**Reviewer's report**

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

**Version:** 2  **Date:** 8 August 2005

**Reviewer:** Maria G G Guzman

**Reviewer’s report:**

The aim of this study is to investigate the variability in flavivirus IgM and IgG antibody concentrations, using two commercial ELISAs against dengue virus. Authors compare IgM and IgG values in paired sera collected at T0 and T3 in terms of:

a) Comparison of test in two laboratories
b) The decay during storage of serum and filter paper
c) Comparison of serum and blood on filter paper

The study is interesting, however, in the present form is complicated for understanding. There are many data and their presentation is not clear for this reviewer.

Authors don’t present the standardization of IgM and IgG detection between paired samples (serum and filter paper). It should be good to first compare (in a small number of samples) the filter paper dilution corresponding to the serum. This could explain the low antibody correspondence observed in the study into results obtained by blood on filter paper and serum.

Results could be showed in the following form:

1. To determine the filter paper dilution corresponding to the serum both for IgM and IgG determinations (in small number of samples that allow the authors to match serum and filter paper and to confirm that the employed filter paper serum dilution is the adequate).
2. To compare IgM and IgG level of antibodies in a bigger group of samples (serum and filter paper)
3. The same comparison than (2) at specific intervals time after collection. It’s important to comment that the median storage time in days was 283 for T0 and 262 for T3. Particularly, blood on filter paper was stored at air condition temperature. Previous studies have demonstrated that these samples must be stored at –20C in order to avoid the antibody decay.

The results could be presented in two manuscripts, one including IgM results and the other IgG results. If results are shown in only one manuscript, it should be recommended to simplify their presentation.

**Other comments:**

**Introduction:**

Paragraph 5: The present study is not the first one evaluating the use of blood on filter paper for dengue antibody determination by ELISA. Authors must include some references of other authors.

**Objective:** Authors investigate the variability in IgM and IgG antibody concentrations to dengue virus but not for flaviviruses. They only include dengue antigen into the study.

**Material and methods:**

Figure 1 shows the diagnostic classification applied into the study. Authors must include the reference or at least explain why they use this methodology. Is there any previous comparison with hemaglutination Inhibition assay for acute secondary dengue classification in serum samples with negative dengue IgM??

**Final recommendation:** major compulsory revision before acceptance

**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'