients with DF and in 80% (24 of 30) of samples from those with DHF. Interestingly, in the DHF patient samples, five of six primary responses (IgM) were observed in samples from patients 1 year old or younger (Table 2).

DF and DHF have become major global public health problems, particularly in the Americas (4). The full scope of the dilemma is probably grossly underestimated due to poor surveillance that is no doubt closely associated with the lack of diagnostic capabilities in countries with endemic dengue. The introduction of commercially available rapid tests for dengue can assist in resolving this problem by providing standardized, readily available diagnostic tools. Results from the present study indicated that both the MRL IgM test and the PanBio rapid dengue test were effective in detecting dengue antibody

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TABLE 1. DF cases diagnosed at the University of the West Indies Hospital Virology Laboratory, 1992 to 1996 (3)

Year	No. of suspect samples	% (No.) of samples positive for dengue
1992	519	35 (179)
1993	303	12 (36)
1994	238	12 (29)
1995	1,225	48 (593)
1996	296	12 (36)

responses. The PanBio test results were slightly better than the MRL test results (100 versus 98%). However, the PanBio test is run on undiluted sera while the MRL test requires a 1:100 serum dilution, which may have been a factor in the two samples missed by the MRL test. Our results with the PanBio test agree with two other recently published studies on the PanBio rapid dengue test, which reported sensitivities of 99 and 100% for patients tested in southeast Asia (10, 11). These studies included samples from patients with all four dengue serotypes and reported that the majority of patients in each study showed elevated IgM titers within 5 days of onset. To our knowledge, there are no other reported studies on the MRL IgM test for comparison.

An advantage in running the MRL IgM dengue test is that serum samples can be analyzed in batches, since this test uses a 96-well plate format. The PanBio test was performed on individual samples, and results had to be timed since the interpretation needed to be completed in 5 min. This format is excellent for performing rapid point-of-care screening of symptomatic patients rather than waiting for the laboratory to accumulate enough samples to make it economically feasible to run a 96-well ELISA plate. In addition the PanBio test is completed without equipment or supplies and can be utilized in areas lacking extensive laboratory infrastructure or in field situations where electricity is not available. Also, since the PanBio test provides information on the IgG response in addition to detecting IgM, a positive IgG response could suggest to the clinician that the patient may be more susceptible to developing DHF, since one theory on the development of DHF states that it is more likely to occur in those with sequential infections with different dengue serotypes (6). It should be noted that the IgG response detected with the PanBio rapid dengue test is set to detect high levels of IgG (HAI assay; 1:2,560), thus indicating a secondary infection response and not residual antibodies from a previous infection.

TABLE 2. Dengue primary and secondary results by age of patients as determined with the PanBio Rapid Immunochromatographic Test

Patient diagnosis	Age category (total no. of patients)	% (No.) of sera with:	
		Primary response only (IgM only)	Secondary response (IgG with or without IgM)
DF	Total (50)	16 (8 of 50)	84 (42 of 50)
	1 year old or less (0)	NT[a]	NT[a]
	2–15 years old (12)	37.5 (3 of 8)	21.0 (9 of 42)
	16–30 years old (29)	50.0 (4 of 8)	60.0 (25 of 42)
	31–45 years old (6)	12.5 (1 of 8)	12.0 (5 of 42)
	>45 years old (3)	0.0 (0 of 8)	7.0 (3 of 42)
DHF	Total (30)	20.0 (6 of 30)	80.0 (24 of 30)
	1 year old or less (8)	83.0 (5 of 6)	12.5 (3 of 24)
	2–15 years old (11)	17.0 (1 of 6)	42.0 (10 of 24)
	16–30 years old (7)	0.0 (0 of 6)	29.0 (7 of 24)
	31–45 years old (3)	0.0 (0 of 6)	12.5 (3 of 24)
	>45 years old (1)	0.0 (0 of 6)	4.0 (1 of 24)

[a] NT, none tested.

Interestingly, the PanBio test indicated that only 20% (6 of 29) of samples from patients with DHF and 16% (8 of 50) of samples from patients with DF showed primary antibody responses alone, since most samples exhibited evidence of secondary dengue infections (IgG and IgM responses). All primary dengue responses in DF patient samples were in sera from older children and adults, since there were no infant sera in this category. However, five of the six DHF patient primary responses were observed in infants 1 year of age or less (8 to 12 months), while the sixth was from the serum of an 8-year-old child. This finding may confirm a previous report that maternal antibodies provide an initial protection to an infant but can also increase the risk of developing DHF in dengue serotype 2 infections (7).

The results of our study indicate that both of the dengue diagnostic tests, the MRL ELISA and the PanBio rapid card test, provide excellent diagnostic tools. This is important since, in the past, reagents to test for dengue infection were not readily available and most countries still rely on reference laboratories that may be miles away or in another country. Turnaround times for out-of-country reference laboratories can run in excess of 1 month. The commercially available tests described in this study can provide laboratories with readily available methodologies with which to screen suspect dengue samples in 1 day, eliminating the need to send samples to reference laboratories far from patient point-of-care facilities.

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