Rapid Dengue Diagnosis: A Prospective Study using a Commercial Rapid Test*

By

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Abstract

Several commercial test kits are available for the diagnosis of dengue infection but their sensitivity and specificity have not been evaluated extensively. Those that have been evaluated has been done retrospectively using stored serum samples. The PanBio Dengue

* A similar evaluation of rapid ICT (PanBio, Windsor, Australia) has been undertaken in 1998 by Proell et al. (Department of Infectious Diseases and Tropical Medicine, University - Munich, Germany) in comparison to standard immuno fluorescence antibody test (IFAT). In all, 25 serum samples for patients returning from dengue endemic areas, presenting clinical signs or recent histories compatible to DF, 30 patients with diarrhoea, and five persons with no history of illness who had been vaccinated against other flaviviruses were evaluated. The results showed that by IFAT, all the 25 patients with clinically suspected DF developed at least a diagnostic four-fold rise of IgG antibody titres against dengue virus and/or had significant IgM titres. In rapid ICT 23 patients (92%) with compatible symptoms had detectable IgM or seroconversion of IgG antibodies. All samples taken from the diarrhoea or the vaccination group were negative for IgM and IgG. The results showed a sensitivity of 92% and specificity of 100% for ICT when compared to IFAT. -Editor
Immunochromatographic Rapid Test takes five minutes to detect IgM and IgG using a capture assay format, and it is used in this study to assess its usefulness in a clinical setting. Of the 185 patients with severe dengue infection, there was an overall agreement of 91.35% cases based on IgM detection alone and 96.76% if combined with HAI results. Three clinical cases were cited in which the rapid test helped in patient management. However, serological results must be interpreted with caution, taking into consideration clinical and other laboratory findings.

Keywords: DF/DHF, Rapid test kit, case studies, Malaysia.

Introduction
The laboratory diagnosis of dengue infection is based on three approaches, namely, virus isolation, serology, and polymerase chain reaction (PCR). Although there has been significant improvement in the isolation of the virus by using mosquitoes and mosquito cell cultures, it is still a relatively slow and technically laborious process. PCR has become more prevalent as evident from the number of publications in recent years (1,2,3,4), but its wide usage in a routine laboratory is difficult to perceive in developing countries.

In his review of the serological diagnosis of DF/DHF, Gubler(5) has mentioned that enzyme-linked immunosorbent assay (ELISA) for the detection of dengue IgM and IgG is widely used in dengue diagnosis and may replace other existing methods, including the standard haemagglutination inhibition (HI). A number of commercial test kits to detect dengue IgM and IgG are available but the accuracy of most of these tests has not been validated.

The WHO collaborating centre for DF/DHF in Malaysia (Department of Medical Microbiology, University of Malaya, Kuala Lumpur) provides facilities for the diagnosis of dengue for cases in the University Hospital as well as in private hospitals, using a variety of techniques including the haemagglutination inhibition test, in-house dengue IgM ELISA, virus isolation in mosquito cell cultures and reverse transcriptase polymerase chain reaction. The selection of a test or a combination of tests depends on the severity of the clinical disease and other economic factors. Generally, the in-house dengue IgM ELISA which we
developed in 1987\(^{(6)}\) is used routinely for all dengue infections and additional tests such as HI and virus isolation are applied to severe cases. PCR is used sparingly on selected specimens because of its cost.

A preliminary evaluation of the PanBio Dengue Immunochromatographic Rapid Test (RT) by this WHO Collaborating Centre using stored specimens has been published\(^{(7)}\). Data from similar studies conducted in other countries supported our preliminary findings that the assay has a sensitivity of 98% and a specificity of greater than 90%\(^{(8,9,10)}\). However, to assess its usefulness in a clinical setting, we undertook a prospective study using the rapid test on patients with severe dengue infection.

**Materials and methods**

The study period was between 2 January 1997 and 30 July 1998. Tests were conducted on 185 severe dengue infections, 177 of which were DHF/DSS, seven with CNS manifestation and one with congenital infection. The ages of the patients ranged from 30 days to 64 years, with 10 patients under one year of age. The male:female ratio was 1.45:1.

**PanBio Rapid Test (RT):** The PanBio Dengue Immunochromatographic Rapid Test was used in this study. In this test, IgM and IgG were both determined using a capture assay format.

**Haemagglutination–inhibition (HI) test:** HI antibodies against DEN–2 and DEN–3 were determined as described\(^{(11)}\), except that the assay was modified to a microtitre format.

**In–house dengue IgM enzyme–linked immunosorbent assay (ELISA):** The in–house IgM ELISA was performed as described previously. Briefly, 96 well microplates coated with rabbit anti–human IgM were reacted with test sera. The antigen used was DEN–2, prepared in suckling mouse brains and the monoclonal antibody (WRAIR–2 3H5) to DEN–2 was used for detection of bound antigen.

**Clinical cases**

**Case 1**

A 13–year–old Chinese boy had one week of high fever, headache and
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generalized body ache. Gum bleeding was noted on the day of admission. He had previously suffered from dengue shock syndrome (DSS) at the age of 8 years. Physical examination revealed a comfortable child with a pulse rate of 70 per minute and blood pressure 120/70 mmHg. He had palatal petechiae and small cervical and axillary lymph nodes. A soft liver was palpable. His haemoglobin was 108 gm/L, total white count 2.3 x 10⁹/L platelet count 136 x 10⁹/L. A differential diagnosis of dengue fever was considered. Dengue IgM was not detected and Widal Weil Felix was not significant. He was afebrile the next day and was discharged well. Although the clinical picture resembled that of dengue fever, the PanBio rapid test was negative and the final diagnosis was non-dengue viral fever. An early discharge was possible when his temperature returned to normal.

Case 2

A 16-month-old Malay girl who was previously well was admitted after five days of fever, lethargy and deteriorating levels of consciousness. Physical examination showed a starry eyed, dazed child who responded only to pain. Her respiratory rate was 36/minute, heart rate 110/min, blood pressure 90/65 mmHg, and she was warm and well perfused. There was no neck stiffness. A maculopapular rash present over the palms and soles and ulcers were noted on the tongue and buccal mucosa. The liver was 3 cm palpable below the subcostal margin. She was hypotonic but tendon reflexes were increased and planter reflexes were upgoing. Soon after admission she had a generalized seizure which was stopped with intravenous diazepam. After this her breathing was laboured and her peripheral pulses became weak. She was intubated and ventilated with positive pressure. Her perfusion improved after 20 ml per kg of 0.9% normal saline intravenously. The diagnosis of enterovirus encephalitis was made – haemoglobin 130 gm/L, haematocrit 0.37, total white count 11.9 x 10⁹/L, platelet count 21 x 10⁹/L. Serum sodium was 125 mmol/L, potassium 5.4 mmol/L, urea 12.2 mmol/L, and creatinine 55 umol/L. PanBio IgM and IgG was positive. The diagnosis was revised to DSS and the patient was managed accordingly. She had a full
recovery. At discharge, the haematocrit was 0.24.

Case 3
A 5-year-old Indonesian boy was admitted on the fifth day of fever with drowsiness. He had been unresponsive eight hours before admission. On arrival in the Emergency Department he had generalized tonic clonic seizures lasting 10 minutes. Subsequently, he opened his eyes to call, but made no verbal responses. He was cold and clammy, heart rate was 190 per minute, and capillary refill was more than five seconds. Blood pressure was 75/23 mmHg and his temperature was 38.6°C. His breathing was laboured at 90 per minute with good air entry bilaterally. The liver was 4 cm enlarged. His pupils were 5 mm and reacted sluggishly to light. No fundal haemorrhages were noted. His muscle tone was increased and plantar responses were equivocal. He was given 30 ml per kg of 0.9% normal saline. His perfusion improved but his neurologic status remained the same. He was electively intubated and ventilated for cerebral protection. His haemoglobin was 171 gm/L, haematocrit 0.52, platelet count 37 x 10^9 and total white count 22.9 x 10^9/L. PanBio IgM and IgG was positive. A diagnosis of DSS with encephalopathy was made. The patient received blood transfusion but died of gastrointestinal haemorrhage 24 hours after admission.

| Table 1. Comparison of dengue rapid test with in-house IgM ELISA |
|----------------------|------------------|------------------|
| PanBio IgM   | PanBio IgG RT | HI   |
| +     | +     | 113  |
| +     | -     | 11   |
| -     | +     | 20   |
| -     | -     | 36²   |

| Table 2. Comparison of dengue rapid test with HI |
|----------------------|------------------|------------------|
| PanBio IgM   | PanBio IgG RT | HI   |
| +     | +     | 113  |
| +     | -     | 11   |
| -     | +     | 20   |
| -     | -     | 36²   |

Result
Of the 185 specimens confirmed to be positive serologically, 159 were IgM positive by both the ELISA and RT and 10 were negative by both tests, an agreement of 91.3% (Table 1). There were two specimens which were ELISA positive but RT negative and both these specimens had HI titre of
>1:1280. Of the 13 specimens which were RT positive but ELISA negative, 10 had HI titres of >1:1280 or greater, confirming a diagnosis of secondary dengue infection. If these 10 RT and HI positive specimens were added to the concordance result above, the agreement would be 96.76%.

Of the 185 specimens, only 180 were sufficient to perform the HI test for comparison with RT IgG results. Of the 180 specimens tested, 113 were positive by both RT IgG and HI (titre >1:1280 or greater) and 36 were negative by both tests, giving an agreement of 82.78% (Table 2). Twenty specimens were HI–positive but RT–negative, of which 15 were the results of primary infection and therefore not detected by RT, and 5 had HI titre of >1:1280. There were 11 specimens which were RT IgG–positive and HI inconclusive, and eight of these were also RT IgM–positive.

**Conclusion**

A rapid immunochromatographic test which takes only five minutes to perform has been evaluated in several retrospective studies in the last two years. The assay, besides being rapid, does not require sophisticated equipment, and can be decentralized to peripheral hospitals and even clinics. Evaluation of this test by a number of laboratories showed the assay has a sensitivity of 98% and a specificity of >90% in the diagnosis of dengue infection. The sensitivity of the RT is further borne out by the result of this prospective study.

The three clinical cases presented exemplify how the test can be used to support clinical decision and patient management. In Case 1, the clinical course of the disease was very similar to DEN. However, when the rapid test was negative for dengue IgM, the patient was given oral fluids and was discharged as soon as he was well. If dengue had been suspected, the patient would have been kept for an additional 24–48 hours when fever would have subsided. This led to more cost–effective patient management.

In Case 2, the child was admitted during the peak of an enterovirus 71 encephalomyelitis outbreak, and since the patient had hand–foot–mouth lesions and CNS signs and symptoms, it was thought that this was due to EV71 infection. However, the rapid test for dengue was positive and the
haematocrit findings of more than 50% haemoconcentration confirmed a diagnosis of DSS and the patient was managed accordingly.

In Case 3, the clinical picture at presentation was that of encephalopathy with circulatory shock. A rapid diagnosis of dengue was useful in guiding fluid therapy and early blood transfusion. The patient's suboptimal clinical response to crystalloid infusion suggested occult haemorrhage and early blood transfusion was necessary to reverse the shock. Unfortunately, this patient succumbed to gastrointestinal haemorrhage.

Despite the apparent usefulness of rapid diagnosis using this commercial kit, it must be remembered that the serological result should be interpreted with caution in acute dengue infection. The absence of IgM and IgG in the first week of illness may indicate a false negative and a repeat sample should be requested. Serological results must be interpreted alongside clinical history and other laboratory results.

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