Author's response to reviews

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

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Answer to comments on reviewer Huang of 29 November 2005

1. We agree with the reviewer that in an endemic area were both JEB and dengue virus are in circulation seroprevalence studies should be interpreted with caution due to high cross-reactivity. The manuscript discussed this issue on page 15. After the sentences on cross reactivity we wrote: " ...... This study did not address cross reactivity with JEB virus but cases with clinically evident encephalitis were excluded and so the number of JEB virus infections causing cross reactions with the dengue ELISA is probably small, if any."

We now replace these sentences by: "This study did not address cross reactivity with JEB virus but cases with clinically evident encephalitis were excluded. Although the clinical pattern of JEB is different compared to dengue virus infections we cannot completely rule out that some of the reactivity measured in serum and on filter paper was due to the serological cross-reactivity between both viruses".

At the end of the discussion we wrote:
"Thus, with sufficient sample sizes and while taking cross reactivity with other flavivirusses into account, notably JEB virus, dengue virus sero-prevalence studies, .........."

We trust that in this sentence sufficiently emphasizes potential cross reactivity.

2. The reviewer indicates that it is misleading to quote the WHO guidelines to differentiate between primary and secondary infections. The dengue MAC ELISA stands for Dengue IgM Antibody Capture ELISA (Innis et al 1989; Vaughn et al 1999). The dengue GAC (IgG Antibody Capture) ELISA has been described in the past by CDC scientists (Innis et al 1989; Vaughn et al 1999). Miagostovich from the Oswald Cruz Institute in Brasil and Vorndam from the CDC published a paper in 1999 in the Journal of Clinical Virology that the GAC ELISA is relatively insensitive. They also indicate that the direct detection of IgG using dengue antigen bound to a micro well is more sensitive (Miagostovich et al 1999; Chungue et al 1989). The Focus Diagnostic IgG dengue ELISA is based on the direct detection of dengue virus IgG antibodies. In several studies it was shown that using the combination of the MAC ELISA (Focus IgM capture ELISA is in principle the same test as the MAC ELISA described in the WHO report) with the improved direct detection IgG ELISA (Focus Diagnostics) instead of the CAG ELISA allows to differentiate between primary and secondary dengue virus ( Cobelens et al. Trop. Med Int Healt 2002; Koraka et al J. Med Virology, 2003 and 2004). Since this calculation method is a slight modification from the published WHO guidelines this might indeed be confusing. In the paper we refer to Cobelens et al. In this manuscript the modified ratio method (IgM MAC,and IgG direct) was used for the first time (page 9 last lines). The following text was added ".....recognizing that this ratio between the results of a capture IgM ELISA and direct IgG ELISA is slightly different from the method published by the WHO." (end of page 9, start of page 10)