Use of dengue blot in dengue diagnosis: the Malaysian experience

R FANG BSc (Hons.), MSc, M SINNIAH MBBS, MSc and LS KUEN

Division of Virology, Institute for Medical Research, Kuala Lumpur

Abstract

Dengue fever/Dengue haemorrhagic fever (DFPHF) has been a public health problem in Malaysia with an endemic level of about 7 per 100,000 population per year. In 1990, Malaysia experienced its most severe outbreak of DF/DHF with a record total of 5,590 cases referred to the Division of Virology, Institute for Medical Research (IMR). Of these, 1,880 were confirmed serologically to be DF/DHF. The conventional serological procedure, the Haemagglutination Inhibition (HI) test, for the diagnosis of DFPHF is cumbersome and causes delay in diagnosis. Another problem associated with the HI test has been that it has often been difficult to obtain a second convalescent serum sample for an accurate diagnosis. This has raised an urgent need to establish a "rapid" test for diagnosis of DF/DHF. As such the authors recently carried out an evaluation of a newly available commercial rapid test, namely, the Dengue Blot Assay (Diagnostic Biotechnology Singapore Pte Ltd). The test is intended for use in laboratory confirmation of dengue virus infection. The evaluation was to determine if the test could be utilised as a routine laboratory test and to establish its sensitivity and specificity. Over 400 samples were tested against the Dengue Blot Assay. Results were checked against an in-house Dengue IgM ELISA and HI assay. Preliminary results indicate that the sensitivity and specificity of the Dengue Blot is satisfactory. Our results also indicate that the Dengue Blot has a useful role to play in a routine laboratory especially since it provides rapid results on single serum samples thereby reducing the workload in a busy diagnostic laboratory.

Key words: Dengue, technology, evaluation

INTRODUCTION

Dengue fever/Dengue haemorrhagic fever (DF/DHF) is an endemic problem in Malaysia, with an endemic level of about 7 per 100,000 population per year. In 1990, the country experienced its most severe outbreak of DF/DHF to date with a record total of 5,590 cases referred to the Division of Virology, Institute for Medical Research (IMR), Kuala Lumpur.' Of these, 1,880 were confirmed serologically by the Haemagglutination Inhibition (HI) assay[2] to be DF/DHF.

Although a "rapid" assay in the form of the Dengue IgM ELISA[3] has been recently introduced as a routine test at the IMR, the HI assay remains the "gold standard" for the serological diagnosis of DF/DHF. However, the problems associated with the HI are well known, in particular, that associated with obtaining a second convalescent serum sample, the inability of obtaining a definitive diagnosis with a single serum sample, and the time factor associated with turning around results to the referring hospital. This problem is compounded in the Malaysian context by the fact that the IMR currently serves as the only viral diagnostic laboratory for all government hospitals under the Ministry of Health in the country. There, therefore, exists an urgent need for the introduction of a "rapid" and simple test for the diagnosis of DF/DHF, especially for an assay which can be easily used at the hospital level.

Thus, a preliminary evaluation of a newly available commercial "rapid" test, the Dengue Blot (DB) assay (Diagnostic Biotechnology Singapore Pte Ltd), was recently carried out by the IMR. The DB assay utilises a cocktail of all 4 dengue viral antigens bound onto nitocellulose membranes. The antigens used in this assay are raised in cell culture. These membranes are then incubated with sera from suspected dengue patients, and antibodies to dengue, if present, are visualised using Protein A labelled with horse radish peroxidase (HRP).

The evaluation was carried out to determine if the assay could be used as a routine laboratory test in hospital laboratories and to establish its sensitivity and specificity in the local context.

Address for correspondence and reprinf requests: R. Fang. Department of Microbiology. Royal Hobart Hospital. PO Box l06J L, Hobart 700l, Tasmania, Australia.

MATERIALS AND METHODS

Nature of specimens examined

Paired sera from 303 patients were examined. The majority of the acute samples were collected within the first 5 days following infection. The convalescent samples were collected, on average, 5 days after the first sample.

All serum samples were stored at 4°C until tested. No preservatives were added.

All serum samples were also tested by the HI assay and the Dengue IgM ELISA. The HI test utilised dengue 1 (DEN-1), dengue 2 (DEN-2), dengue 3 (DEN-3), dengue 4 (DEN-4), Japanese encephalitis (JE) virus, and Sindbis antigens. All these antigens were prepared by suckling mouse brain (smb) extraction using sucrose-acetone. A smb DEN-2 antigen was used for the Dengue IgM ELISA. The procedure for the IgM assay was as described by Lam and colleagues.[3]

No virus isolations were attempted on the acute samples due to the inappropriateness of the samples received.

Dengue Blot assay

Paired sera were tested under code in strict accordance with the manufacturer's instructions. All reagents were supplied with the kit.

Briefly, sera were diluted 1:100 with diluent buffer prior to incubation with nitrocellulose membranes for 1 hour at room temperature (RT) on a rocking platform. After incubation, the membranes were washed with washing buffer. Protein A conjugated with HRP was then incubated with the membranes for 1 hour at RT. The membranes were then washed and reacted with substrate and colour development solution. Results were only considered as valid if the positive and negative controls (provided with the kit) gave an acceptable reading in accordance with the manufacturer's criteria. Interpretation of results were in strict accordance with the manufacturer's instructions, and were reported off as either presumptive positive, inconclusive, negative or invalid. An inconclusive result was noted when the colour intensity of the spot developed from the test serum was less than that given by the positive control serum provided. Interpretation of results was carried out independently by two personnel.

RESULTS

The patients examined were classified into 5 groups on the basis of their HI results and the World Health Organisation (WHO) criteria.[4]

These groups were: Negative, No rise in tire, No significant rise in Titre, Presumptive Positive to Flavivirus, and Positive to Flavivirus Infection. The IMR uses a titre of of 1:1280 or greater, instead of 1:2560, to determine a "Presumptive Positive to Flavivirus Infection". The reason for this was that previous studies have indicated that a titre of 1:1280 is acceptable in the Malaysian context as a base line for secondary infections.[5]

Of the 303 patients, 96 were classified, as "Negative" on the basis of the HI test. Of these, the DB assay correctly identified 90, with 4 patients being rated as Inconclusive and with sera from 2 patients giving Invalid results (Table 1).

95 patients were reported as "Positive to Flavivirus infection" on the basis of the HI result. DB results correlated with 71 of the patients' results, with 9 being reported as Inconclusive and with 13 reported as Negative. There were 2 pairs of Invalid results (Table 1).

A further 10 patients were classified as "Presumptive Positive" by the HI. All 10 patients were also classified as Dengue Positive by the DB assay.

The remaining patients were either classified as "No Rise in Titre" (n=48) or as "No significant Rise in Titre" (n = 54). Correlation of DB results to these two groups is provided in Table 1.

Sera from 5 out of the 303 patients tested gave invalid results by this assay, i.e., the serum antibodies reacted with non-dengue cell culture constituents in the negative control antigen dot on the nitrocellulose membrane.

No discrepancy in the interpretation of results was noted between the laboratory personnel involved in the evaluation.

DISCUSSION

The DB assay is a fairly simple, straight forward test to use as all important reagents and test materials were provided with the kit. All other equipment required for the test should normally be readily available in the laboratory. One possible shortcoming in the packaging of the kit was that only one tray (required to contain the test samples) was supplied. This limited the initial test run to 8 samples with one positive and one negative control. However, it is felt that this is not an insurmountable problem if a larger number of samples are to be tested as additional trays can be provided by the local agent.

As this assay is technically simpler to per-

TABLE 1: Comparison of Dengue Blot with the Haemaglutination Inhibition Assay

Haemagglutination inhibition assay	Dengue Blot			
	Positive	Negative	Inconclusive	Invalid
Negative n=96	0	90	4	2
Positive n=95	71	13	9	2
Presumptive positive n=10	10	0	0	0
No rise in titre n=48	5	24	18	1
No significant rise in titre n=54	4	35	15	0

Total sample size: 303 paired sera.

form than the HI or the IgM ELISA, it is the authors' impression that this assay has the potential to be used at the government hospitals in the Malaysian context. It is felt that the assay could be used by a skilled medical laboratory technologist at the state government hospital level with a minimum of fuss. Some additional specific training would, however, be required prior to the establishment of the assay.

However, it should be noted that the results obtained indicated that the DB assay has a sensitivity of 77.14% (based on a sample size of 105 paired sera) with a specificity of 93.75% (based on a sample size of 96). This compares with a sensitivity of 87.61% and a specificity of 91.66% for the Dengue IgM ELISA which is currently in routine use at the IMR. (The sensitivity and specificity of the ELISA is based on the same sample population as that for the Blot as shown in Table 2).

Previous reports have indicated that this assay is particularly useful in detecting secondary infections to dengue[6,7,8] and that the kit would be of use in areas where the majority of dengue infections are of the secondary type. However, in this study, the majority of positive samples obtained were primary infections (95 out of 105 patients). The Dengue Blot picked a total of 71 of these patients as positive, with an additional 9 patients' samples giving inconclusive results. This meant that with primary infections the Blot had a sensitivity of 74.73% compared with a sensitivity of 86.32% for the IgM ELISA which

is in routine use at the IMR. The IMR intends to carry out a more comprehensive study on the suitability of the assay for secondary infections.

Our data also indicate that the potential for false negative results exist and that care should be taken in the interpretation of negative results. The present evaluation did not take into consideration potential cross reactivity of the kit with other infections. However, assuming that specificity is a measure of the ability of a kit to detect all true negative cases as negative, this study showed that the Blot has a specificity of 93.75%. Data from other studies indicate that little cross reactivity occurs.

TABLE 2: Comparison of Dengue IgM ELISA with the Haemagglutination Inhibition Assay

HI	IgM negative	IgM positive
Negative (n=96)	88	8
No rise in titre (n=48)	38	10
No significant rise in titre (n=54)	33	21
Presumptive positive (n=10)	0	10
Positive (n=95)	13	82

Total sample size: 303 paired sera

The results of this study also indicated that neither the DB nor the IgM ELISA significantly confirmed more positive cases on acute serum samples alone. As such, the DB assay cannot be taken to serve as a acceptable rapid diagnostic test to confirm dengue infection upon hospitalisation. There appears to be no single assay at this point in time which can fulfill this role, and a battery of tests, including virus isolation, is needed to accurately confirm dengue infection.

However, inspite of its limitations, it is felt that the Dengue Blot assay could be useful as a first line screen in Malaysian hospitals in view of its simplicity and ease of use. This would be especially so in hospital laboratories where there are problems in establishing the IgM ELISA assay. In such situations, where the DB assay is utilised, any problem sera or paired sera that are negative or inconclusive should then be referred to the IMR or the WHO Collaborating Laboratory for Arbovirus Infections at the University of Malaya for confirmation and a definitive diagnosis.

ACKNOWLEDGEMENTS

REFERENCES

1. Institute for Medical Research, Annual Report, 1991, IMR, Kuala Lumpur, Malaysia.
2. Clarke DH, Casals J. Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. Am J Trop Med Hyg 1958; 7: 561-73.
3. Lam SK, Devi S, Pang T. Detection of specific IgM in dengue infection. Southeast Asian J Trop Med Pub Hlth 1987; 18 (4): 532-7.
4. Technical Advisory Group on Dengue Haemorrhagic Fever/Dengue Shock Syndrome. Dengue Haernorrhagic Fever Diagnosis, Treatment and Control, 1986; World Health Organisation, Geneva, Switzerland.
5. Lim TW. Laboratory diagnosis of dengue. In: Rudnick A, Lim TW, Eds: Dengue Fever Studies in Malaysia, Bulletin No.23, IMR, 1986: 169-80.
6. Lai OF, Chan YC, Ngoh BL, Tan HC, Chan L. Rapid diagnosis of dengue by a commercial kit (Dengue Blot). Proceedings of the VIII International Congress of Virology, Berlin, August 26 - 31, 1990.
7. Chan YC, Lai OF, Ngoh BL, Tan HC, Chan L. Dengue diagnosis using a commercial kit (Dengue Blot): a prospective study. Proceedings of the VIII International Congress of Virology, Berlin, August 26-31, 1990.
8. Cardosa MJ. Diagnosis and surveillance of dengue virus infections: gold Standards and new directions. Malays J Pathol 1989; 11: 7-10.