COMPARATIVE EVALUATION OF VARIOUS COMMERCIAL ASSAYS FOR DIAGNOSIS OF DENGUE FEVER

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Abstract. Dengue fever (DF) is endemic in India and dengue hemorrhagic fever (DHF) has been reported with increasing frequency in the last decade. We evaluated three commercial assays for detection of antibodies to dengue virus, to assess their performance in a diagnostic laboratory. Sera from 58 patients collected during a febrile outbreak in New Delhi in 1997 were studied. The methods evaluated were MRL Diagnostic Dengue Fever Virus IgM Capture ELISA, Pan Bio Dengue Duo IgM and IgG Capture ELISA and Pan Bio Rapid Immunochromatographic test. The MRL ELISA correctly identified 97.8% (43 of 44) of samples as dengue positive while the Pan Bio Duo ELISA and Pan Bio RIT identified 95.45% (42 of 44). The sensitivities of both Pan Bio Duo ELISA and Pan Bio RIT for primary dengue and secondary dengue were 100% and 93.54% respectively. The specificity of three assays were MRL IgM ELISA 100%, Pan Bio Duo ELISA 92.8% and Pan Bio RIT 85.7%.

INTRODUCTION

Dengue fever (DF) is self limiting viral disease distributed throughout the tropical areas of the world with approximately 2.5 billion people living in dengue endemic area area (Halstead, 1980). Currently dengue fever is responsible for significant morbidity and mortality that is much more than any other arboviral disease. While occurrence of classical dengue fever has been known in India for decades with occasional reports of hemorrhagic manifestations, dengue hemorrhagic fever (DHF) have been reported with increasing frequency in India only since 1988 (ICMR, 1980). Delhi has witnessed recent epidemic of DHF in 1996 (Seth *et al*, 1996). The increasing importance of dengue fever has underlined the importance of early detection as an aid to control of spread and management of the disease. Virus isolation methods are slow and labor intensive. Moreover, they are helpful in the first week of illness, when there is viremia (Gubler and Clark, 1995).

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Antidengue immunoassays are more likely to be useful to confirm a clinical diagnosis of infection after this period as appearance of dengue antibody generally coincides with end of viremia (Innis *et al*, 1989). Traditionally hemagglutination inhibition (HAI) and plaque reduction neutralization have been used. These tests are of diagnostic value if a seroconversion or four fold or greater rise in titers between acute and convalescent phase sera can be demonstrated. Very often the collection of paired sera is not well spaced to show a rise in titers of diagnostic value or only single specimen is collected. In addition, the variable potency of reagents made in different laboratories has limited their wide spread use. Several commerical ELISA kit have been developed for rapid diagnosis of dengue fever (DF) as well as for classification of serological response in dengue infection based on the ratio of IgM and IgG in a single specimen. We have evaluated three commercial assasys for detection of antibodies to dengue virus, the MRL Diagnostic Dengue Fever Virus IgM Capture ELISA. (Cypress, CA, USA), Pan Bio Rapid Immunochromatographic Test (Brisbane, Australia) and Pan Bio Dengue Duo IgM and IgG. Capture ELISA (Brisbane, Australia) on serum samples collected during a febrile outbreak in New Delhi in 1997. All the above three assays

were compared to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in-house ELISA which was used as a gold standard.

MATERIAL AND METHODS

Serum samples

Fifty-eight serum samples were chosen at random from sera collected during a dengue outbreak. We selected 44 samples from patients with DF comprising primary dengue virus infection (n=13) and secondary dengue virus infection (n=31). Fourteen samples were collected from those who were dengue negative. Serum samples were obtained on average of 7-10 days after DF symptoms had appeared. Clinically suspected cases of dengue were defined as having symptoms of fever of 5-6 days duration, arthritis, rash with or without hemorrhagic manifestation. The diagnosis of dengue fever was established by in-house AFRIMS ELISA and/or virus isolation and sera were stored at -70ºC until further evaluated. Dengue cases from this outbreak were attributed to DEN-1 (Vajpayee *et al*, 1999).

AFRIMS ELISA

The in-house ELISA performed was described previously (Innis *et al*, 1989). For single specimens, forty units of IgM antibody to dengue virus (with dengue IgM antibody titer greater than JEV IgM antibody titer) was considered evidence of dengue virus infection. A dengue IgM: IgG ratio equal to or greater than 1.8:1 was defined as primary dengue infection. A ratio less than 1.8:1 was defined as secondary dengue infection.

Pan Bio Dengue Duo ELISA

IgM-IgG capture ELISA was performed using dengue Duo ELISA test (Pan Bio Australia) as per the manufactuerer's instruction. Results were expressed as the ratio of absorbance in test sample divided by the absorbance of calibrator sera. The recommended interpretation of the test was as follows:

a. Primary dengue was defined when IgM > 1.0 and IgG : IgM < 0.5

b. Secondary dengue was defined when IgG > 1.0 and IgG : IgM > 1.0

c. Suspected secondary dengue was defined when IgM < 1.0 and IgG > 1.0.

Dengue IgM and IgG rapid immunochromatographic test (RIT)

In the dengue rapid test (Pan Bio Australia) IgM and IgG antibodies to dengue virus are determined simultaneously by a rapid colloidal gold based immunochromatographic test for the separate determination of IgM and IgG antiboides in a capture assay format. The results were interpreted as per manufacturer's instructions (Vaughn *et al*, 1998). Samples were classified as negative, primary dengue, secondary dengue and suspected secondary dengue.

MRL Diagnostics Dengue Fever IgM capture ELISA

IgM capture ELISA was performed using MRL Diagnostics Dengue Fever Virus IgM Capture ELISA (CA, USA) as per the manufacturer's instruction. Results were expressed as index value relative to the cut off calibrator which is obtained by dividing specimen optical density (OD) value by the means of corrected cut off calibrator absorbance value. The recommended interpretation of the test was index value >1.00 considered presumptive for the presence of IgM antibodies to dengue fever virus and index value <1.0 indicated no IgM antibodies to dengue fever virus detected.

RESULTS

Sensitivity and specificity of MRL IgM capture ELISA

MRL test showed sensitivity of 97.8% (CI 93.47-100.00) as it correctly identified 43 out of 44 of the dengue samples as positive. The 14 negative contorl sera were negative with MRL showing 100% specificity.

Sensitivity and specificity of Pan Bio Duo ELISA and Pan Bio RIT

The overall sensitivity for detection of dengue positve sample by Pan Bio Duo ELISA

and RIT was 95.45% (CI 89.21-100.00) as compared to MRLs ELISA (97.8%). But the use of separate IgG and IgM results allowed the infections to be classified as primary or secondary dengue virus infection. The infection in all patients with primary dengue virus infection (n=13) was correctly classified (IgM positive only) by both Pan Bio Duo ELISA and RIT indicating 100% sensitivity. The infection in 29 out of 31 patients with secondary dengue virus infection was correctly classified (IgG positivity with or without IgM positivity) showing senstivity of 93.54% (CI 84.58 -100.0). The specificity of Pan Bio Duo ELISA and RIT were 92.8% and 85.7% respectively (Tables 1 and 2).

DISCUSSION

Dengue fever continues to be major public health problem. The severe form of the disease is a leading cause of hospitalization and death among children in many Southeast Asian countries (WHO, 1985) inlcuding India. There is a need for good, rapid diagnostic tests for dengue survillance in these countries where dengue is endemic. The diagnosis of dengue infection can be improved by new commercially available tests which provides standardized reagents thereby reducing inter-laboratory variation. All the three assays evaluated were useful in confirming the clinical diagnosis of dengue infection. The sensitivity of MRL IgM Capture ELISA (97.8%) compares well with recently published study (Palmer *et al*, 1999). The specificity of 100% observed in our study also correlated with earlier study (Palmer *et al*, 1999; Branch and Levett 1999). The overall senstivity (95.45%) of Pan Bio Duo ELISA and Pan Bio RIT for detection of dengue positive sample was less than MRL IgM capture ELISA (97.8%) which is observed in earlier study also (Cazzubbo *et al*, 1999; Branch and Levett 1999). But Pan Bio Duo ELISA and Pan Bio RIT have added advantage of detecting both IgM and IgG in the serum and thus are able

Table 1
Sensitivity and specifity of Pan Bio Duo ELISA.

	Diagnosis by Pan Bio Duo ELISA				
Diagnosis by AFRIMS ELISA (No. of specimens 58)	Negative	Primary dengue infection	Secondary dengue infection	SN	SP
Negative (14)	13	1	0		13/14 (92.85%)
Primary dengue virus infection (13)	0	13	0	13/13 (100%)	
Secondary dengue virus infection (31)	2	0	29	29/31 (93.54%)	

Table 2
Sensitivity and specificity of Pan Bio RIT.

	Diagnosis by Pan Bio RIT				
Diagnosis by AFRIMS ELISA (No. of specimens 58)	Negative	Primary dengue infection	Secondary dengue infection	SN	SP
Negative (14)	12	2	0		12/14 (85.7%)
Primary dengue virus infection (13)	0	13	0	13/13 (100%)	
Secondary dengue virus infection (31)	2	0	29	29/31 (93.54%)	

to distinguish primary infection from secondary infection. This information is important as secondary infection with a different serotype is associated with an increased risk of DHF (Halstead, 1981) and more relevant in contest of endemic area like India. The senstivity of both the above tests were 100% in primary infection. Our result correlates with (Lam and Devine, 1998; Sang *et al*, 1998; Vaughn *et al*, 1998) which reported sensitivities of 98% and 100% for primary infection. The sensitivities for secondary infection was 93.54% for both Pan Bio Duo ELISA and Pan Bio RIT which is comparable to earlier studies for evaluation of kits (Lam and Devine 1998; Sang *et al*, 1998; Vaughn *et al*, 1999). The Pan Bio Duo ELISA has added benefit of an operational time of less than 3 hours made possible by simultaneous incubation of antigen and peroxidase labelled anti-dengue monoclonal antibody (Vaughn, 1999) while MRL IgM capture ELISA took 4 hours to complete. The most rapid of above three commerical assays evaluated were Pan Bio RIT which took 5 minutes to complete but these kits, because of their formats, are most suitable for small numbers of patients.

In conclusion, all the three tests appear to be useful in confirming the clinical diagnosis of dengue infection. There is a need for standardization. ELISA kit for diagnosing large number of patients at hospitals in endemic area which do not have facilities for preparing their own reagents and have to send samples to far off reference centers. All the above three assays evaluated appears to be comparable in sensitivity and specificity and can provide excellent diagnostic tool for diagnosis of dengue infection. Pan Bio Duo ELISA and Pan Bio RIT have and advantage over MRL IgM capture ELISA for distinguishing between primary and secondary infection.

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