

COMPARATIVE SENSITIVITY OF LABORATORY METHODS TO DIAGNOSE DENGUE VIRUS INFECTIONS AT HUSADA HOSPITAL, JAKARTA

Ratna Tan¹, Herman Kurniawan², Sri Hartati², Susana Widjaja², and GB Jennings²

¹Husada Hospital; ²US naval Medical Research Unit No.2, National Institute of Health Research, Jakarta, Indonesia

Abstract. Several methods are available for diagnosis of dengue virus infections including a new commercially available dengue blot IgG assay. We conducted a study to compare the sensitivity of the dengue blot with the conventional diagnostic methods. Serum samples from suspected dengue patients were collected for virus isolation and the following serological assays: the hemagglutination-inhibition assay, an IgM/IgG enzyme-linked immunosorbent assay, and the dengue blot. When suspected dengue samples were tested by all methods, viral isolation detected the fewest dengue infections (10.5%), while the IgM/IgG ELISA was the most successful (46.3%) in diagnosing dengue infections. In a specific comparison between the IgM/IgG ELISA and the dengue blot, the dengue blot had an overall sensitivity of 48.8%, with a specificity of 88.7%. When patients were classified by their serological response, the dengue blot had a sensitivity of only 1.7% in those patients with a primary or recent dengue infection, however in secondary infections, the sensitivity of the dengue blot improved to 93.5%. Testing convalescent samples from patients with primary infections, only slightly changed the sensitivity of the dengue blot. The diagnosis of dengue is needed rapidly by clinicians to insure prompt treatment of patients. The dengue blot provides a rapid and easily performed assay, especially sensitive in secondary dengue infections which are most common in hospitalized cases in Asia.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a major health problem of Indonesia, with thousands of cases occurring each year (Masyhur and Wiryowidagdo, 1989). Mortality in DHF can be as high as 25% in patients who do not receive proper therapy, but overall mortality has declined to less than 2% since 1980 in Indonesia (Samsi *et al*, 1990). Quick and accurate diagnosis is vital to insure prompt treatment of these critically ill patients. The principal means of diagnosis of dengue infections are virus isolation and serological assays. Virus isolation provides the definitive dengue diagnosis, but the necessary requirements to perform virus isolation, limit the usefulness of this technique to a few laboratories (Lam *et al*, 1987). Although virus isolation is important for epidemiologic information, few laboratories have

access to adult mosquitoes, cell culture facilities, and/or a fluorescence microscope. Another problem with this method is that it is a time consuming procedure, thus limiting its usefulness for management of the patients.

The hemagglutination-inhibition (HI) assay is the gold standard of dengue serologic assays (Kuno *et al*, 1991) but there exist some disadvantages in its application. It requires acute and convalescent serum samples, at least 7 days apart. The serological diagnosis of dengue infections in the HI assays depends on demonstrating a rising dengue titer between the acute and convalescent sera, or the presence of high titer dengue antibodies (WHO, 1986). The need for the two serum samples, prevents the assay from providing clinicians with rapid information about the diagnosis. The collection of properly paired sera is often difficult, because patients are discharged from the hospital before the required 7 days. In addition, cross reactivity with other arboviruses, such as Japanese encephalitis virus (JEV), can give false positive results (Cardosa, 1989).

Innis *et al*, (1989) designed an enzyme-linked

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immunoassay (ELISA) for the detection of dengue infections which overcame the disadvantages of the HI assays. The detection of anti-dengue IgM in an acute sample provides a rapid dengue diagnosis, and sera can be tested simultaneously to differentiate anti-dengue and anti-JEV IgM and IgG antibodies. However, the necessity for special reagents and supplies limit this ELISA to laboratories with sophisticated equipment.

Recently, another immunoassay, dengue blot, was introduced and is the only commercially available diagnostic kit for dengue (Wuriyadi, 1991). The assay is easily performed, and requires no special equipment. It takes only a few hours to complete, thus giving the treating physician a prompt diagnosis. In this study, we diagnosed dengue viral infections at Husada Hospital by the above mentioned assays: virus isolation, HI, IgM/IgG ELISA and dengue blot IgG. The purpose of the study was to determine which assay is the most useful to detect dengue virus infections, and compare the sensitivity of the new dengue blot with the conventional assays.

MATERIALS AND METHODS

Patients

Suspected dengue infected patients were selected from the Pediatric Department of Husada Hospital between August 1990 and December 1991. An acute blood sample was collected at admission to the hospital and a convalescent blood sample at discharge from the hospital (range 5 - 10 days post-admission).

Laboratory assays

Acute blood samples were processed for virus isolation (Gubler *et al*, 1984) in C6/36 cell culture, an *Aedes albopictus* cell line (Igarashi, 1978), provided by the Centers for Disease Control Fort Collins, USA. Dengue virus was identified by serotype specific monoclonal antibody using an indirect fluorescent antibody assay (Gubler *et al*, 1984).

The HI was performed in microtiter plates as described previously (Clark and Casals, 1958). Based on WHO criteria (WHO, 1986), a fourfold or greater antibody rise or persistently high antibody titer ($\geq 1:1,280$) in convalescent sera is requi-

red for classification as dengue positive. The IgM/IgG ELISA was performed and interpreted as described by Innis *et al* (1989). Briefly, results are expressed as units, with 40 units the lower limit of positive in this assay. For dengue infection, a ratio of anti-dengue IgM to IgG (if either test result is ≥ 40 units) of ≥ 1.8 is typical of a primary infection, while a ratio of < 1.8 , indicates a secondary infection. Patients with an anti-dengue IgM value of ≥ 40 units are diagnosed as recent dengue infections. The dengue blot was performed according to the manufacturer's directions, samples are classified as positive or negative only.

Statistics

Sensitivity and specificity were calculated using the equations: sensitivity = $TP/TP + FN \times 100\%$ and specificity = $TN/FP + TN \times 100\%$. (TP = truepositives; FP = false-positives; TN = true-negatives; FN = false-negatives) (Lilienfeld and Lilienfeld, 1980).

RESULTS

Sera from 95 dengue patients with acute and convalescent sera were tested by all methods: virus isolation, HI, IgM/IgG ELISA, and dengue blot. Table 1 presents an overall comparison of the laboratory methods used in diagnosis of dengue virus infections. Virus isolation detected the fewest dengue infections (10.5%) although only acute samples were assayed. When paired sera were tested, the IgM/IgG ELISA was the most successful (46.3%) of the serological assays in diagnosing dengue infections. The HI assay was less reliable since 17.9% of the HI results were inconclusive due to the tested paired sera being collected < 7 days apart.

A specific comparison was made between the IgM/IgG ELISA and the commercially available dengue blot using acute sera from 263 patients (Table 2). The overall sensitivity of the dengue blot was 48.8%, with a specificity of 88.7%, but if patients were classified by serological response, a marked difference was detected in the sensitivity (Table 3). If the patient had a primary or recent dengue infection, the sensitivity of the assay was only 1.7%. However, if the patient had a secondary dengue infection, the sensitivity of the den-

Table 1

Comparison of laboratory methods in diagnosing dengue virus infections (n = 95).

Laboratory method	No. of positives	% Positives
Isolation ^a	10	10.5
HI ^b	40	42.1
ELISA IgM/IgG ^b	44	46.3
Dengue blot	22	23.2

^a Acute samples only

^b Paired sera

Table 2

Comparison of IgM/IgG ELISA with dengue blot (Acute samples, n = 263).

Dengue blot	IgM/IgG ELISA	
	+	-
+	59 (TP)	16 (FP)
-	62 (FN)	126 (TN)

gugue blot IgG increased to 93.5%. When the dengue blot negative/ELISA positive patients (N = 62) were assayed again using the convalescent sample, 6 (9.7%) additional dengue patients were identified.

DISCUSSION

Viral isolation provides the definitive diagnosis

of dengue infections, while the HI assay is considered the gold standard in dengue serology (Cardosa, 1989). The recent introduction of the IgM/IgG ELISA has challenged the role of the HI as the gold standard (Cardosa, 1989; Innis *et al*, 1989; Kuno *et al*, 1991). The ability to detect dengue infections with a single sample has improved the laboratory's diagnostic capability. In this study, we demonstrate the usefulness of the ELISA, and introduce the utility of the commercially available dengue blot assay. Viral isolation and the HI assay suffered from the limitations described previously (Cardosa, 1989; Innis *et al*, 1989) Kuno *et al*, 1991; Lam *et al*, 1987). The low isolation rate (10.5%) was mostly likely due to the admission of the patients late in the course of illness when antibody levels have developed and viremia levels had fallen. Many (17.9%) of the HI assay results were inconclusive, since the convalescent samples were collected earlier than the required 7 days.

The new dengue blot assay performed well in our study when compared to the more accurate IgM/IgG ELISA. False-negatives occurred with the assay primarily due to sample collection before the development of an anti-dengue IgG response. This also would explain the large difference in sensitivity seen between primary and secondary dengue infections. This difference was only slightly affected by repeating the dengue blot on a second sample collected later during hospitalization, possibly due to the fact that most of the second samples were collected less than 7 days apart. Therefore the clinician should be aware that a dengue infection could still be possible in a patient with a negative dengue blot, especially if a primary infection is suspected.

Table 3

Dengue virus infections by serological response (Acute samples, n = 263).

Serological response	IgM/IgG ELISA	Dengue blot (+)	Dengue blot (-)
Primary infection	9	0	9
Secondary infection	62	58	4
Recent infection	50	1	49
Not dengue	142	16	126

False positives rarely occurred with the dengue blot IgG. False positives could be due to cross-reactive antibodies, however acute and convalescent samples from active JEV infections were all negative when tested with the dengue blot (unpublished data). False positives can also occur due to high levels of anti-dengue IgG antibodies which can persist for several months after infection (Innis *et al*, 1989). Serum samples from previous dengue patients, when tested by the dengue blot have been found to remain positive for as long as 5 months after an acute dengue infections (unpublished data). Therefore, with a suspected dengue patient the clinician should feel confident with a positive dengue blot if he has ruled out a history of recent dengue illness.

In a clinical/hospital setting, the diagnosis of dengue is urgently required by the treating physician. While the IgM/IgG ELISA provides a rapid diagnosis, the specialized requirements for the assay are not routinely available in a clinical laboratory. If one understands the above-mentioned limitations of the assays, the commercially available dengue blot overcomes the need for specialized reagents, and gives the clinician a rapid dengue diagnosis.

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