Reviewer's report

Title: Variation in dengue virus plaque reduction neutralization testing: systematic review and pooled analysis

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Reviewer: Stephen Whitehead

Reviewer's report:

The authors sought to investigate the sources of variation in the PRNT by reviewing the published assay results from human studies, including both natural infections and vaccination. Using this data they test two models to account for the observed variability: assay strain selection and inter-laboratory differences. It is well appreciated in the field that the PRNT is highly variable and is dependent on the method used. This fact was reaffirmed in the current study, but could not be further defined since only limited details of the PRNT assay were consistently disclosed in the body of literature that was accessed. Inter-lab differences, which reflect method differences, were shown to account for about half of the observed variance. Remarkably, assay strain selection only accounted for a small fraction (8%) of the observed variance. Several issues remain to be addressed by the manuscript and are detailed below.

Discretionary revisions:

1. Page 6. Unfortunately, only eight vaccine studies are included in the analysis. It is not clear if vaccine studies were limited to live attenuated vaccines or a single dose of vaccine. There are certainly many human vaccine studies reported in the literature. What were the most frequent reasons for excluding so many vaccine studies? Lack of individual data? Lack of PRNT assays to all four serotypes (was this an exclusion criterion)? Were authors asked to provide individual data if only summary data was presented in their publication.

2. Page 6. Data abstraction. What about the use of complement in the PRNT assay? I think some labs still use complement in their PRNT and this may contribute to inter-lab differences. Also the method for plaque enumeration (direct staining vs. foci immunostain) is likely important and is probably a significant driver for the selection of virus strains since not all strains perform well by direct staining.

3. Page 11. In the first two paragraphs under the heading “Patterns of Reported PRNT Titers in Primary and Secondary Exposure” the authors show that primary dengue exposure leads to a principally homotypic antibody response and secondary exposure leads a more broad heterotypic response. This has been demonstrated repeatedly in the literature as early as Sabin’s work. What new information does the current study provide?

4. Page 13. Paragraph 1. There appears to be a mistake in the strain reported to
give a titer of 2.89. From Figure 3, strain CH53489 appears to be closer to 2.89 than strain 116/00.

5. Page 14. Lines 1 and 2 and Table 3. I am confused by the data presented in Table 3 and the conclusion that vaccination titers were 0.91 that of titers from natural exposure. If this is actually true, vaccine developers will be very happy, although I'm not sure if the majority of vaccines included in this analysis are still viable vaccine candidates. In Table 3, I interpreted the relative titers presented for the different time categories to be calculated relative to unexposed titers ("reference" designation for unexposed titers). If this is the case, then peak PRNT titers are reached 12 – 30 dpi and are 8 – 7 fold higher than unexposed (background) titers. By a year, titers have decreased to 2 – 3 fold above background. However, vaccination titers are listed as 0.8 – 0.9 compared to unexposed, which does not seem correct and is probably not what the authors intended to convey. Am I misinterpreting the data? As you can see, Table 3 needs to be improved. Probably best to divide it into at least three tables with proper headings for each column.

6. Page 13. Middle paragraph. The authors point out that assay strains DENV2 PR-159 and DENV4 Dominica yield lower titers? Why do the authors think this is the case? What factors may be involved?

7. Page 16. The authors observe that PRNT titer increases with each 10% increase in neutralization stringency. They admit that this is counterintuitive and may be influenced by their sampling. Given the data that they have collected, they should be able to test if the association between titer and PRNT stringency was influenced by the reported lower titers for PRNT90 and reported higher titers for PRNT50.

8. Page 17. The conclusions are brief. What new insights does this study present to the reader? Do the authors believe that vaccine-induced protection can be inferred from PRNT titers? Is the PRNT a relevant correlate of protection? I believe vaccine protection will be determined by carefully designed Phase III clinical studies. Comparison of PRNT titers from different labs will always be a challenge, even with full disclosure of the methods and assay parameters. Why do the authors believe that comparability between laboratories is actually necessary? It seems like an arduous goal without a truly compelling purpose. Why advocate for standardized strains when strain selection only accounts for 8% of the variance? What other factors are likely to contribute to the observed variability? How can these factors be elucidated.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests