Reviewer's report

Title: Performance of Commercial Dengue NS1 ELISA and Molecular Analysis of NS1 Gene of Dengue Viruses Obtained during Dengue Surveillance in Indonesia

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Reviewer: David W Smith

Reviewer's report:

This is a very interesting and informative paper that presents a large and detailed analysis of dengue diagnostic assays in Indonesia. The data on NS1 nucleotide and amino acid sequences is an important contribution.

Major Compulsory Revisions

1. The diagnosis of dengue is complex, especially in endemic/epidemic areas where there is a mixture of primary and secondary infections occurring in populations with different background immunity. It would be better if the authors gave this greater emphasis as a possible cause for variable findings about NS1 and IgM sensitivity reported in different populations. The sometimes conflicting information in the literature makes it difficult for laboratories to develop confident interpretations of test results in possible secondary infections, and readers need to understand why these will take some time to resolve! The need for local studies is stated in the final sentence of the manuscript, but the reasons why this is necessary could be more strongly emphasised in the discussion.

2. Page 6, Methods: It doesn’t say where the specimens were tested – in each of the centres or at a centralised laboratory? I can’t see where the specimen storage and transport methods are stated, which is particularly important if samples were sent to a central laboratory. If the samples were tested in the regional laboratories, how did they ensure uniformity of quality? Could this account for some of the geographical variation?

3. Page 9: Neither the results nor the methods state the time after onset of illness for the collection of samples? Was this data collected? If it was, was there a broad distribution of times and, if so, how would that have affected the results? If not, the authors should discuss the possible impact of time of collection on their results.

4. Page 6, line 126: The separation of primary and secondary dengue cases is critical in assessing dengue diagnostic tests. This line states that the separation of primary and secondary dengue was done “according to the manufacturer’s instructions”. I was unable to find this information through the product support pages. It would be better to actually state what the criteria were and give a link. Also there should be references that justify the use of this definition instead of the WHO laboratory criteria, and it should be included in the discussion.

5. Page 9, line 200/201: The specificity analysis did not include patients with other flavivirus infections. Is there a reason for that such as unavailability of
samples? As a minimum other publications looking at specificity across flaviviruses should be referenced to reassure the readers.

6. Page 10/11: Did the authors consider delays in specimen transport or variations in laboratory testing as possible causes of variations in sensitivity of the tests?

7. Page 15: The discussion of the NS1 Ag variation and test sensitivity is very interesting, but there doesn't appear to be a clear statement about why they think the assay was much less sensitive for DENV-4 NS1. It doesn't appear to be due to antigenic changes. There should be a definite statement that they don’t know the cause for that and it may be due to virus characteristics that they haven’t noticed, higher rates of immune complex bound antigens, differences in test performance in the various laboratories, specimen handling and/or transport differences, etc.

Minor Essential Revisions
1. Page 4, line 79; “accurate diagnosis with the broad spectrum” would be clearer as “accurate diagnosis due to the broad spectrum”
2. Page 4, line 80: “dengue diagnosis tools” should be “dengue diagnostic tools”
3. Page 4, line 80/81: “NS1 antigen has become the basis for commercial diagnostic assays” is a bit unclear as IgM and IgG detection are the basis for many commercial diagnostic assays. If the intent is to say that laboratories are increasingly using NS1 detection as the preferred diagnostic test, which is true, then it should say that.
4. Page 5, line 91: the phrase “geographical regions of the study site” doesn’t seem right. Should it be “study sites in different geographical regions”?
5. Page 5, line 96: “used and becoming” should be “used and are becoming”
6. Page 6, line 114: “written consents” should be “written consent”
7. Page 6, line 122: Does this mean that the NS1 ELISA was repeated, or an antibody ELISA? Please be explicit.
8. Page 7, line 141: Please specify the modifications
8. Page 7, line 205: “assessed” should be “assess”.

Discretionary Revisions
1. The outlier in NS1 performance was the DENV-4 positives, which drags down the overall sensitivity of the test. It is mentioned briefly in the discussion, but it would be better to include some data about how the NS1 test performed if DENV-4 was excluded.
2. The sensitivity of the NS1 assay is discussed in several places. Did the authors look at the S/CO ratios? It would be useful to see comparative data showing the distribution of S/CO ratios for the different dengue serotypes

**Level of interest:** An article of importance in its field
Quality of written English: Needs some language corrections before being published

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'