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Multicentre evaluation of dengue IgM dot enzyme immunoassay

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Abstract

Background: The traditional methods used in the diagnosis of dengue infection do not lend themselves to field application. As such, clinical specimens have to be sent to a central laboratory for processing which invariably leads to delay. This affects patient management and disease control. The development of the dengue IgM dot enzyme immunoassay has opened up the possibility of carrying out the test in peripheral health settings.

Objectives: This multicentre study was conducted to evaluate a new, commercial nitrocellulose membrane based IgM capture enzyme immunoassay.

Study design: The sensitivity and specificity of the test were compared with in-house dengue IgM enzyme-linked immunoassays routinely performed by each of the selected centres. Known positive and negative dengue specimens, as well as specimens from non-dengue cases, were included in the evaluation.

Results: Based on 402 specimens tested by the six centres, the sensitivity was 92.1% and specificity 88.1%, with an overall agreement of 92.8% when compared with IgM EIA assays performed on microplates.

Conclusions: The results suggest that this commercial kit has a role to play in the diagnosis of dengue infection, especially in peripheral health settings.

Keywords: Dengue; IgM capture dot enzyme immunoassay; Laboratory diagnosis

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1. Introduction

Methods which are currently available for the laboratory diagnosis of dengue infections do not lend themselves easily to field application. As such, clinical specimens have to be despatched to central laboratories for processing. This will invariably create problems and lead to delay in diagnosis, which in turn will affect patient management and disease control. Cardosa et al. (1988b) reported the development of a dot enzyme immunoassay for the detection of dengue antibodies. This formed the basis of a commercial dengue blot kit in which the viral antigens are bound to nitrocellulose membranes instead of microplates, and the antibodies are visualized using Protein A which is labelled with the enzyme horseradish peroxidase. This kit has been evaluated by various workers and was found to be particularly useful in detecting secondary dengue infections (Cardosa et al., 1988a; Fang et al., 1992) and less so for primary dengue infections.

Using the same principle, Cardosa et al. (1995) developed a nitrocellulose membrane based IgM capture enzyme immunoassay (MAC DOT) for diagnosis of dengue virus infections. The MAC DOT was tested on several sample sets including a retrospective study of 119 patients from Thailand with confirmed dengue infection. The sensitivity of the test was shown to be 94% taking only admission sera into consideration but rose to 99% when both an admission and a discharge specimen were considered. Other sample sets confirmed the high sensitivity and a study of 494 unselected febrile children showed that the specificity of the MAC DOT was 98%.

A multicentre evaluation of the MAC DOT was undertaken at the request of the World Health Organization, Geneva, and coordinated by the WHO Collaborating Centre for DF/DHF, Kuala Lumpur, Malaysia.

This is the report of the evaluation study prepared by the WHO Collaborating Centre.

2. Materials and methods

2.1. Participating centres

Laboratories located in the following six centres participated in the evaluation: Malaysia (Centre 1), Singapore (Centre 2), Tahiti (Centre 3), Japan (Centre 4), Puerto Rico (Centre 5) and Thailand (Centre 6).

3. MAC DOT kits

The MAC DOT kits were provided free by the manufacturer, Venture Technologies (Malaysia), through the courtesy of Prof. M.J. Cardosa, University Malaysia Sarawak, Malaysia. The MAC DOT is similar to the IgM capture ELISA or MAC ELISA (Lam et al., 1987; Innis et al., 1989) with the important difference being the use of nitrocellulose membranes instead of 96 well microtitre plates. The dengue antigens used in the MAC DOT consist of all four dengue serotypes prepared from C6/36 infected cells and a dengue group reactive monoclonal antibody was used for the detection of bound antigens. The full details of the test are contained in the paper by Cardosa et al. (1995).

3.1. In-house dengue IgM ELISA

The in-house dengue IgM ELISA used by Centre 1 has been published (Lam et al., 1987). Briefly, 96-well microplates coated with rabbit anti-human IgM were reacted with test sera. The antigen used was dengue 2, prepared in suckling mouse brains and the monoclonal antibody (WRAIR-2 3H5) to dengue 2 was used for detection of bound antigen.

In Centre 2, the method of Cardosa et al. (1995) was used. The main difference between this IgM ELISA format and that used in Centre 1 was the use of a cocktail of all four types of dengue antigens prepared in C6/36 cells. The bound antigen was detected by a group reactive monoclonal 4G2 antibody obtained from the American Type Culture Collection hybridoma line.

In Centre 3, an in-house dengue IgM antibody capture ELISA was used (Chungue et al., 1989). Dengue 1 and dengue 3 antigens prepared in suckling mice were used and the detecting anti-

body was homologous mouse hyperimmune ascitic fluid.

Centre 4 used the method described by Bundo and Igarashi (1985). Dengue 2-infected C6/36 cell culture fluid was used as antigen and the detecting antibody was a conjugated anti-flavivirus human IgG.

Both Centres 5 and 6 used the method of Innis et al. (1989). Tetravalent dengue antigen diluted in phosphate buffered saline plus 20% acetone extracted normal human serum was used followed by an overnight incubation step. The detecting antibody was horseradish peroxidase conjugated human anti-flavivirus IgG also diluted in 20% acetone extracted normal human serum.

3.2. Specimens

3.2.1. Centre 1

The serum specimens used in the Malaysian study consisted of 87 samples from 70 patients. Of these 87 samples, 52 were dengue IgM positive and 35 IgM negative. Among the dengue IgM negative samples, 2 each were from confirmed rubella, measles and typhoid fever cases.

3.2.2. Centre 2

The serum specimens used in the Singapore study consisted of 72 specimens. Of these, 39 were dengue IgM positive by ELISA and 33 were IgM negative.

3.2.3. Centre 3

The serum samples used in the Tahiti study consisted of 68 samples. Of the 68 samples, 49 were dengue IgM ELISA positive including 12 which were weakly positive and 19 were IgM negative.

3.2.4. Centre 4

The serum samples used in the Japan study consisted of 63 samples from Myanmar. Of these, 58 were IgM positive and 5 were IgM negative.

3.2.5. Centre 5

The serum specimens used in the San Juan study consisted of 40 samples. Of these, 20 were low to medium dengue IgM ELISA positive and 20 were dengue IgM negative. This was the only centre where the specimens were not tested blind.

3.2.6. Centre 6

The serum specimens used in the Thailand study consisted of 72 samples from 41 patients. Of these, 46 were IgM positive and 26 IgM negative. Among the negative samples, there were 7 from confirmed Japanese encephalitis and 4 were malaria positive samples.

3.3. Sensitivity and specificity

The sensitivity and specificity of the MAC DOT over IgM ELISA were calculated by dividing the number of MAC DOT positive or negative samples by the total positive or negative samples, respectively. The overall agreement was the sum of the positive and negative samples by both methods divided by the total number of samples tested.

4. Results

Table 1 presents a summary of the results from each of the six centres and Table 2 summarizes the sensitivity, specificity and overall agreement of the MAC DOT compared with the IgM ELISA.

Of the 87 samples tested in Centre 1 (Malaysia), 51 were both MAC DOT and IgM ELISA positive and 30 were MAC DOT and IgM ELISA negative, including two samples each from confirmed rubella, measles and typhoid fever. One sample was IgM ELISA positive and MAC DOT negative, while 5 were MAC DOT positive and IgM ELISA negative. Of these 5 samples, 4 were false negative by IgM ELISA since they were found subsequently to be presumptive positive by haemagglutination inhibition with HI titres greater than 1:1280. The overall agreement was 93.1% with a sensitivity of 98.1% and specificity of 85.7% (Table 2). If we take into consideration the 4 false negative samples, the specificity would be higher.

Table 1
Comparison of MAC DOT with IgM ELISA

Centre	MAC DOT	ELISA		
		Positive	Negative	Total
1	Positive	51	5	56
	Negative	1	30	31
	Total	52	35	87
2	Positive	37	0	37
	Negative	2	33	35
	Total	39	33	72
3	Positive	42	1	43
	Negative	7	18	25
	Total	49	19	68
4	Positive	57	2	59
	Negative	1	3	4
	Total	58	5	63
5	Positive	16	0	16
	Negative	4	20	24
	Total	20	20	40
6	Positive	44	3	47
	Negative	2	23	25
	Total	46	26	72

Of the 72 samples tested in Centre 2 (Singapore), 37 were both MAC DOT and IgM ELISA positive and 33 were MAC DOT and IgM ELISA negative. There were 2 samples which were IgM ELISA positive but MAC DOT negative. The overall agreement was 97.2% with a sensitivity of 94.9% and specificity of 100%.

Of the 68 samples tested in Centre 3 (Tahiti), 42 were both MAC DOT and IgM ELISA positive and 18 were both MAC DOT and IgM ELISA negative. Seven samples were IgM ELISA positive and MAC DOT negative and one was MAC DOT positive but IgM negative. The overall agreement was 88.2% with a sensitivity of 85.7% and specificity of 94.7%. Discrepant results occurred mostly with specimens which were weakly positive by either IgM ELISA or by MAC DOT.

Of the 63 samples tested in Centre 4 (Japan), 57 were both MAC DOT and IgM ELISA positive and 3 were MAC DOT and IgM ELISA negative. There was 1 sample which was IgM ELISA positive and MAC DOT negative and 2 which were MAC DOT positive but IgM negative. The overall agreement was 95.2% with a sensitivity of 98.3% and specificity of 60%. The low value for specificity was due to the small sample size of negative specimens.

Of the 40 samples tested in Centre 5 (Puerto Rico), 16 were both MAC DOT and IgM ELISA positive and 20 were MAC DOT and IgM ELISA negative, 4 samples were IgM ELISA positive but MAC DOT negative. The overall agreement was 90% with a sensitivity of 80% and specificity of 100%. The sensitivity could have been higher if the sample size included strongly positive specimens.

Of the 72 samples tested in Centre 6 (Thailand), 44 were both MAC DOT and IgM ELISA positive and 23 were MAC DOT and IgM ELISA negative. Among the 23 negative samples, there were 7 confirmed Japanese encephalitis cases and 4 malaria positive cases. Of the 2 samples which were IgM ELISA positive but MAC DOT negative, one was a definite false negative since the sample was also positive by HI and the other was a false dengue IgM ELISA positive due to cross-reaction with JE IgM ELISA in a confirmed JE

Table 2
Summary of results from the six centres

Centre	No. tested	% Sensitivity	% Specificity	% Agreement
1	87	98.1	85.7	93.1
2	72	94.9	100.0	97.2
3	68	85.7	94.7	88.2
4	63	98.3	60.0	95.2
5	40	80.0	100.0	90.0
6	72	95.7	88.5	93.1
Total	402	92.1	88.1	92.8

infection. Of the 3 samples which were IgM ELISA negative and MAC DOT positive, follow-up specimens from the three patients were subsequently IgM ELISA positive, indicating that MAC DOT was able to detect dengue IgM earlier than IgM ELISA. The overall agreement was 93.1% with a sensitivity of 95.7% and specificity of 88.5%. If we take into consideration the three patients whose early samples were IgM ELISA negative and became positive on follow-up samples, the overall agreement would then be 97.2% with a sensitivity of 95.9% and a specificity of 100%.

Table 2 summarizes the results from the six centres based on a total of 402 samples. The sensitivity of MAC DOT as compared with the IgM ELISA ranges from 80 to 98.3% with an average of 92.1%. The specificity ranges from 60 to 100% with an average of 88.1%. Two centres had 100% specificity and Centre 4 (Japan) had a specificity of 60% based on only 5 negative samples. The overall agreement ranges from 88.2 to 97.2% with an average of 92.8%. The only centre which was below 90% was Centre 3 (Tahiti).

5. Discussion

All six centres did not encounter any difficulty with the technical performance of the MAC DOT. There were some problems with the interpretation of the colour change since the reading was subjective and the colour was quite faint, especially with weak IgM ELISA positive samples. The MAC DOT was more labour intensive when handling many specimens as compared to the conventional IgM ELISA. It also took slightly longer to carry out than some of the IgM ELISA formats used in this study as it required an overnight incubation step. Shorter incubation could result in loss of sensitivity and specificity (M.J. Cardosa, pers. comm.).

Although the six centres used slightly different dengue IgM ELISA format, with different reagents, incubation times and cut-off criteria, the results obtained were in fairly close agreement. Four of the centres achieved sensitivity equivalent to that reported by Cardosa et al. (1995). Centre 5 (San Juan) reported a lower sensitivity since the samples tested consisted of only low to medium dengue IgM ELISA positive samples. Four centres reported specificity of over 80%. Centre 4 (Japan) reported specificity of only 60% but this was based on very few negative samples. There was no false positive with non-dengue cases such as Japanese encephalitis, rubella, measles and typhoid fever. Four malaria positive samples tested in Centre 6 (Thailand) were MAC DOT negative but of 30 malaria positive blood samples tested in Centre 1 (Malaysia) by MAC DOT, 7 (23.3%) were found to be weakly reactive (data not included) and negative by IgM ELISA. The reason for this cross-reactivity is not apparent.

The MAC DOT appeared to be more sensitive than dengue IgM ELISA since three confirmed dengue cases tested in Centre 6 (Thailand) were IgM ELISA negative in the acute samples but were positive by MAC DOT. In Centre 1 (Malaysia), 4 out of 5 high-titred HI dengue samples were positive by MAC DOT but negative by IgM ELISA. Since there are a number of such single samples submitted for dengue diagnosis, the MAC DOT has an advantage over the IgM ELISA used in Centre 1.

With the development of this commercial kit, it should now be possible to decentralize the diagnostic service to peripheral health settings where laboratories processed relatively few specimens.

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