Reviewer’s report

Title: Enzyme-linked immunoassay for dengue virus IgM and IgG antibodies in serum and filter paper blood

Version: 3 Date: 12 September 2005

Reviewer: Jyh-Hsiung Huang

Reviewer’s report:

General
1. This study analyzed 781 pairs of acute and convalescent sera and 161 corresponding pairs of filter paper blood spots using Focus ELISA kits from Vietnamese febrile patients. The results showed that ELISA in serum is subject to inter-laboratory variation and ELISA on filter paper suffers from random variability.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. Page 2, Conclusion: The conclusion is misleading and should be revised. Based on the results presented in this study, ELISA on filter papers suffers from random variability and should not be used for diagnostic confirmation and assessment of approximate sero-prevalence.
2. Page 3, Background, line 10: This sentence needs revision. Recent advances have shown that dengue IgM capture ELISA is highly specific to dengue although IgG ELISA is not. Indeed, dengue IgM capture ELISA had been successfully used to distinguish the four dengue serotypes of primary infection.
3. Page 4, last paragraph: “In this study we investigated the variability in flavivirus IgM and IgG antibody concentration----”. Similar to above question, the specificity issue should be clarified. If the dengue IgM capture ELISA is not specific to dengue, how can one confirm the dengue virus infection using serological method based on commercial kit?
4. P7, paragraph 1, last four lines: Algorithm for the serological diagnosis of dengue in Figure 1 is confusing and revision is needed. (1) The criteria used to differentiate primary and secondary dengue virus infection is problematic since “Focus” ELISA kits are not designed to do so using IgM/IgG ratio (both kits are for qualitative use); (2) Four fold increase of serially diluted pair sera (t3 vs. t0) should be used as a reliable diagnostic measurement of flavivirus cross-reactive IgG antibodies instead of IgG-IgG-IgG-IVt3/IgG-IVt0 ratio; (3) For those dengue patients classified as “acute secondary dengue” based on the criteria of IgG-IgG-IgG-IVt3/IgG-IVt0 ?2 and negative IgM, it would be most appropriate classified as “acute secondary flavivirus” infection.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
5. Page 15, line 2: Why outbreak surveillance should focus on outbreaks of hemorrhagic dengue instead of dengue fever?

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Discretionary Revisions (which the author can choose to ignore)
6. P3, Background, line 1-2: The sentence could be revised to “both molecular and serological tests can be used to confirm the clinical diagnosis.”
7. P12, Discussion: Although the interpretation of serological tests is often difficult, the significant inter-laboratory variation presented in this study suggests that quality control should be improved in the performance of ELISA. Most of the potential sources of variation mentioned in this discussion
section could be avoided if the assay kits were performed according to the instruction. The discussion can be further focused and reduced in length.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No