Evaluation of a Capture Screening Enzyme-Linked Immunosorbent Assay for Combined Determination of Immunoglobulin M and G Antibodies Produced during Dengue Infection

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A commercially available enzyme-linked immunosorbent assay (ELISA) (PanBio Dengue Screening ELISA) that utilized both immunoglobulin M (IgM) and IgG capture in the same microtiter well for the diagnosis of dengue infection was evaluated. Sensitivity in primary and secondary dengue was 95%, while specificity was 94%.

Dengue is one of the most important mosquito-borne diseases in the world but still remains very much underreported, especially in developing countries where diagnostic facilities are inadequate (12). Serology is a useful aid in the diagnosis of dengue infections, with hemagglutination inhibition (HAI) assays traditionally used (24). Enzyme-linked immunosorbent assay (ELISA) for immunoglobulin M (IgM) and IgG has also been reported to be useful in dengue serology (1, 8, 10, 23), and a number of commercial tests have recently been reported (5, 9, 13, 14, 18, 20, 22, 25). One of these, the Dengue Duo ELISA (PanBio Pty Ltd, Brisbane, Australia), is novel in that it utilizes both IgM and IgG in the diagnosis of dengue infection, with the cutoff in the IgG ELISA set to detect high levels of IgG present in secondary but not primary or past dengue infections (14, 20, 22). This diagnostic strategy has the advantage of detecting secondary infections that may be missed through the use of IgM alone (22). Furthermore, it has demonstrated specificity superior to that of assays using IgM alone since the cutoff in the Dengue Duo IgM ELISA is set higher, as the IgG assay detects secondary cases (4). The PanBio Dengue Duo ELISA can also distinguish between primary and secondary dengue infection by comparison of the IgM and IgG results (22). One disadvantage of the PanBio Dengue Duo ELISA has been the need to run two assays (IgM and IgG), posing a financial burden in less developed regions where dengue is endemic and where accurate diagnosis is important. Consequently, some countries have not been able to afford the assay since it is effectively twice the cost of other assays. To overcome this problem, PanBio has released a Dengue Screening ELISA (DSC-500) that combines the IgM and IgG capture assays into one well. This effectively halves the cost of diagnosis and retains the advantages of using both IgM and IgG in the diagnosis of dengue infection. In the PanBio Dengue Screening ELISA, both anti-human IgG and anti-human IgM are applied as a coating to the same assay well. The level of the anti-human IgG applied as a coating to the well is set to detect high levels of IgG characteristic of secondary but not primary or past dengue infections (6), while the level of anti-human IgM has been set to maximize sensitivity and specificity.

In this study, the PanBio Dengue Screening ELISA was evaluated using sera collected in Malaysia from patients with and without dengue infections. Specimens from patients with clinically suspected cases of dengue infection were selected retrospectively from a bank of frozen sera collected after hospital admission. Paired sera from 18 patients with dengue infection (nine primary and nine secondary) were tested, as well as 20 single sera from patients with dengue infection (nine primary and nine secondary) were tested, as well as 20 single sera with positive in-house IgM ELISA and known HAI titers. The in-house IgM ELISA was performed as described previously (10), while titers of HAI antibodies against dengue type 2 and dengue type 3 were determined as described previously (3), except that the assay was modified to a microtiter plate format. The first of the paired sera was collected during acute infection, while the follow-up serum was collected within 2 weeks. Dengue diagnosis was based on in-house IgM ELISA and HAI using World Health Organization criteria, with an HAI titer of $\geq 1:1,280$ used to define a secondary infection (24). All sera used in this study were tested for Japanese encephalitis (JE) by HAI, and none were found to be positive. In addition, 10 sera from patients with clinical presentation of dengue infection but no laboratory evidence of disease (IgM enzyme immunoassay [EIA] negative and HAI titer of $<1:400$) and 24 sera from patients with serologically confirmed malaria, measles, rubella, mumps, or Chikungunya infections were tested.

The PanBio Dengue Screening ELISA (DSC-500) was performed according to the manufacturer’s instructions. Serum diluted 1:100 in the diluent provided was added to the assay plate, which contained a mixture of anti-human IgM antibody and anti-human IgG antibody attached to the surface of the wells, and incubated at 37°C for 60 min. Concurrently, peroxidase-labeled antiflavivirus monoclonal antibody conjugate was added to the vials containing lyophilized dengue virus types 1 to 4, which resuspended the antigen and allowed formation of antigen-antibody complexes. After residual serum was removed from the assay plate by washing, antigen-antibody com-
the onset of infection and that IgG appears to be a more sensitive marker than IgM in secondary dengue (8, 11, 19). Consequently, the PanBio Dengue Screening ELISA, which combines the use of IgM and IgG, may lead to the earlier diagnosis of dengue infections. Similar results showing improvement with the combination of IgM and IgG have been reported previously (8, 19, 20, 22).

One of four samples from malaria cases showed cross-reactivity in the PanBio Dengue Screening ELISA. Due to the low sample numbers used, it would be desirable to test a larger number of specimens to determine the exact level of cross-reactivity. This reactivity may have been due to co-infection, polyclonal reactivation, or cross-reactivity between homologous regions of the different microorganisms. Consequently, it would be important to rule out these other infections to ensure confident diagnosis of dengue infection. High levels of antibody cross-reactivity in patients with dengue and other flavivirus infections such as JE have been reported previously (8, 16), and 50% of patients with JE have been shown to give false-positive cross-reactivity in the Dengue Duo ELISA, which utilizes the same reagents as does the PanBio Dengue Screening ELISA (22). Consequently, one would expect high levels of cross-reactivity with JE patient samples in the PanBio Dengue Screening ELISA, and caution should also be used in interpreting tests that are positive in areas where dengue virus cocirculates with other flaviviruses. However, most cases of JE can be differentiated from dengue on clinical grounds, despite unusual cases of dengue encephalopathy being reported previously (7, 15).

Unlike ELISAs that determine IgM and IgG separately (8, 19, 20, 22), the PanBio Dengue Screening ELISA cannot distinguish between primary and secondary infection. However, this would not be a problem in regions of endemicity where cost is a key driver in the decision to use the assay for disease control and management. In these regions, the majority of cases are secondary infections and patient management is dictated by the patient’s age and other clinical factors (17). For example, a study in Thailand revealed that most children had acquired a dengue infection by school age (5 years), with the majority of these having been asymptomatic (21).

The combined detection of IgM and IgG in the one well reduces assay cost. Total assay time for the PanBio Dengue Screening ELISA is just over 2 h, which is quick relative to other dengue ELISAs that have been reported (1, 8, 10). One factor contributing to the speed of this test is the incubation of antigen. This format has been reported to decrease the number of assay steps and to speed up diagnosis of dengue (2). Another advantage of the PanBio Dengue Screening ELISA is that antigen is provided in a stable dried form and all reagents are provided ready to use.

### Table 1. Sensitivity of PanBio Dengue Screening ELISA (DSC-500)

| Serum                                      | No. positive/no. total (%) |
|--------------------------------------------|----------------------------|
| Primary dengue (paired sera)               |                            |
| Serum 1 (n = 9)                            | 6/9 (67)                   |
| Serum 2 (n = 9)                            | 9/9 (100)                  |
| Secondary dengue (paired sera)             |                            |
| Serum 1 (n = 9)                            | 9/9 (100)                  |
| Serum 2 (n = 9)                            | 9/9 (100)                  |
| Single serum; in-house IgM EIA positive;   |                            |
| HAI titer of >640 (n = 10)                 | 10/10 (100)                |
| Single serum; in-house IgM EIA positive;   |                            |
| HAI titer of <640 (n = 10)                 | 10/10 (100)                |
| Total                                      | 53/56 (95)                 |

### Table 2. Specificity of PanBio Dengue Screening ELISA (DSC-500)

| Serum                                      | No. negative/no. total (%) |
|--------------------------------------------|-----------------------------|
| No evidence of dengue infection (in-house IgM EIA negative; HAI titer of <640 (n = 10)) | 9/10 (90)                  |
| Measles IgM positive (n = 5)               | 5/5 (100)                   |
| Rubella IgM positive (n = 5)               | 5/5 (100)                   |
| Mumps IgM positive (n = 5)                 | 5/5 (100)                   |
| Chikungunya infection IgM positive (n = 5) | 5/5 (100)                   |
| Malaria                                    | 3/4 (75)                    |
| Total                                      | 32/34 (94)                  |
FIG. 1. Individual assay values for the PanBio Dengue Screening ELISA in different diagnostic groups. The cutoff for the ELISA (10 PanBio Units) is shown by a broken line. Three sera from patients with primary infections (each the first [S1] serum of a pair) were negative on the PanBio Dengue Screening ELISA and the in-house IgM capture ELISA. HI, HAI titer; pos, positive; neg, negative.

The PanBio Dengue Screening ELISA is best suited for routine diagnostic laboratories where large numbers of samples are tested or where cost is an issue and it is not necessary to distinguish between primary and secondary infections.

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