

Development of IgM-capture Enzyme-Linked Immunosorbent Assay for Serodiagnosis of Dengue using Beta-propiolactone-inactivated Dengue Viral Antigens

by

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Abstract

Dengue viral antigen was treated with beta-propiolactone (BPL) and used in immunoglobulin M (IgM)-capture enzyme-linked immunosorbent assay (IgM-capture ELISA) for the serodiagnosis of dengue. Dengue viruses lost infectivity after treatment with 0.2% BPL at 37°C for 10 minutes and longer. Forty-eight human serum samples, including both positives and negatives, were tested by IgM-capture ELISAs with untreated and BPL-treated dengue antigens. The results obtained by IgM-capture ELISA with BPL-treated dengue antigens were consistent with those obtained by ELISA with untreated dengue antigens. This IgM-capture ELISA system with BPL-inactivated dengue antigens is safe and more suitable for use in diagnostic laboratories.

Keywords: Dengue, IgM-capture ELISA, Beta-propiolactone.

Introduction

Dengue fever (DF)/dengue haemorrhagic fever (DHF) is a serious public health problem in many tropical and subtropical areas of the world⁽¹⁾. In recent years, epidemics of DF/DHF have dramatically increased in the Western Pacific, South-East Asia and South America because of rapid

population growth, uncontrolled urbanization and increased frequency of air travel⁽²⁾. DF/DHF will continue to be a major public health problem in the world unless effective control measures are taken. Dengue surveillance is essential to monitor epidemics, both at national and international levels. Laboratory diagnosis is necessary to confirm dengue virus infections. We have

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previously reported about dengue IgM-capture ELISA with a high sensitivity⁽³⁾.

Four types of dengue viruses were combined and used as a tetravalent dengue antigen in the IgM-capture ELISA. Dengue viruses are a human pathogen, and the use of infectious viruses as antigens can be a potential problem in the assay. In the present study, we used beta-propiolactone (BPL)-inactivated dengue viruses as antigens in IgM-capture ELISA to access its specificity and sensitivity *vis-à-vis* infectious viruses as antigens.

Materials and methods

IgM-capture ELISA was performed as previously reported^(3,4). Forty-eight serum specimens from 33 Japanese dengue-confirmed cases were used in the study. The sera were obtained in clinics and hospitals in Japan from 1998 to 2000 and sent to the Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan. The sera were stored at -80°C before use. The serum samples (0.1 ml) diluted at 1:101 were subjected to ELISA. The result was obtained with a positive to negative (P/N) ratio: P/N ratio = A_{492} , reading with the viral antigen/ A_{492} , reading with uninfected control antigen.

IgG purified from pooled dengue patients sera (H-IgG) and the flavivirus-specific monoclonal antibody, D1-4G2-4-15 (4G2)⁽⁵⁾, were used as the detection antibodies. IgG was purified by protein G affinity column chromatography (Immuno-Pure IgG Purification kit[®], Perbio Science,

Erembodegen-Aalst, Bergium), and then conjugated with horseradish peroxidase (EZ-Link Plus Activated Peroxidase kit[®], Perbio Science).

Prototype dengue viruses were inactivated by incubation with BPL at a final concentration of 0.2% (v/v) for 30 min at 37°C . The infectivity of the virus was titrated by the focus-forming method in Vero cells⁽⁶⁾.

Results and discussion

Prototype dengue virus strains: type 1, Hawaii; type 2, New Guinea C; type 3, H87 and type 4, H241, were propagated in mosquito cell clone, C6/36, and then inactivated by BPL. The infectivity of the viruses obtained before and after BPL treatment is presented in the Table. Dengue viruses lost infectivity after treatment with BPL for 10, 30 and 60 minutes (Table).

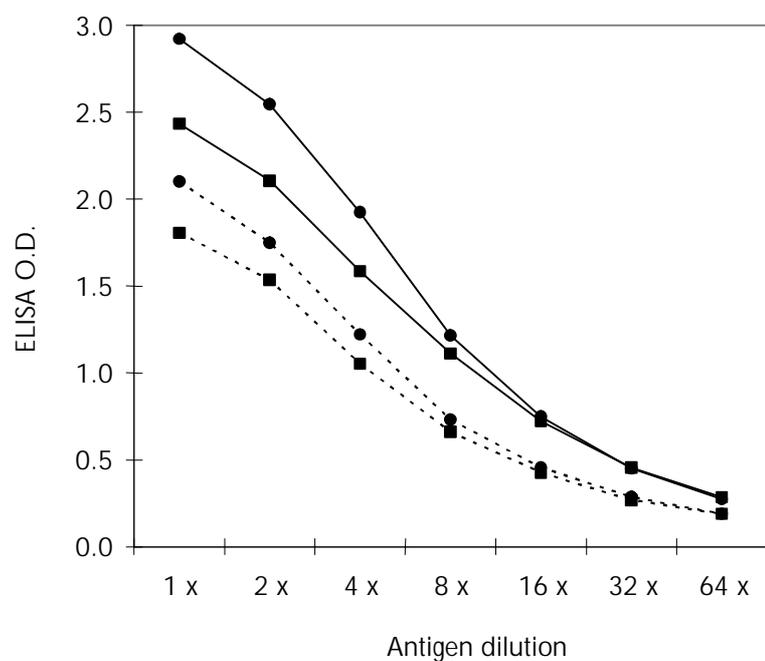
In order to prepare the assay antigen, BPL-treated antigen titration was carried out by an antibody sandwich ELISA according to the previously described method⁽³⁾. (data not shown). The tetravalent antigen was prepared by mixing equal volumes of each monovalent antigen according to the method described previously^(3,4).

The reactivity to H-IgG and 4G2 IgG was compared between the untreated and BPL-treated dengue antigens (Figure 1). The reactivity of the BPL-treated virus to H-IgG was low, while that to 4G2 IgG was high enough to be used as antigen in IgM-capture ELISA.

Table. Effect of BPL on infectivity of dengue viruses

Dengue virus serotypes	Original virus titres FFU/0.025 ml	Incubation time (min)	No BPL treatment FFU/0.025 ml	0.2 % BPL FFU/0.025 ml
DEN-1	6.9×10^5	10	6.9×10^5	$< 1.0 \times 10^2$
		30	6.2×10^5	$< 1.0 \times 10^2$
		60	5.9×10^5	$< 1.0 \times 10^2$
DEN-2	1.2×10^5	10	1.2×10^5	$< 1.0 \times 10^2$
		30	1.1×10^5	$< 1.0 \times 10^2$
		60	1.0×10^5	$< 1.0 \times 10^2$
DEN-3	1.3×10^4	10	1.3×10^4	$< 1.0 \times 10^2$
		30	1.3×10^4	$< 1.0 \times 10^2$
		60	1.3×10^4	$< 1.0 \times 10^2$
DEN-4	4.8×10^4	10	4.6×10^4	$< 1.0 \times 10^2$
		30	4.6×10^4	$< 1.0 \times 10^2$
		60	4.6×10^4	$< 1.0 \times 10^2$

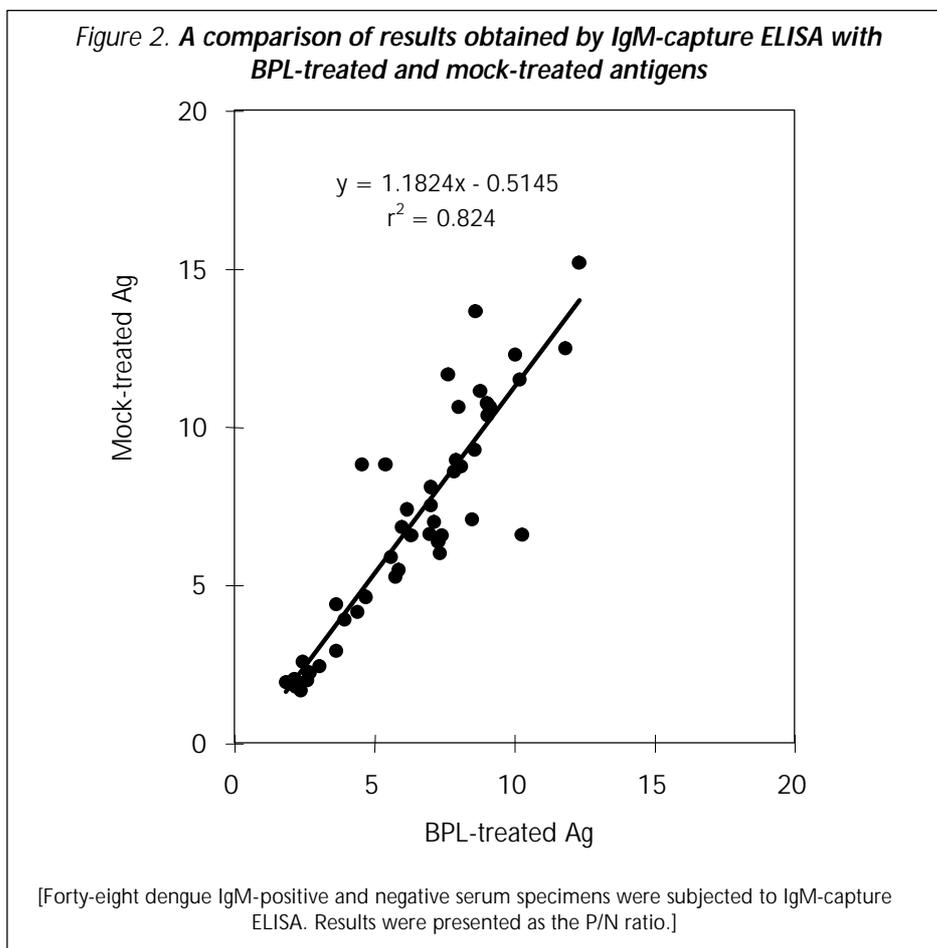
FFU: focus formin unit

Figure 1. Reactivity of the tetraivalent BPL-treated dengue viral antigen

[Dengue antigen captured on the solid phase was detected by horseradish peroxidase-conjugated H-IgG (2.5 µg/ml, square) and 4G2 IgG (2.5 µg/ml, circle). Solid lines indicate mock-treated antigen, and broken lines indicate BPL-treated antigen.]

Forty-eight human samples including positives and negatives were tested by IgM-capture ELISA with untreated and BPL-treated antigens (Figure 2). Positive(P)/negative(N) ratios obtained by the ELISA with untreated dengue antigens were somewhat

higher than those obtained by the ELISA with BPL-treated antigens. However, the P/N ratios of these IgM-positive and negative serum samples were correlated ($r^2=0.824$) between these two ELISA systems.



BPL induces alkylation of the proteins and inactivates viruses⁽⁷⁾. The reaction is completed by incubating the viruses with BPL at 37°C, and degrades *per se* depends on hydration during the incubation period. In the present study, BPL inactivated dengue viruses, but did not change the reactivity to

the monoclonal antibody, 4G2. The results obtained by the IgM-capture ELISA with BPL-treated antigen were similar to those obtained by the IgM-capture ELISA with untreated antigens. Thus, the newly-developed ELISA system maintains high specificity and sensitivity. The new system

also has the advantage in the prevention of possible laboratory infections. The IgM-capture ELISA with BPL-inactivated dengue antigen is, therefore, suitable for use in diagnostic laboratories in developing countries.

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References

1. McBride WJ and Bielefeldt-Ohmann H. Dengue viral infections: Pathogenesis and epidemiology. *Microbes Infect*, 2000, 2: 1041–1050.
2. Gubler DJ and Melzer M. Impact of dengue/dengue haemorrhagic fever on the developing world. *Adv Virus Res*, 1999, 53: 35–70.
3. Nawa M, Takasaki T, Yamada K, Akatsuka T and Kurane I. Development of dengue IgM-capture enzyme-linked immunosorbent assay with higher sensitivity using monoclonal detection antibody. *J Virol Methods*, 2001, 92: 65–70.
4. Nawa M, Yamada K, Takasaki T, Akatsuka T and Kurane I. Serotype-cross-reactive immunoglobulin M responses in dengue virus infections determined by enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol*, 2000, 7: 774–777.
5. Gentry MK, Henchal EA, McCown JM, Brandt WE and Dalrymple JM. Identification of distinct antigenic determinants on dengue-2 virus using monoclonal antibodies. *Am J Trop Med Hyg*, 1982, 31: 548–555.
6. Despres P, Frenkiel MP and Deubel V. Differences between cell membrane fusion activities of two dengue type-1 isolates reflect modifications of viral structure. *Virology*, 1993, 196: 209–219.
7. *Microbiology*, 4th ed., pp 146–147. Edited by Prescott LM, Harley JP and Klein DA. (1999), WCB McGraw-Hill, London.