Reviewer's report

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

Version: 6 Date: 16 October 2005

Reviewer: Jyh-Hsiung Huang

Reviewer's report:

General
1. Since no author’s response letter describing the changes made to the reviewer in PDF format was found, I simply make this review based on the revised manuscript.
2. The revised manuscript was extensively rewritten with significant improvement. However, the authors have not adequately answered several important issues, which are critical to this paper.
3. Most important, the criteria used for diagnostic classification of dengue virus infection are not reliable and should be revised.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Page 3, Conclusion: I would not recommend the ELISA on filter paper method for seroprevalence study since 67 out of 161 samples gave discrepant results comparing to method based on ELISA on serum. This problem will not disappear even with sufficiently large sample size.
2. For serological diagnosis, serial (paired) specimens are required to confirm or refute an acute flavivirus or dengue virus infection. Otherwise, it is impossible to distinguish between acute and recent infection. This problem has to be addressed in the classification of diagnosis of dengue virus infection shown in Figure 1. For example, why for IgM all subjects with positive IgMt3 are expected to have acute primary dengue and thus increasing IgM index values from t0 to t3? (Page 10, line 4). The classification based on the ELISA results of IgMt3 and IgMt3/IgGt3 without IgMt0/IgGt0 might not be able to distinguish between acute and recent dengue virus infection.
3. The algorithm used in this study for the serological diagnosis of dengue in Figure 1 is confusing and changes are needed. (1) The criteria used to differentiate primary and secondary dengue virus infections are problematic since “Focus” ELISA kits are not designed to do so using IgM/IgG ratio (both kits are for qualitative use). Had the criteria used in this study been evaluated properly to support its usefulness in the diagnostic classification of dengue virus infection? (2) Would it be better if the algorithm shown in Figure 1 start with differentiation of positive or negative acute (and recent) dengue virus/flavivirus infection based on the increase and decrease of both IgM and IgG antibodies from paired serum samples instead of IgMt3? Indeed, the best time to collect convalescent sera should be days 14 to 20 after the onset of illness to avoid the decline of IgM antibodies.
4. For most reliable result, apparent serum conversion or four fold increase of IgM and/or IgG antibody in serially diluted pair sera (t2 vs. t0) should be used to define acute flavivirus infection. Therefore, apparent increase of flavivirus cross-reactive IgG antibodies defined by IgG-IVt3/IgG-IVt0 >=2 would be significant and the cases classified as acute flavivirus infection.
5. Page 14, line last 5: “The distinction with other flavivirus can be improved by applying higher cut off values, with the risk of losing some sensitivity.” This statement is not correct in that one can not distinguish various flavivirus by the increase or decrease of cut off value, this can only be made by testing against a panel of flavivirus antigens simultaneously.
6. Page 17, line 5: “The proportion of IgG positive cases in a survey, the seroprevalence will not change significantly by———”. There are two problems with this statement: 1) this assay is not
dengue-specific, and 2) the rate of inaccurate data derived from filter paper method will not be
changed if large samples are analyzed. Therefore, result from this study suggested that ELISA on
filter paper should not be used for dengue diagnosis and seroprevalence study.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the
author can be trusted to correct)
7. Page 16, last line: Why outbreak surveillance should focus on outbreaks of dengue hemorrhagic
fever and dengue shock syndrome instead of dengue fever? A good surveillance systems should be
able to detect all clinical cases with various disease severity

Discretionary Revisions (which the author can choose to ignore)
None

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

Declaration of competing interests:
'I declare that I have no competing interests'