Author’s response to reviews

Title: Evaluation of the performances of six commercial kits designed for dengue NS1 and anti-dengue IgM, IgG and IgA detection in urine and saliva clinical specimens.

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Author’s response to reviews:

Reviewer #1:

1. Lines 72-75

The authors should adhere to the original terms used in the 1997 WHO Dengue classification. They should not classify according to "non-severe" and "severe" as this may be easily confused with the 2009 WHO Dengue classification.
We thank Reviewer 1 for pointing this mistake. The two sentences lines 72-75 were modified and it now reads: “Fifty patients presented with mild symptoms defined by the 1997 WHO criteria as classical dengue fever (DF) and 36 patients experienced severe symptoms compatible with the diagnostic of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [12]”.

2. Lines 84 and 91

Replace "medium" with "averagely" to maintain consistency between the prose and tables.

- We thank Reviewer 1 for pointing this inconsistency between the prose and the tables. We amended the text, which now reads lines 88-92 “The samples of the antibody panel were classified into three categories based on whether the corresponding plasma sample collected at the same time point was weakly, averagely or highly positive in antibody based on the optical density (OD) measured by ELISA. Similarly, the samples included in the NS1 panel were classified into weakly, averagely or highly positive based on the concentration of NS1 measured by ELISA in the corresponding plasma specimen.” and lines 97-99 “Weakly, averagely and highly positive plasma samples corresponded to samples with a result lower than the 1st quartile, between the 1st and the 3rd quartile and greater than the 3rd quartile, respectively.”.

3. Line 106

For the benefit of readers, the authors may wish to describe the accuracy of their IPC ELISAs here in brief.

- We thank Reviewer 1 for this comment. We chose not to give details of IPC ELISAs because the accuracy of these tests have already been fully describe our paper entitled Value of Routine Dengue Diagnostic Tests in Urine and Saliva Specimens (Andries A-C, Duong V, Ly S, Cappelle J, Kim KS, Lorn Try P, et al. Value of Routine Dengue Diagnostic Tests in Urine and Saliva Specimens. PLoS Negl Trop Dis. 2015;9:
e0004100. doi:10.1371/journal.pntd.0004100). We amended the text as following: “These ELISAs and their performances were previously described in details by Andries et al. [11].”.

4. Page 9

The authors may wish to tabulate the results reported on this page into a table such that readers can follow them more easily.

- We are grateful to Reviewer 1 for this constructive comment. A new Additional File (Additional file 2) has been created in order to help readers to follow the results reported page 9.

5. Line 173

The authors may wish to specify the "direct relationship" between the level of antibodies in plasma samples and the percentage of positive urine and saliva samples tested.

- The text was amended in order to specify the direct relationship we mentioned line 181. The paragraph now reads: “For each of the three different antibody isotypes, there was a direct relationship between the level of antibodies in the plasma samples and the percentage of positive urine and saliva samples tested by IPC ELISAs and RDTs (Table 3). The higher the level of antibodies was in the plasma sample, the higher the probability was for the corresponding urine or saliva specimen to test positive.”

6. Page 10

The authors must ensure that the results reported in the prose (both in the Results and Discussion sections) correspond to those reported in the tables. There have been numerous circumstances where the numbers reported in the prose differed from those reported in the tables.
- We thank Reviewer 1 for pointing this problem of discrepancy between numbers reported page 10 in the prose and those in Table 4. All numbers were checked and corrected in the text where necessary.

7. Lines 199-204

The authors discussed that by combining the IgM and IgG results, the overall diagnostic sensitivity in saliva can be increased. The authors may wish to discuss further the rationale behind combining the IgM and IgG results either here on in the Discussion section.

- We thank the Reviewer for this comment. We added now in the discussion section the following sentences to further clarify the value of combining IgM and IgG tests: “IgM levels peak in the serum about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life. During a secondary dengue infection IgG is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life. Early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used. Combining IgM and IgG test therefore offers the possibility to serologically detect a dengue infection during a larger window of time. In addition, the comparison of IgM and IgG results distinguishes primary and secondary dengue infections [2]. As a result, diagnostic kits combining both anti-dengue IgM and IgG antibodies became increasingly popular”

8. Line 245

During the review, I was not able to identify the 9 urine samples tested positive with RDT but negative with IPC ELISA from the tables.

- We are apologize for this unfortunate oversight. The 9 urine samples that tested positive with the NS1 RDT but negative with the NS1 IPC ELISA are now clearly identifiable in the Additional file table 2a.
9. Line 248

The authors should either delete the phrase "of the conjugate" or explain what this conjugate is, as it was not mentioned prior to this sentence.

- We thank Reviewer 1 for pointing out this problem in our paper. We added a short explanation of what a conjugate is and the sentence now reads “The reduced specificity observed with the RDT in saliva specimens could be a consequence of a non-specific adhesion of the detector colloidal particles, which are referred to as conjugates, to the nitrocellulose membrane.”

10. Line 251

Please start a new paragraph with "The IgG RDT demonstrated...".

- We followed the advice of Reviewer 1.

11. Lines 251-253

For the benefit of the readers, the authors may wish to state the cut-off value for kappa coefficients in order for two tests to be considered in good agreement.

- The text was amended in order to state the cut-off value for a good agreement. The text now reads: “The IgG RDT demonstrated a good agreement, defined by a kappa coefficient ≥ 0.61 [26], with the in-house reference IgG indirect ELISAs, in both saliva (kappa coefficient: 0.62) and urine (kappa coefficient: 0.71) specimens.”.

12. Lines 253-255

This statement contradicts Table 3 whereby it was shown that the urine IgA RDT had a detection rate of 71.4%.
We are sorry for this inaccuracy in the text and we thank Reviewer 1 for pointing it out. The text was amended lines 268-270 as following: “IgM and IgA RDTs performed less well in both saliva and urine. The agreement of these tests with the corresponding IPC ELISAs was only fair (0.21 ≤ kappa coefficient < 0.41) to moderate (0.41 ≤ kappa coefficient < 0.61). Sensitivities of the RDT for IgM and IgA detection in saliva were lower than 50% even in patients with high levels of the corresponding antibody isotype in the plasma specimens collected at the same time-points.”

13. Lines 277-278

The authors may wish to further discuss the limitation on the use of samples from healthy individuals as negative controls, instead of samples from febrile, non-Dengue patients. This is important as non-specific binding in the saliva RDTs may become more pronounced in samples from febrile, non-Dengue patients, compared to healthy individuals.

We thank Reviewer 1 for this constructive comment. We amended the text lines 295-297 in order to add this limitation: “Moreover we used only samples from febrile, non-dengue patients to evaluate the specificity. Non-specific binding with the saliva RDTs might have been more pronounced in these samples than it would have been with samples from healthy individuals.”

Reviewer #2:

The aim of this study was to evaluate the performances of rapid diagnostic test for Dengue targeted to the detection of NS1 antigen and anti-dengue IgG, IgM and IgA in urine and saliva. Authors used a sample of urine and saliva specimens collected sequentially from 86 patients with a confirmed dengue infection. More than one specimen per patients were used since a total of 291 saliva and 440 urine samples were included in the NS1-evaluation panel and 530 saliva and 528 urine specimens for antibody RDT tests. The reference used in this assessment of performance was an in house ELISA. The development of Dengue rapid test for clinical practice, particularly for severe form of dengue is of interest and welcome. However, the performance obtained in this study is far from being convincing, in particular in the light of low to very low and sometimes extremely poor sensitivity.
The paper also suffers of several weaknesses

1) The abstract does not give the major results. Nothing is said there on the sensitivity.

- We thank Reviewer 2 for this useful comment. The results section of the abstract was amended as following: “The RDTs demonstrated an overall sensitivity of 15.5%/27.9%/10.7% for NS1/IgG/IgA detection in urine samples and 20.4%/34.8%/11%/6.2% for NS1/IgG/IgM/IgA detection in saliva samples. Compared to the in-house NS1 ELISA, the results obtained with the NS1 RDT demonstrated a good correlation with urine samples (kappa coefficient: 0.88) but not with saliva specimens (kappa coefficient: 0.28). RDTs designed for antibody detection in saliva and urine were extremely specific (100%), but less sensitive than the in-house ELISAs (i.e. reduction of the overall sensitivity by 12.2% for the RDT designed for IgG detection in urine and by 23.7% for the RDT detecting anti-DENV IgM in saliva). IgM were not detected in urine, either by RDT or ELISA.”

2) The results are presented in a very confusing way.

- We are sorry that Reviewer 2 found the presentation of the results confusing. A new Additional table had been added in order to help the readers to follow the description of the comparison between the RDTs and the in-house ELISAs.

3) The study is based on 86 patients with repeated specimen. The analysis has not taken into account the repeated nature of the data which have a significant impact on the variance.

- We thank reviewer 2 for this comment. We made a mistake in our Methods section as only non parametric tests (McNemar test) were performed in order to avoid the problem of variance. We amended the Methods as following: “Statistical differences between various categorical groups were detected using McNemar’s test.”.
4) The conclusion which says "…would benefit from some further improvement…" is excessively optimistic with such levels of sensitivity obtained and not acceptable. This is also true for the conclusion of the abstract.

- We thank Reviewer 2 for this remark. Both conclusions were modified. The conclusion of the abstract now reads: “Although the RDTs evaluated here offer an apparently attractive approach for dengue diagnosis, this study suggests that these new commercial kits would require further improvement to increase the sensitivity.”

- Whereas the conclusion of the text reads: ”Although urine and oral fluid-based rapid diagnostic tests offer an attractive apparent alternative option to blood for dengue diagnosis, this evaluation suggests that this first series of diagnostic tools developed by a commercial company really need further improvement especially in a context where the body fluids explored are already known to perform less well compared to blood specimens for the diagnosis of dengue infection”

5) The authors say that he In House ELISA was used as a reference. This is not true and misleading for the reader since the reference is a confirmed dengue case based on RT-PCR and/or the detection of the NS1 protein and/or an IgM seroconversion and/or a fourfold antibody titer increase measured by hemagglutination inhibition assay in paired plasma of patients presenting with symptoms suggestive of a dengue infection.

- We are grateful to Reviewer 2 for pointing out this inaccuracy in our manuscript. The real reference is indeed a confirmed case based on RT-PCR and/or the detection of the NS1 protein and/or an IgM seroconversion and/or a fourfold antibody titer increase measured by hemagglutination inhibition assay in paired plasma of patients presenting with symptoms suggestive of a dengue infection. Nevertheless as the RDTs evaluated in this study are designed for dengue diagnosis in urine and saliva we didn’t want to compare them to tests performed in plasma. Thus we preferred to compare the RDTs to our in-house ELISAs. In order to clarify this, we removed the word “reference” when we are referring to our in-house ELISAs. This has been further clarified in the revised version of the manuscript and the result section was amended as follow: “The RDTs evaluated in this study were compared to in-house ELISAs and we will refer to them as “Institut Pasteur Cambodia (IPC) ELISAs” throughout the manuscript. A capture ELISA was used to detect NS1 in plasma, urine and saliva. Anti-DENV IgG were detected in plasma, urine and saliva by an indirect ELISA whereas anti-DENV IgM and IgA were detected by capture ELISAs (MAC-ELISA and AAC-ELISA, respectively). These ELISAs and their performances were previously described in detail by Andries et al. [11].”
6) The differential positivity rates between the IPC and RDT tests that they used in the abstract and results sections are therefore not appropriate. While comparing a RDT test with another in-house test that does not perform well in term of sensitivity in urines and saliva.

- Please kindly refer to the answer of the previous comment that clarifies and justifies our methodological approach.

7) The authors do not refer to the clinical perspective for which their rapid test would be used.

- We thank Reviewer 2 for this comment. We amended the text in that section that now reads: “Saliva and urine specimens provide interesting advances for dengue diagnosis as their collection is non-invasive and thus well accepted by patients. In addition, it does not require medically-trained staff and the samples are easy to process as it does not require on-site laboratory for centrifugation. In a previous study, we demonstrated that urine and saliva samples were interesting alternatives to venous blood specimens for dengue diagnosis in all situations when blood collection was difficult [11]. In this recent study, in-house ELISAs for NS1 antigen and anti-DENV antibodies detection were developed to assess the value of urine and saliva specimens for dengue diagnosis. We showed that antibody detection in saliva and urine was useful for instances such as outbreak investigations or in young children, in addition to the all situations when blood could not be easily collected (e.g., lack of phlebotomist, refusal of procedure, etc.). Good salivary- and urinary-based RDTs could be valuable tools for dengue diagnosis as they combine easy sampling methods with rapid, equipment-free testing. Such kits could be used by nurses, at the patient’s bedside, in hospital settings but also by general practitioners in private clinics or by epidemiologists during outbreak investigations and field studies.”