Evaluation of a New Commercially Available Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assay for Diagnosis of Japanese Encephalitis Infections

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A new commercial enzyme-linked immunosorbent assay (ELISA) for the diagnosis of Japanese encephalitis virus infections showed a sensitivity of 88% with sera and 81% with cerebrospinal fluid and a specificity of 97% with sera from patients with primary and secondary dengue virus infections. Specificity was 100% when samples from nonflavivirus infections were tested.

Japanese encephalitis (JE) is a major public health problem in Asia, with approximately 35,000 cases and 10,000 deaths occurring annually throughout Asia (2, 3, 6). The case fatality rate of JE virus infections is approximately 25%, with 50% of survivors developing permanent neurological and psychiatric sequelae (4). In areas of endemicity, the majority of the population has sustained infection by young adulthood, and the ratio of apparent infections to clinically apparent infections has been estimated to be between 50:1 and 300:1 (12).

As JE and dengue viruses cocirculate in many regions of southern and eastern Asia, it is often necessary to distinguish between these two flaviviruses when diagnosing infection (7, 8). Most of the population in areas of endemicity have been exposed to at least two flavivirus infections during early childhood, making definitive diagnosis difficult due to the cross-reactive antibodies produced in secondary flavivirus infections. The majority of the cross-reactivity occurs at the immunoglobulin G (IgG) level, whereas IgM is generally specific for the infecting virus (1, 8, 9). Consequently, assays detecting IgM may be more useful in the diagnosis of active flavivirus infections than assays detecting IgG or total antibody.

In this study we assessed the ability of the PanBio JE IgM enzyme-linked immunosorbent assay (ELISA) to diagnose active infection and to differentiate between dengue and JE virus infections. JE virus infection was defined as a febrile illness associated with a decrease in consciousness and virus isolation in the cerebrospinal fluid (CSF) or the presence of IgM to JE virus in the CSF by using the reference ELISA. In children experiencing a febrile illness consistent with dengue fever or dengue hemorrhagic fever, dengue virus infections were defined as the isolation of a dengue virus, the detection of IgM to dengue virus in excess to IgM to JE virus in the reference enzyme immunoassay, or a fourfold rise in dengue virus hemagglutination inhibition titer (5, 11).

Paired serum samples from 19 patients with suspected JE virus infections were collected at the time of hospital admission (S1) and at the time of discharge (S2), and single serum samples (n = 10) and CSF samples (n = 43) were collected at various times after the onset of symptoms from patients presenting at the Centre for Tropical Diseases in Ho Chi Minh City, Vietnam, with JE virus infection. Serum samples from patients with suspected dengue virus infections were collected at the time of hospital admission and at the time of discharge at either the Queen Sirikit National Institute of Child Health (Bangkok Children’s Hospital) or the Kamphaeng Phet Provincial Hospital, Bangkok, Thailand. Paired serum samples from 40 patients with dengue (20 primary and 20 secondary infections) and single serum samples from 26 patients with dengue were used in this study. Serum samples from 20 patients with suspected dengue but no laboratory evidence of infection (clinical symptoms similar to dengue virus infection but negative by hemagglutination inhibition, ELISA, and virus isolation) were also tested. A panel of sera from nonflavivirus infections which included cases of typhoid from Malaysia (n = 15, positive by Widal felix test), cases of rickettsial scrub typhus from Thailand (n = 15, positive by indirect immunoperoxidase assay), and cases of leptospirosis from Australia (n = 15, positive by microscopic agglutination test) was also included. Twenty CSF specimens collected from patients with other neurological conditions undergoing lumbar puncture or myelogram treatment were also tested. All samples were frozen at −70°C prior to assay.

The PanBio JE IgM ELISA (JEM-200) was performed according to the manufacturer’s instructions. Diluted sample (1:100 for sera and 1:10 for CSF) that contained anti-human IgM antibody attached to the surface of the wells was added to the assay and incubated at 37°C for 60 min. Concurrently, peroxidase-labelled anti-flavivirus monoclonal antibody conjugate was added to the vials containing lyophilized inactivated JE virus, which resuspended the antigen and allowed formation of antigen-antibody complexes. After the residual serum was re-
moved from the assay plate by washing, antigen-antibody complexes were transferred from the antigen vials to the assay plate. After a further 60-min incubation at 37°C, the assay plate was washed and tetramethylbenzidine substrate was added. After 10 min the reaction was stopped by the addition of 1 M phosphoric acid, and absorbances were read at 450 nm. Positive, negative, and calibrator control sera used in each kit were tested in parallel with the diluted serum and CSF samples. Positivity was determined by comparison with the absorbance of the reference serum provided (cutoff calibrator). A sample was defined as positive if the sample/calibrator absorbance ratio was $\geq 1.0$ and as negative if the ratio was $< 1.0$.

The assay results obtained for each group of patients are shown in Fig. 1. The JE IgM ELISAs had a sensitivity of 88% (42 of 48 samples) for the diagnosis of active JE virus infections with serum samples and 81% (35 of 43 samples) with CSF samples (Table 1). Paired serum samples were available from 19 patients with acute JE virus infection. Of the admission sera (S1), the JE IgM ELISA correctly diagnosed 17 of the 19 samples (89%), while all of the 19 serum samples collected at the time of hospital discharge (S2) were positive (Table 1).

None of the 40 serum samples from patients with primary dengue virus infection were positive, and only 3 of 66 serum samples from patients with secondary dengue virus infection produced a positive result. Of these three serum samples, two had ratios just above the cutoff value (1.03 and 1.08). The PanBio JE IgM ELISA results were negative for all 65 serum samples collected from patients with nonflavivirus infections, including those with scrub typhus, leptospirosis, and typhoid. Twenty CSF specimens collected from patients with other neurological conditions undergoing lumbar puncture or myelogram treatment were also negative in the ELISA, and these showed much lower values than the CSF collected from patients with JE virus infection (Fig. 1). Consequently the overall specificity for the PanBio JE IgM capture ELISA with sera or CSF was 98% (188 of 191 samples).

![FIG. 1. PanBio JE IgM ELISA ratios for sera and CSF collected from patients with JE virus infection, CSF collected from patients without JE virus infection, sera from patients with dengue virus infections, and sera from patients with nonflavivirus infections. The cutoff ratio (1.0) for the PanBio ELISA is shown by a broken line.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>JE diagnostic group</th>
<th>No. of positive samples/no. tested (% positive) by JE IgM ELISA</th>
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<tr>
<td></td>
<td>AFRIMS</td>
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<tr>
<td>Paired sera</td>
<td></td>
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<tr>
<td>S1</td>
<td>19/19 (100)</td>
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<tr>
<td>S2</td>
<td>19/19 (100)</td>
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<tr>
<td>Total sera</td>
<td>43/48 (90)</td>
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<tr>
<td>CSF</td>
<td>36/43 (84)</td>
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<tr>
<td>Total sensitivity</td>
<td>79/91 (87)</td>
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</table>
Very good correlation was observed between the results of the PanBio JE IgM ELISA and those of the in-house JE IgM ELISA performed at the Armed Forces Research Institute of Medical Sciences (AFRIMS) (5) with both sera (Pearson’s $r = 0.9198$, $P < 0.0001$) and CSF (Pearson’s $r = 0.9116$, $P < 0.0001$) (Fig. 2). Combining the serum and CSF results, the JE IgM ELISA showed a sensitivity of 85%, which was similar to the sensitivity observed with the AFRIMS ELISA (87%) based on the testing of individual samples. With paired sera, the sensitivity of both assays was 100% (Table 1).

A commercially available nitrocellulose membrane-based IgM capture enzyme immunoassay (MAC DOT) for the diagnosis of JE virus infection has also been described recently (10). This assay has utility in the diagnosis of JE in field settings, where relatively small numbers of samples are tested. Good sensitivity in the diagnosis of JE virus infections was reported. Apart from the larger number of samples that can be accommodated, the PanBio JE IgM ELISA also has the advantage of an assay time of less than 3 h, while the MAC DOT test includes an overnight incubation. Furthermore, interpreting the PanBio JE IgM ELISA results is more objective than interpreting those of the MAC DOT test, which involves comparing dot intensity.

The combined use of ELISAs for the detection of specific IgM produced during JE and dengue virus infections has been reported to be useful in distinguishing between these two diseases (5). In this study, only three serum samples from patients with secondary dengue virus infection produced false-positive reactions (specificity, 97%), while the PanBio Dengue IgM ELISA has previously been reported to show no cross-reactivity in sera from patients with JE virus infections (11). Consequently, the PanBio JE IgM ELISA and PanBio Dengue IgM ELISA may be used concurrently to distinguish between active JE and dengue virus infections with a high predictive value. In addition, these ELISAs use a common assay method and the same diluted sera can be used in each assay.

This study indicates that the PanBio JE IgM ELISA is a reliable, rapid, sensitive, and specific serological test for the diagnosis of JE virus infections and that it can be used in conjunction with the PanBio Dengue IgM ELISA to differentiate between dengue and JE virus infections. Further studies are needed to determine the reactivity of the JE IgM ELISA with other flavivirus infections.

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