Evaluation of Four Methods for Detection of Immunoglobulin M Antibodies to Dengue Virus

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Dengue has become hyperendemic in many islands of the Caribbean region. The performance in a diagnostic laboratory of four commercial assays for detection of immunoglobulin M (IgM) antibodies was evaluated. Sera from 62 patients with dengue virus infection were studied. These included 18 patients from whom dengue virus type 2 was isolated in a 1997 outbreak (specimens collected a mean of 14 days after onset of symptoms), 8 patients with dengue hemorrhagic fever (mean time after onset, 11 days), and 36 patients in whom dengue was previously confirmed by serology (mean time after onset, 10 days). Thirty serum specimens from blood donors in a country where dengue is not endemic were used as negative controls. The methods evaluated were two IgM-capture enzyme-linked immunosorbent assays (ELISA) (MRL Diagnostics, Cyprus, Calif., and PanBio, Queensland, Australia), a dot ELISA dipstick assay (Integrated Diagnostics, Baltimore, Md.), and a rapid immunochromatographic assay for dengue IgG and IgM (PanBio IC). IgG antibodies were also detected by an ELISA method (MRL Diagnostics). The sensitivities of the four assays were as follows: MRL Diagnostics IgM ELISA, 98.4%; PanBio IgM ELISA, 85.5%; Integrated Diagnostics IgM dot ELISA, 96.8%; and PanBio IC, 83.9%. The specificities of all tests were 100%. Evidence of secondary dengue was found in all patients with dengue hemorrhagic fever and in 83% of the remaining patients. The MRL Diagnostics IgM ELISA appears to be more sensitive than the PanBio IgM ELISA, and this may be significant when IgM titers are low, particularly in patients with secondary dengue infections. The dot ELISA dipstick assay is equally sensitive and may be more appropriate for use in laboratories with lower workloads.

Dengue fever is one of the most common infectious diseases in tropical and subtropical regions. Dengue fever is caused by the four serotypes of dengue virus, but infection with any one type is not protective against subsequent infection with any of the other types, which may then result in the manifestation of severe disease known as dengue hemorrhagic fever (DHF). The risks of DHF are greatly increased when the disease is hyperendemic, with the simultaneous circulation of multiple serotypes of dengue virus within a population. The incidence of dengue fever has increased globally over the past 20 years (1, 2). Within the Americas, the Caribbean region has been no exception, and dengue has become hyperendemic (5). In the southern and eastern Caribbean, several islands have experienced large outbreaks of dengue fever in recent years, and DHF has been reported for the first time (9, 11). In Barbados there have been outbreaks caused by dengue virus type 1 (1995) and dengue virus type 2 (1997), with attack rates of approximately 800/100,000 population. With the return of dengue to popular tourist destinations in the Western Hemisphere, there is an increased risk of importation of dengue (and DHF) to countries where the virus and the disease are not endemic (3, 8).

Dengue fever is diagnosed by isolation of the virus, by serology, or by reverse transcription-PCR (1). Diagnostic laboratories in most developing countries lack the facilities for the diagnosis of dengue by any means other than serology. Detection of immunoglobulin M (IgM) antibodies is a sensitive method, but until recently, IgM assays were not widely available for use in nonspecialized laboratories. Several assays for the detection of dengue virus antibodies are now available commercially (4, 6, 10, 12). We evaluated four such assays.

**MATERIALS AND METHODS**

**Human sera.** A panel of 62 serum samples from patients with laboratory-confirmed dengue virus infection during the 1997 outbreak was studied. This included 18 patients from whom dengue virus type 2 was isolated (specimens for serology collected a mean of 14 days after onset of symptoms), 8 patients with DHF (mean time after onset, 11 days), and 36 patients in whom dengue was previously confirmed by serology (mean time after onset, 10 days). Thirty serum specimens from blood donors in a country where dengue is not endemic (the United States) were used as negative controls. All sera were stored at −20°C until they were thawed for testing.

**Dengue IgM-capture ELISA.** IgM antibodies were detected by two microplate enzyme-linked immunosorbent assays (ELISA) (PanBio IC) obtained from MRL Diagnostics (Cyprus, Calif.) and PanBio (Queensland, Australia). Each assay was performed with 10 μl of serum according to the manufacturer's instructions. For both assays optical density readings at 450 nm were compared with reference cutoff readings to determine positivity. The results were expressed in each case as an IgM ratio or index, with a value of greater than 1.0 taken as a positive result. The MRL and PanBio assays required approximately 5 and 3 h, respectively, for completion.

**Dengue dot ELISA dipstick assay.** IgM antibodies were detected by a commercial semiquantitative dot ELISA dipstick assay (Integrated Diagnostics, [INDX], Baltimore, Md.). All steps were carried out at 50°C. In this assay, 10 μl of serum and 40 μl of goat anti-human IgG absorbent (proSorb G) were diluted in 2 ml of sample diluent, and the mixture was incubated at 50°C for 10 min prior to the addition of the assay strip. Alkaline phosphatase-conjugated goat anti-human IgM (μ chain) was used as described previously (12). The dot ELISA dipstick assay was also used to detect both IgM and IgG. In this format, the IgG absorbent was omitted and the conjugate used was alkaline phosphatase-conjugated goat anti-human IgG (heavy and light chains) and IgM (μ chain). After the assay strips were allowed to dry, the test was scored on a scale of from 1 to 4 dots (5 dots for the combined IgM and IgG dipstick assays); positive dots were gray to blue in color with distinct borders against a white background. Each assay strip contained positive and negative control dots. The assay required approximately 2 h for completion.

**Immunochromatographic test for dengue virus IgM and IgG.** A rapid immunochromatographic card test (PanBio IC) was used to detect IgM and IgG antibodies. In this assay, 30 μl of serum and 2 drops of buffer were added to a membrane. During a 5-min incubation at room temperature, IgM and IgG antibodies were captured by anti-human IgM or anti-human IgG antibodies and...
were reacted with a complex of dengue virus antigen with colloidal gold-labelled anti-dengue virus monoclonal antibody. The presence of IgM and/or IgG was indicated by the presence of purple bands. Each assay card also contained a control band which indicated the correct functioning of the test.

**IgG ELISA.** IgG antibodies were detected by a commercial microplate ELISA (MRL Diagnostics). A mixture of dengue virus types 1 to 4 was used as the antigen. The assay was performed with 10 μl of serum according to the manufacturer’s instructions. Optical density readings at 450 nm were compared with reference cutoff readings to determine positivity.

**Test reading.** To negate reader bias, samples tested by microplate assays were tested in a random order. The results were then collated by a different investigator. The dipstick and rapid card tests were each read independently by two investigators, the second of whom was blinded to the sample source.

**RESULTS**

The results of serological tests with sera from 36 patients with dengue who were previously confirmed to be positive by a monoclonal antibody-capture ELISA at a regional reference laboratory are shown in Table 1. These specimens were collected a mean of 10 days (standard deviation [SD] ±3.5 days) after the onset of symptoms. IgM antibodies were detected in all sera by at least two assays. Both microplate ELISAs and the dipstick assay detected IgM in more than 90% of the serum specimens, while the rapid immunochromatographic method was less sensitive.

Sera from 18 patients whose dengue was confirmed by isolation of dengue virus type 2 were collected for serology a mean of 14 days (SD, ±3.8 days) after the onset of symptoms. IgM antibodies were detected in 17 (94%) of these serum specimens by both the MRL microplate ELISA and the INDx IgM dipstick assay and in 16 (89%) of these specimens by the PanBio microplate ELISA and the PanBio IC assay. Eighty-three percent (15 of 18) of the serum specimens were IgM positive by all four assays, while one was IgM negative by all four assays.

Eight patients with DHF were studied. Four patients died; specimens were taken a mean of 11 days (SD, ±4 days) after the onset of symptoms, while specimens from the survivors were taken a mean of 11 days (SD, ±4 days) after the onset of symptoms. IgG antibodies were detected in all eight patients with DHF. Positive test results were obtained for all specimens by the MRL microplate ELISA and the INDx dipstick assay. The PanBio microplate IgM ELISA was the least sensitive assay for this group, detecting DHF only in four patients, while the PanBio rapid immunochromatographic assay detected either IgG or IgM, or both, in seven of eight (88%) serum specimens. Semiquantitative IgM data shown in Table 2 suggest that the PanBio IgM microplate ELISA is less sensitive when small amounts of IgM are present.

IgM results for all three groups of sera are summarized in Table 3. Both the MRL microplate ELISA and the INDx IgM dipstick assays had high sensitivities (98 and 97%, respectively), whereas the PanBio microplate ELISA and the PanBio rapid immunochromatographic assay had lower sensitivities (85 and 84%, respectively). Thus, a negative result by either the MRL microplate ELISA or the INDx IgM dipstick assay effectively ruled out dengue virus infection in a high proportion of patients in our study. However, all four assays were highly specific and thus were associated with high positive predictive values.

There was a high correlation between the IgM ratios and indexes derived from the MRL and PanBio microplate IgM ELISAs ($r = 0.88; P < 0.0001$), but the IgM ratios calculated for the PanBio ELISA were consistently lower than the IgM indexes calculated for the MRL ELISA.

IgG antibodies were detected in 53 of 62 (85%) specimens by the microplate ELISA and 40 of 62 (65%) specimens by the rapid immunochromatographic assay. The combined IgM and IgG dipstick assay was strongly positive (4 or 5 dots were positive) for 57 of 62 (92%) serum specimens, equivocal for 3 serum specimens, and negative for 2 serum specimens. One of these specimens was also negative by all other assays. When the rapid immunochromatographic assay results for IgM and IgG were combined, the number of positive serum specimens increased to 54 (87%). Of these, 14 showed evidence of IgM only and 2 showed evidence of IgG only.

IgM antibodies were not detected in any of the 30 control serum specimens from blood donors. However, two specimens were IgG positive by the microplate ELISA and a third was positive by the combined IgM and IgG dipstick assay. IgG antibodies were not detected in these specimens by the rapid immunochromatographic assay.

**DISCUSSION**

Of the four commercial assays for the detection of dengue virus IgM evaluated in this study, two were clearly more sensitive. The MRL microplate IgM-capture ELISA and the INDx IgM dipstick assay were found to be highly sensitive (sensitivity, ≥97%), while the PanBio microplate IgM-capture ELISA and the PanBio rapid immunochromatographic assay were less sensitive. The sera from patients with confirmed dengue that we tested were in three groups and were collected a mean of 10 to 14 days after the onset of symptoms, well into the period when IgM seropositivity would be expected.

A high correlation was found between the results obtained by the two microplate IgM-capture ELISAs, but the IgM ratios obtained by the PanBio assay were lower, which translated into a lower sensitivity, particularly when IgM concentrations were near the threshold. This is most likely to occur when specimens are tested early in the course of the disease and also when specimens are from patients with secondary dengue virus infection. In this study, we were able to examine sera from eight patients with DHF, four of whom died. All eight patients had

### Table 1. Detection of IgM antibodies in 36 patients diagnosed by serology

| No. of patients | MRL IgM ELISA | PanBio IgM ELISA | INDx IgM dipstick assay | PanBio IC IgM assay |
|----------------|--------------|-----------------|------------------------|---------------------|
| 31             | +            | +               | +                      | +                   |
| 1              | +            | +               | –                      | –                   |
| 3              | +            | –               | +                      | –                   |
| 1              | +            | +               | –                      | –                   |
| No. (%) positive | 36 (100) | 33 (92) | 35 (97) | 31 (86) |

### Table 2. IgM reactivity with sera from eight patients with DHF

| Patient no. | MRL IgM index* | PanBio IgM ratio* | No. of INDx dipstick dots |
|-------------|----------------|------------------|--------------------------|
| 9           | 7.7            | 6.8              | 3                        |
| 10          | 2.4            | 0.8              | 1                        |
| 17          | 9.0            | 5.5              | 3                        |
| 18          | 5.8            | 2.9              | 2                        |
| 32          | 2.0            | 0.7              | 1                        |
| 34          | 2.4            | 0.9              | 1                        |
| 38          | 3.2            | 0.9              | 1                        |
| 69          | 10.2           | 11.2             | 3                        |

*Values of MRL IgM index and PanBio IgM ratio were determined as described in the text. In each assay, an index of ratio ≥1.0 is regarded as a positive result.
IgG antibodies, consistent with a secondary dengue virus infection. All were IgM positive by the MRL microplate ELISA and by the INDX dipstick assay, but only four were IgM positive when they were tested by the PanBio microplate ELISA, again reflecting a lack of sensitivity of the PanBio assay. When the IgM indexes and ratios for sera from patients with DHF were compared, four were strongly positive by both the MRL and PanBio assays, while four were less strongly positive by the MRL assay and were negative by the PanBio assay (Table 2). Similarly, four of the serum specimens reacted strongly by the INDX IgM dipstick assay, while four gave weakly positive results.

IgG antibodies were detected by the microplate ELISA in a large proportion (85%) of serum specimens, suggesting that many of the cases of dengue in the 1997 outbreak in Barbados were secondary infections. Detection of IgG antibodies may become more important as the number of secondary infections and cases of DHF occurring in this population increases (1). All the assays studied were highly specific with specimens from patients with DHF and cases of DHF occurring in this population increases (1). In the Caribbean region other flavivirus infections are generally absent, so the confounding effect noted in studies from southeast Asian countries (10) was not applicable here.

Recently, several studies have evaluated the rapid immunochromatographic assay in southeast Asian populations (4, 7, 10), with sensitivities reported to be 99 to 100%. In our study performance.

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REFERENCES
1. Gubler, D. J. 1998. Dengue and dengue hemorrhagic fever. Clin. Microbiol. Rev. 11:480–496.
2. Halstead, S. B. 1998. Emergence mechanisms in yellow fever and dengue. In W. M. Scheld, W. A. Craig, and J. M. Hughes (ed.), Emerging infections 2. American Society for Microbiology, Washington, D.C.
3. Laboratory Centre for Disease Control. 1998. A point source dengue outbreak in Canadian tourists in Barbados. Can. Commun. Dis. Rep. 24:161–164.
4. Lam, S. K., and P. L. Devine. 1998. Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgG antibodies produced during dengue infection. Clin. Diagn. Virol. 18:75–81.
5. Pan American Health Organization. 1997. Re-emergence of dengue in the Americas. Epidemiol. Bull. 18:1–6.
6. Sang, C. T., A. J. Cuzzubbo, and P. L. Devine. 1998. Evaluation of a commercial capture enzyme-linked immunosorbent assay for detection of immunoglobulin M and G antibodies produced during dengue infection. Clin. Diagn. Lab. Immunol. 5:7–10.
7. Sang, C. T., L. S. Hoon, A. Cuzzubbo, and P. Devine. 1998. Clinical evaluation of a rapid immunochromatographic test for the diagnosis of dengue virus infection. Clin. Diagn. Lab. Immunol. 5:407–409.
8. Shirlcliffe, P., E. Cameron, K. G. Nicholson, and M. J. Wiselka. 1998. Don’t forget dengue! Clinical features of dengue fever in returning travellers. J. R. Coll. Phys. London 32:235–237.
9. Teelucksingh, S., A. S. Mangray, S. Barrow, N. Jankey, P. Prabhakar, and M. Lewis. 1997. DHF/DSS: an unwelcome arrival in Trinidad. West Indian Med. J. 46:38–42.
10. Vaughn, D. W., A. Nisalak, S. Kalayanarooj, T. Solomon, N. M. Dung, A. Cuzzubbo, and P. L. Devine. 1998. Evaluation of a rapid immunochromatographic test for diagnosis of dengue virus infection. J. Clin. Microbiol. 36:234–238.
11. Villedieu, L., J. M. Mansuy, J. F. Magnaval, and L. Schlegel. 1998. Aspects de la dengue a la Martinique en 1995–1996. Med. Trop. 58:145–148.
12. Wu, S. J., R. Hanson, R. Paxton, A. Nisalak, D. W. Vaughn, C. Rossi, E. A. Henchal, K. R. Porter, D. M. Watts, and C. G. Hayes. 1997. Evaluation of a dipstick enzyme-linked immunosorbent assay for detection of antibodies to dengue virus. Clin. Diagn. Lab. Immunol. 4:452–457.