Author's response to reviews

Title: Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses

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Author's response to reviews: see over
Dear Editors,

In addition to the editorial requests (in particular, the addition of a sentence in the background part of the Abstract and the addition of Conclusions, Competing interests, Authors’ contribution and Acknowledgements sections), we have tried to addressed the vast majority of all the referees’ comments.

Major compulsory revisions from referee #1:

I agree with these findings and conclusions of this paper. However, the presentation of the results in tables 2, 3, 4 and in the text is not very clear. The authors compare sensitivity of dengue RDT’s in these tables: BR-NS1, SD-NS1, SD NS1 and/OR IgM, SD NS1 and/OR IgM and/OR IgG. If the authors use the statement “SD NS1 OR IgM OR IgG”, I understand that they consider a patient with a positive result for IgM detection with negative NS1 detection as a dengue confirmed case. The same comment could be underline for a positive IgG detection. IgM can persist several weeks/months and this is well underlined in the discussion. IgG persist after a primary infection: an isolated positive detection of IgG cannot be associated with a confirmed diagnosis of dengue infection. Moreover antibody cross-reactivities with other flaviviruses exist. The exact significance of “SD NS1 and/OR IgM” and “SD NS1 and/OR IgM and/OR IgG” must be clearly defined in the manuscript. It refers to algorithm previously published in ref 11, but did the authors use the second serum for general conclusion on dengue status? The authors must clarify.

Authors response:

As suggested, we have changed the phrasing in the manuscript and tables 2, 3, 4 to “SD NS1 or IgM”, “SD NS1 or IgG” and “SD NS1 or IgM or IgG. The “dengue” patients in the study were clinically suspected to have dengue and in reference laboratory tests all patients were RT-PCR positive with either confirmatory or highly suggestive serology using paired plasma samples. This has been made clear in the Materials and Methods section. The reviewer makes the point that a positive IgM or IgG result with a negative NS1 result in the rapid test could reflect recent infection in the past weeks or possibly months, rather than acute infection. We entirely agree and had highlighted this limitation in the Discussion. Nevertheless, the reality is that rapid tests that detect DENV- IgM and IgG are widely available and in some settings frequently used. In the context of a patient in whom the differential diagnosis includes dengue, the finding of positive IgM or IgG in a rapid test is frequently interpreted as sufficient for a presumptive diagnosis by clinical staff. One of the aims of the manuscript is to determine whether such interpretation is reasonable when using the SD Duo rapid test. In response to the reviewers comments, we have modified the manuscript and tables 2, 3 and 4 to highlight that inclusion of IgM or IgG results is not sufficient for a definitive diagnosis of dengue. In tables 3 and 4 we have deleted reference to the IgM and IgG results in the assay.
The reviewer #1 makes an important point and this relates to the statements “SD NS1 and/or IgM” and “SD NS1 and/or IgM and/or IgG” used in the text and in the Tables 2, 3 and 4. We do agree that these statements can be confusing and the referee’s suggestion to use instead “SD NS1 and/or IgM” and “SD NS1 or IgM or IgG” is good. By using “and/or” we wanted to highlight that for example “SD NS1 and/or IgM and/or IgG” positive samples can be NS1 positive or NS1 and IgM positive or NS1 and IgG positive or IgM positive or IgG positive and in all these cases can be considered as positive. But to avoid any confusion, we have added a warning sentence in order to clarify the exact meaning of these statements in page 7.

Another concern raised by the referee #1 is about patients with a positive result for IgG detection but negative NS1 detection. One of the challenges in serological diagnosis of dengue is that some patients with RT-PCR confirmed DENV infections make very robust IgG responses but weak or un-measurable IgM responses. In the study panel described in Table 2, 3 and 4, there were 21 patients who were IgG positive but IgM and NS1 negative in the SD rapid test. Of these 21 patients, 20 were DENV RT-PCR positive. Therefore the inclusion of an IgG positive result on the rapid test was a useful marker of acute DENV infection. Caution is needed however as a positive IgG alone could also represent infection anytime in the previous few months. The potentially for poor specificity was highlighted in that one patient with no laboratory acute evidence of dengue had a positive IgG test result alone (Table 2, last row). We have made comments to this effect in the Discussion page 13 and highlighted the problems in interpreting a positive IgG test result alone.

Concerning the algorithm used for lab-confirmed dengue diagnosis and if the second samples have been used, we have added the description of the algorithm (as asked by the referee #2) in the Materials and Methods section and explained how the second samples are used in order to confirm or not the dengue diagnosis (please, see page 6).

Minor essential revisions from referee #1

1- Introduction:
Line 2: replace (DENV1-4) by (DENV-1 to DENV-4)
At the end of the introduction, the sentence “Our findings suggest that…” is a summary of the results. This part should be removed from the introduction.

Authors response: “DENV1-4” has been replaced by “DENV-1 to DENV-4” and the sentence at the end of the introduction has been moved to the Conclusions section.

2- Discussion, page 12:
“It is possible these factors might account for the differences between the study findings, It may also reflect chance differences associated with relatively small sample sizes.” Replace the comma by a point after “findings”.

Authors response: comma has been replaced by a point.

3- Page “figure 3”, replace figure 3 by figure 3A and page “figure 4” replace figure 4 by figure 3B to be consistent with the text.

Authors response: now the figures 3A and 3B are on the same page “figure 3” and the page “figure 4” has been deleted.

Minor essential revisions from referee #2:

1- Page 6 paragraph 2: The algorithm used in this study should be described in M & M and not referred in other publication.

Authors response: The algorithm is now described in the Materials and Methods section page 6. The basis for the diagnosis in the case population has been clarified in the M&M section.

2- Page 6 paragraph 2: The author states that the ELISA test used to detect IgM and IgG was the one defined by Innis et al as IgM and IgG antigen capture ELISA. Please check it out because I think it is IgM and IgG capture ELISA.

Authors response: That’s correct. The word “antigen” has been replaced by “antibody”.

3- Figure 3 and 4: They are complex and it is not clear how the author establish the difference between NS1 positive and negative. It is necessary to be more explicit to convince.

Figures 3 A and B are indeed complex and it is why they are only used to illustrate the relationship between the detection of NS1 (by the BioRad rapid tests), time since illness onset and in particular, the interfering effect of DENV-reactive IgG in the test sample (as it is written described in on page 11). Because the presence of DENV-reactive IgG in the test samples is a major factor associated with a negative NS1 result, and the IgM and IgG titers are expected to rise with the time since illness onset, we believe that these figures provide an elegant summary of the dynamics of the NS1 detection window, worth to be displayed.

We sincerely hope that our responses would satisfy editors and reviewers. We would like to emphasize that both referees have found our work of relevant importance in the dengue field. Because dengue fever is a major public health concern and its rapid, easy and effective diagnosis is crucial, we still believe our manuscript represents a timely and significant piece of work and we would be very happy to have this paper accepted for publication in BMC Infectious Diseases.

Sincerely,

Vianney Tricou.