

## Evaluation of a MRL Diagnostic IgM Capture ELISA Kit for Dengue Diagnosis

**R. Rodriguez-Roche, S. Vazquez and M.G. Guzman**✉

*Virology Department, PAHO/WHO Collaborating Center for the Study of Dengue and its Control, Institute of Tropical Medicine "Pedro Kouri", Autopista Novia del Mediodía, Km 6, PO Box 601 Marianao 13, Havana, Cuba*

Classical dengue along with its more serious forms, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), is currently the most important arboviral disease of humans, being endemic in many tropical and subtropical countries with approximately 2.5 billion people at risk of infection. The four serotypes (DENV-1 to -4) are estimated to cause up to 100 million infections annually<sup>[1-4]</sup>.

Dengue surveillance systems with laboratory support are essential for early detection and adequate response to prevent or control dengue epidemics. Laboratory methods for dengue diagnosis with adequate sensitivity and specificity, therefore, become crucial<sup>[5,6]</sup>.

Primary, secondary and even tertiary dengue infections can be detected taking into account the existence of four serotypes. During a primary infection, individuals develop IgM antibodies 5–6 days after the onset of illness lasting 30 to 90 days. IgG levels rise after IgM and are detectable for life<sup>[7]</sup>. During a secondary infection IgM levels are lower and in some cases absent. In contrast, IgG levels rise rapidly to higher levels than observed in primary or past dengue infection. Consequently, it is useful to combine the detection of specific IgM

and IgG for serological diagnosis of dengue virus infection<sup>[8]</sup>.

There are several laboratory tests that can be used for routine serological diagnosis of dengue infection. The capture IgM ELISA is the most useful procedure currently available and it is widely recommended for serological surveillance<sup>[5,9,10]</sup>.

The aim of this study is to evaluate the commercially available MRL diagnostic kit designed to detect anti-dengue virus IgM antibodies in human serum samples. Dengue IgM, an in-house kit developed by the Arbovirus Laboratory at "Pedro Kouri" Tropical Medicine Institute (IPK) was considered as the gold standard. This system has been previously evaluated and applied with excellent results<sup>[9,11,12]</sup>.

Both captures ELISA were compared in parallel by using a panel of 51 serum samples from patients with known and documented clinical history for dengue illness during the 1997 outbreak of Santiago de Cuba. Laboratory confirmation of dengue virus infection was done. The patients from whom DENV-2 was isolated in C6/36-HT cells<sup>[13]</sup> or had positive PCR to dengue virus<sup>[14]</sup> were included.

✉lupe@ipk.sld.cu; ☎ 53-7-2020450, Fax: 53-7-2046051



Specimens for serology were collected at 5 to 7 days after the onset of fever.

Thirty-five serum samples received through the dengue surveillance system during a non-epidemic period (1992–1995) with evidence of non-circulation of dengue virus were included as negative samples<sup>[3]</sup>. Neither seroconversion nor virus isolation or positive PCR were obtained from these patients. Finally, most of them were diagnosed as cases of influenza or leptospirosis. (data not shown).

Sera were diluted 1:100 when using the MRL diagnostic kit and the procedures were performed as per the manufacturer's instructions. For dengue IgM the sera were diluted 1:20, and the procedure followed was according to Vazquez et al., 1998, 2003<sup>[11,15]</sup>. In both assays, samples were tested in duplicate. The results are shown in the Table.

**Table.** ELISA performance by MRL diagnostics and dengue IgM

	Dengue IgM positives	Dengue IgM negatives	Total
MRL diagnostics positives	51	5	56
MRL diagnostics negatives	0	30	30
Total	51	35	86

Statistical analysis was used to estimate the sensitivity and specificity in relation to the dengue IgM kit. The concordance between dengue IgM and MRL diagnostic kits was calculated by means of the Kappa index ( $\kappa$ ). All values are expressed with the corresponding confidence intervals of 95% (95% CI) and were calculated using Epidate statistics software (version 2.0, 1997).

The MRL test correctly identified 100% (51 of 51) of dengue samples as positive. On the

other hand, this test showed a specificity of 85.7% (95% CI, 74.1–97.3). The concordance was 94.2%,  $\kappa=0.88$  (95% CI, 0.77–0.98). Positive predictive value (PPV) and negative predictive value (NPV) were estimated as PPV=91.1% (95% CI, 83.6–98.5) and NPV=100% respectively.

Results from the present study indicated that MRL diagnostic was effective in detecting dengue antibody responses and constituted a useful diagnostic tool. In an earlier evaluation of the MRL diagnostic kit, Palmer et al., 1999, reported lower sensitivity as compared to the PanBio rapid immunochromatographic test<sup>[16]</sup>. However, Branch et al., 1999, found that MRL diagnostics was more sensitive than both Panbio system ELISA and Rapid immunochromatographic test<sup>[17]</sup>. With regard to the specificity, these authors recognized that both MRL and PanBio assays were highly specific and both were associated with high PPV. In the present study, despite the fact that the sensitivity was 100%, MRL diagnostic kit showed lower specificity. Researchers suggest that low specificity when comparing different ELISA methods is due to longer incubation periods. Here, incubation times were identical for both assays. Moreover, the inverse relationship between sensitivity and specificity has been well documented; therefore, it is important to set the correct cut off to maximize sensitivity and/or specificity taking into consideration the target disease<sup>[18]</sup>. The reasons why specificity diminished for the MRL diagnostic kit was not investigated here. This variation may be also related to the different method of preparing dengue antigens for the assays.

To make possible appropriate diagnosis, it is essential to have some important information with regard to the origin of the sample, the vaccination status and the travel history of the patients. However, some problems with false positive results can be resolved using an IgG



detection system, if pairs of samples are available<sup>[5,19]</sup>. Alternatively, it is known that all serological results must be correlated with clinical history, epidemiological data and other data available to the attending physician in making the diagnosis of dengue fever infection.

## References

- [1] Guzman M, Kouri G, Diaz M, Llop A, Vazquez S, Gonzalez D, Castro O, Alvarez A, Fuentes O, Montada D, Padmanabha H, Sierra B, Perez A, Rosario D, Pupo M, Diaz C and Sanchez L. Dengue, one of the great emerging health challenges of the 21st century. *Expert Rev Vaccines*. 2004; 3: 511-520.
- [2] Guzman MG and Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *J Clin Virol*. 2003; 27(1): 1-13.
- [3] Guzman MG and Kouri G. Dengue: an update. *Lancet Infect Dis*. 2002; 2(1): 33-42.
- [4] Gubler DJ. The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects for the future. *Ann Acad Med Singapore*. 1998; 27(2): 227-234.
- [5] Pan American Health Organization. *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control*. Scientific publication no.548. Washington, PAHO, 1994.
- [6] Guzman MG and Kouri G. Dengue diagnosis, advances and challenges. *Int J Infect Dis*, 2004; 8(2): 69-80.
- [7] Innis B. Antibody responses to dengue virus infection. In: Gubler D and Kuno G (Eds). *Dengue and dengue haemorrhagic fever*. New York: CAB International, 1997: pp 221-243.
- [8] Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P and Hoke CH. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg*. 1989; 40(4): 418-427.
- [9] Guzman MG and Kouri G. Advances in dengue diagnosis. *Clin Diagn Lab Immunol*. 1996; 3(6): 621-627.
- [10] Kuno G, Gomez I and Gubler DJ. An ELISA procedure for the diagnosis of dengue infections. *J Virol Methods*. 1991; 33(1-2): 101-113.
- [11] Vazquez S, Saenz E, Huelva G, Gonzalez A, Kouri G and Guzman M. [Detection of IgM against the

## Acknowledgements

We thank Prof. Ernest A. Gould at CEH, Oxford, UK, for his useful suggestions and comments regarding the manuscript.

- dengue++ virus in whole blood absorbed on filter paper]. *Rev Panam Salud Publica*. 1998; 3(3): 174-178.
- [12] Vazquez S, Lemos G, Pupo M, Ganzon O, Palenzuela D, Indart A and Guzman MG. Diagnosis of dengue virus infection by the visual and simple AuBioDOT immunoglobulin M capture system. *Clin Diagn Lab Immunol*. 2003; 10(6): 1074-1077.
- [13] Rodriguez-Roche R, Alvarez M, Guzman MG, Morier L and Kouri G. Comparison of rapid centrifugation assay with conventional tissue culture method for isolation of dengue 2 virus in C6/36-HT cells. *J Clin Microbiol*. 2000; 38(9): 3508-3510.
- [14] Rosario D, Alvarez M, Diaz J, Contreras R, Rodriguez R, Vazquez S and Guzman MG. Reaccion en cadena de la polimerasa para la deteccion rapida y determinacion del serotipo de virus del dengue en muestras clinicas. [Polymerase chain reaction for rapid detection and serotyping of dengue virus in clinical samples]. *Rev Panam Salud Publica*. 1998; 4(1): 1-5.
- [15] Vazquez S, Valdes O, Pupo M, Delgado I, Alvarez M, Pelegrino JL and Guzman MG. MAC-ELISA and ELISA inhibition methods for detection of antibodies after yellow fever vaccination. *J Virol Methods*. 2003; 110(2): 179-184.
- [16] Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M and Ager AL. Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the PanBio Rapid Immunochromatographic Test for diagnosis of dengue fever in Jamaica. *J Clin Microbiol*, 1999; 37(5): 1600-1601.
- [17] Branch SL and Levett PN. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. *Clin Diagn Lab Immunol*, 1999; 6(4): 555-557.
- [18] Greiner M, Sohr D and Gobel P. A modified ROC analysis for the selection of cut-off values and the definition of intermediate results of serodiagnostic tests. *J Immunol Methods*, 1995; 185(1): 123-132.
- [19] Vazquez S, Bravo JR, Perez AB and Guzman MG. [Inhibition ELISA. Its utility for classifying a case of dengue]. *Rev Cubana Med Trop*, 1997; 49(2): 108-112.

