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

**Version:** 3  **Date:** 18 October 2013

**Reviewer:** SUNETHRA GUNASENA

**Reviewer's report:**

Discretionary Revisions / Minor Essential Revisions

1. **Title**
   Comment: Change unnecessary upper case in the words
   Length of the title suggest to be shorter

2. **Abstract**
   **Background**
   Line 26: Omit “various” at the beginning of the sentence
   Para 2: Use the word assay instead of kit

3. **Method**
   Line 36:
   Suggest: 188 samples were confirmed for dengue by RT-PCR and/or virus isolation.

4. **Results:**
   Need to change as per the text following corrections

5. **Conclusions:**
   Need to change as per the text following corrections

6. **Introduction**
   **Para 1:**
   Line “undifferentiated fever (Dengue Fever, DF)” change to undifferentiated fever and dengue fever
   Line 71: “with aid the of” replace with the word “by”
   **Para 2**
   Line 80: “the detection of virus NS1 antigen” change to “the detection of virus encoded NS1 antigen”
   Line 82 to line 83:
change to “Assays have been developed to diagnosed DENV infections by the detection of NS1 protein in blood during the acute phase”

Para 3
Line 87: “accuracies” change to “accuracy”

Line 89 – 92 : change to “A multi-country evaluation study reported that the best performing NS1 assay had only a moderate sensitivity (median 64%, range 34-76%), with 100% specificity. The poor sensitivity of the evaluated assay has been related to geographical regions of study site suggesting the need for further assessment [10].”

Para 4
Line 90: “becoming” change to “has been”

Line 99: “isolated” change to “collected”

7. Materials and Methods
“Sample collection and dengue detection” change to “Sample collection and detection of dengue”

Para 1
Line 114 - 115: Change to “febrile patients with clinically suspected dengue based on WHO-SEARO 2011 guidelines were enrolled upon obtaining written consents”
Line 116: Omit “Detection of”

Line 118 - 122: change to “Dengue infection was confirmed with the results of conventional RT-PCR … as the gold standard”
Comment: Authors need to clarify of different molecular methods used

RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)
Line 133 - 136: rewrite as “RNA extraction and PCR preparation/reaction procedures were performed at the Good Clinical Laboratory Practice (GCLP)-certified laboratory at the Eijkman Institute. Strict control measures were adopted to prevent cross contamination between samples”

8. Results
Sub topic, Sensitivity of NS1 and IgM tests “referred to” genome detection/virus isolation
Comment: Change to “Sensitivity of NS1 and IgM tests with reference to genome
detection/virus isolation”

Line 193 to line 197:
Comment: Change to “Of the samples tested 106 were positive for NS1 antigen and 188 samples were positive by dengue genome detection and/or by virus isolation”

Line 199 - 201: change to “None of the 43 confirmed non-dengue cases and 20 healthy individuals’ samples were positive by NS1 ELISA giving specificity of 100%”

Line 202 - 204:
Change to “140 (73.7%) of samples detected positive by Panbio Dengue Duo IgM & IgG Capture ELISA. If both NS1 and IgM positive results were combined, the detection rate was increased to 89.4”.

Para 2
Line 229 - 230: Comment: Omit as it is given in the methodology

Major compulsory revisions

1. Materials:

Line 122: “To confirm the accuracy of the detection results, ELISA was repeated on NS1-negative samples”
Comment: meaning not clear, did authors repeat NS1 ELISA assay on negative samples?

Line 123 - 126:
Comment: Authors have used molecular assays and/or virus isolation as the gold standard. Detection of IgM and IgG in a single sample is not confirmatory and cannot use as support the diagnosis in PCR positive samples. However it is useful in identifying primary infection from Secondary infections according to the manufacturer’s guidelines

Para 1: Re writing recommended as it has included methodology. This para should contain only results. Should not have repetition with methodology

2. Results:

Line 197: Using the molecular detection results as the gold standard, the sensitivity of NS1 ELISA kits was assayed. For all Indonesian samples, the sensitivity of NS1 ELISA reached 56.4% (Table 1)

Comment 1: Did the authors use dengue genome detection and/or by virus isolation as gold standard or only the dengue genome detection as mentioned in the methodology? Clarify
Comment 2: Sensitivity of NS1 ELISA only the average value is valid as some centers has only few positive samples.
Sentence corrected as “Sensitivity of NS1 ELISA was 56.4% when genome detection and / or by virus isolation used as the gold standard”

Line 201: As the IgG test could not be used to distinguish between current and past dengue infection, in this study we did not assessed its performance
Comment: Did the manufacturer mention that an IgM result by itself is valid for this Duo test? Authors need to clarify

Sub topic: Comparison of the sensitivity of NS1 test and IgM test

Para1:
Comments: 1. Different geographical region cannot be compared to each other, in some regions there is not sufficient number of samples
2. Performance of NS1 ELISA and IgM ELISA cannot be compared directly as these two markers detect diagnostic markers at two time frames (acute Vs convalescent) of the dengue illness. Performance should be checked independently against the duration of illness

Line 211:
Comment: Without the duration of illness these observations are not valid and may be incorrect. According to authors observations, IgM positivity in secondary infections is higher than in primary infections which is in contrast to published information

Sub topic: NS1 sensitivity in relation to geographical regions, virus serotypes, disease severity, and infection status

Line 216:
Comment: Not correct as in some regions only few samples included

Line 220 -227:
Comment: Better to give the result and then the explanation
A total of 23 out of 24 samples from Samarinda that were positive for DENV-4 by RT-PCR, which included two mixed infections with DENV-1 and -2 (data not shown) were negative for NS1 contributed to the low sensitivity of the NS1 ELISA in detecting DENV-4. Confirmation with IgM and IgG ELISA detected 20 of them positive for dengue

Line 230:
Comment: Statistical significance has been calculated comparing the two tests not the different component of the said variable. Authors should have separate tables giving the statistical significance of variables they mentioned

Line 231 - 232:
Comment: Statement cannot be applied to DSS group as there not enough subjects in this group

Line 234 - 238:
Comment: Not valid for geographical regions as some regions sample size is not sufficient

Sub topic” IgM sensitivity in relation to geographical regions, virus serotypes, disease severity, and infection status

Line 242 - 245:
Comment: Not valid for geographical regions as some regions sample size is not sufficient

3. Discussion
Para 1
Line 274: word “kit” change to “assay” in all places

Line 275 - 278:
Comment: Authors mentioned use of PCR and / or virus isolation as the gold standard in the methods and in results

Line 278 - 281:
Suggest: Samples positive by RT-PCR and/or virus isolation were subjected to NS1 gene sequencing and then analyzed according to the NS1 positivity by ELISA. Give the number of samples that were PCR positive but repeatedly negative by NS1 ELISA

Line 283: Overall, our results revealed the sensitivity of the NS1 kit reached 56.4%.
Suggest: Our results detected overall sensitivity of 56.4% in the NS1 assay used.

Line 286 -288:
Suggest: Panbio Dengue Duo IgM & IgG ELISA assay detected IgM in 140 of the 188 samples tested with sensitivity of 73.7%
Comment: Authors cannot claim “this data suggest the better detection rate of IgM than that of NS1” without comparing the duration of illness.

Line 292 to Line 325:
Comment: Discussion should follow the correct statistical analysis of the variables. Statistical analysis performed comparing the two tests rather than the components of the variable

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: Not applicable