

Comparative Assay on Dengue IgM–ELISA Using Reagents From 2 Sources

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Abstract: In order to distribute dengue IgM–capture ELISA to the peripheral laboratories, supply of key reagents should be established by local production. In this study, comparative IgM–capture ELISA was carried out on a total of 237 sera using key reagents from 2 sources. The current set consisted of suckling mouse brain (SMB)–derived dengue type 2 (D2) antigen and a D2 monoclonal antibody (MAb) supplied from the United States. While an alternative set was supplied by one of the authors, consisting of tissue culture–derived dengue antigen (TCA) and MAb established in USM.

When the IgM–ELISA results were compared with those by the hemagglutination–inhibition (HI) test as a gold standard, the sensitivity was 50.0% by the current set and 75.0% by the alternative set of reagents, respectively. While the specificity was 95.2% by the current set and 90.3% by the alternative set of reagents. The results showed that the alternative set of reagents can effectively be used in dengue IgM–ELISA and is significantly more sensitive than the current set of reagents (Chi SQR=12.26, $P < 0.01$) at less than 1% risk. However there is no significant difference in specificity between the two reagents (Chi SQR=2.45, $0.5 > P > 0.1$) at 10% risk.

Key words: Dengue, IgM–ELISA, reagents

INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) have been major health problems in many tropical countries especially in Southeast Asia (Halstead, 1966; 1980; 1992; World Health

Organization, 1966). Till the advent of modern serological techniques, the haemagglutination–inhibition (HI) test was used in the laboratory diagnosis of dengue (Clarke and Casals, 1958; Shope and Sather, 1979; World Health Organization, 1986). This test, which is still used as the gold standard, is relatively laborious, time–consuming and requiring paired serum specimens. Introduction of the IgM–capture ELISA was a significant advance in the dengue serology (Burke, 1983; Bundo and Igarashi, 1985; Lam *et al.*, 1987; Innis *et al.*, 1989). However, it still needs improvements especially in the supply of key reagents such as assay antigens and dengue monoclonal antibodies (MAb), before the test can be routinely used in the peripheral laboratories. A simplified version of dengue antibody assay in a dot–blot test is currently commercially available, but it appears to lack sensitivity to detect primary dengue cases and is also quite expensive (Fang *et al.*, 1992).

In the Virology Division, Institute for Medical Research (IMR), Malaysia, in–house dengue IgM–capture ELISA was established according to the method of Lam *et al.* (1987), which greatly increased efficiency and sensitivity in the serological diagnosis on dengue. Two major reagents in this test are dengue antigens and dengue MAb. The antigens are prepared from infected suckling mouse brains (SMB) by sucrose–acetone extraction (Clarke and Casals, 1958), and dengue MAb is supplied on a limited basis by kind courtesy from Centres for Disease Control and Prevention, Division of Vector–Borne Infectious Diseases, Ft. Collins, CO, USA. The limitation in the supply of these 2 reagents has made it difficult to decentralize the dengue IgM–ELISA to peripheral laboratories in Malaysia.

One of the authors to this paper (Jane Cardoso), raised the dengue antigen in tissue culture (TCA), and dengue MAb in her own laboratory. If these reagents can be utilized as an alternative set in the dengue IgM–ELISA, the results will greatly facilitate the decentralization of the test, leading to the better efficiency in the laboratory diagnosis and epidemiological surveillance, and eventually to the effective control of dengue.

MATERIALS AND METHODS

Antigens: Two sources of antigens were used: suckling mouse brain–derived antigen (SMB), and tissue culture–derived antigen (TCA). The SMB antigen of dengue type 2 virus (D2) was prepared in the Division of Virology, IMR, by sucrose–acetone extraction method (Clarke and Casals, 1958). The TCA was prepared as a cocktail of 4 types of dengue virus antigens prepared in the infected C6/36 cell cultures (Igarashi, 1978).

Monoclonal antibody (MAb): Two kinds of MAb used in this study were: anti–D2 MAb 3H5–1–21–B71541 kindly provided by Dr Nick Karabatsos, CDC, Ft. Collins, CO, and dengue cross–reactive MAb MF4/5/A5/C3–3 provided by one of the authors (Jane Cardoso).

Serum specimens: A total of 237 paired sera were previously examined by the HI test according to the standard method (Clarke and Casals, 1958; Shope and Sather, 1979; World Health Organization, 1986), showing 92 positives and 145 negatives. The convalescent phase sera from each pair were used in this study of IgM–ELISA after storage at –20°C.

IgM–capture ELISA: The modified method of Lam *et al.* (1987) was used with reaction period

of 1 hour at 37°C except the last step. A 96-well U-bottom plate was sensitized with anti-human IgM (Dakopatts, Denmark). After washing, the plate was reacted with test sera diluted 1:100 in PBS-Tween in duplicate wells for each specimen, along with a standard positive and a standard negative specimens. After incubation and washing, the plate was successively reacted with dengue antigen, followed by dengue MAb, then horseradish peroxidase-conjugated anti-mouse IgG (Bio Rad, Canada), which is free from cross reaction to human immunoglobulins. After the last washing, peroxidase reaction was carried out for 30 minutes using a substrate solution of *o*-phenylenediamine and hydrogen peroxide. The peroxidase reaction was stopped by adding 4N sulphuric acid, and absorbance at 490 nm was measured by a Dynatech MR5000 ELISA reader using 630 nm as a reference wavelength.

Interpretation of the results: The 92 HI positives were classified into the following categories according to the WHO criteria;

1. Primary infection = 4-fold rise or more in titre, interval between specimens of ≥ 7 days, maximum titre of ≤ 1280 .
2. Secondary infection = 4-fold rise or more in titre, interval between specimens any number of days, maximum titre of ≥ 2560 .
3. Presumptive secondary infection = No 4-fold rise in titre, interval between specimens any number of days, maximum titre of ≥ 2560 .
4. Definite infection = 4-fold rise or more in titre, interval between specimens of < 7 days, maximum titre of ≤ 1280 .

The positive to negative ratio (P/N ratio) was calculated by dividing the average absorbance in duplicate sample wells by the average absorbance in the negative standard. Any specimens with P/N ratio equal to or greater than 2.0 were considered positive. The sensitivity and specificity of the tests were calculated by the following formula:

$$\text{Sensitivity} = \frac{\text{Number of true positive}}{\text{Number of true positive} + \text{false negative}}$$

$$\text{Specificity} = \frac{\text{Number of true negative}}{\text{Number of true negative} + \text{false positive}}$$

RESULTS AND DISCUSSION

The results of comparative IgM-ELISA using 2 sets of reagents were shown in Tables 1 and 2. The sensitivity and specificity of the tests were calculated to see the significance of their difference. The alternative set of TCA antigen and MF4-MAb showed sensitivity of 75.0%, which was higher than the value (50.0%) for the set of SMB antigen and D2-MAb which is currently used in IMR. This difference was statistically significant (CHI SQR = 12.26, $p < 0.01$) at less than 1% risk. While the TCA antigen and MF4-MAb showed specificity of 90.3% which is less than the value (95.2%) for SMB antigen and D2-MAb. However this difference was not statistically significant (CHI SQR = 2.45, $0.5 > P > 0.1$) at 10% risk.

Table 1 Comparison of the IgM-ELISA using SMB antigen and D2-MAb with the HI as standard.

		IgM-ELISA with SMB antigen and D2-MAb		
		positive	negative	total
HI	positive	46	46	92
	negative	7	138	145
total		53	184	237

Sensitivity = $46/92 \times 100 = 50.0\%$ Specificity = $138/145 \times 100 = 95.2\%$ **Table 2** Comparison of the IgM-ELISA using TCA antigen and MF4-MAb with the HI as the standard.

		IgM-ELISA with TCA antigen and MF4-MAb		
		positive	negative	total
HI	Positive	69	23	92
	negative	14	131	145
total		83	154	237

Sensitivity = $69/92 \times 100 = 75.0\%$ Specificity = $131/145 \times 100 = 90.3\%$

There is a significant difference in the percentage of positives for definite infection (see Table 3) between SMB antigen/D2-MAb, 45.9% and, TCA/MF4-MAb, 71.6% (CHI SQR=10.07) at less than 1% risk. Although the day of interval between samples were mostly not available to us, the inability of SMB antigen/D2-MAb to pick up more definite infection in this case is that most of the samples were probably primary infection. The limitations being that the SMB antigen is a D2 antigen and the monoclonal antibody used was D2 specific monoclonal antibody which fails to pick up primary infection of other serotypes. However there is no significant difference in the percentage of positives for secondary infection between the two reagents; SMB antigen/D2-MAb, 70.6% and TCA antigen/MF4-MAb, 88.2% (CHI SQR = 1.62, $0.5 > P > 0.1$) at 10% risk.

Table 3 Classification of HI positive samples.

	Total Number	SMB antigen/ D2-MAb	TCA antigen/ MF4-MAb
Definite infection	74	34 (45.9%)	53 (71.6%)
Secondary infection	17	12 (70.6%)	15 (88.2%)
Presumptive secondary infection	1	0 (0%)	1 (100%)
Total	92	46	69

A suitable laboratory test for early confirmation of symptomatic dengue or undifferentiated fever cases has been shown to be an effective first line screening test (Burke, 1983; Bundo and Igarashi, 1985; Lam *et al.*, 1987; Innis *et al.*, 1989). However, its use has been confined to reference laboratories since some reagents were not easily available. Our study showed that the TCA antigen and MF4–MAb can be used with a better sensitivity without losing significant specificity of currently used SMB–derived D2 antigen and MAb solicited from the USA. The next step in this study will be to use a cocktail of antigen produced in IMR since all four types of dengue antigen are produced here and a cross–reacting monoclonal antibody. With strong possibility to produce these reagents in quantities locally, the modified dengue IgM–capture ELISA could be introduced to the peripheral health centres. This decentralization, when carried out through technical transfer from the central reference laboratory such as IMR, will greatly enhance laboratory diagnosis, surveillance and control of DF/DHF in Malaysia.

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