Author’s response to reviews

Title: Detection of dengue viruses using reverse transcription-loop-mediated isothermal amplification

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Author’s response to reviews: see over
Dear Editor,

Thank you for giving us the opportunity to revise the manuscript 8332620219503180. Herewith, please find the point-by-point response to all the reviewers’ comments. We have carefully revised the manuscript to fully address all the relevant comments. We have added additional sentences, where necessary to explain further areas which were unclear. We hope that these changes meet both yours and the reviewers’ expectations.

All the authors have read the revised manuscript and agreed to its submission and are responsible for its content. Please do not hesitate to contact us shall you require further clarifications.

Thank you in advance.

Sincerely Yours,

Sazaly Abu Bakar
Corresponding Author
Reviewer: 1

Comment 1

The title is rather long and it should be shortened to read: Detection of Dengue viruses Using Reverse-transcription Loop-mediated Isothermal Amplification.

Reply: The title has been revised as suggested. Please see page 1, line 1-2.

Comment 2

The RT-LAMP assay have already been developed and evaluated for detection of Dengue virus as mentioned in the reference list (Reference Number 32; Parida et al., 2005; Reference number 33 Li et al., 2011). However, in the present study a large sample size (n=305) was considered. For this reason, I believe this manuscript should be condensed (shortened) accordingly and published as a short communication.

Reply: Thank you for the suggestion. We have tried to keep the manuscript as succinct as possible. Do note that the current manuscript not only used larger sample size but also used a re-designed set of primers which would require the whole complete evaluation to ensure high reproducibility. As such, our RT-LAMP assay is sufficiently different from those previously reported (please see page 13, line 303-320) that we suggest it to remain as full research article. In this study, we presented a different strategy for primer design which enable single tube system for simultaneous detection of all four DENV serotype by targeting at the most conserved region of viral genome (please see Table 1 and Additional file 1). In addition, our developed assay was the first to be evaluated against the various DENV genotypes common in the region.
Reviewer: 2

Comment 1
The authors present a new methodology with the advantage of exquisite PCR sensitivity, and simplicity allowing field use. They compare it with standard ELISAs for IgG and IgM. The comparison which is missing however, is with results using serology assays in the format of immunochromatography which are very simple, completely field compatible except for conservation conditions, and sensitive early during the acute episode especially when including detection of NS1 antigen (e.g. Panbio Dengue Duo and Rapid). Indeed, this is the test which the authors should demonstrate that LAMP is superior to. Otherwise, there will be no incentive to displace immunochromatographic assays.

Reply: The immunochromatographic NS1rapid format test is indeed one of the simple and rapid tests for early detection of DENV infection. However, its utility has been limited because reduced sensitivity of detection of the test has been reported in the secondary infection (please see page 4, line 91-94, or please refer to References 24-27 on page 18). In endemic regions where sequential DENV infection is common, the rapid NS1 test results could contribute to high false negative results. In Malaysia, for instance, previous report has shown high dengue IgG seropositivity (91.6%) among the healthy blood donors who might at risk of secondary infection (Muhammad Azami et al., 2011). A NS1 negative assay result, thus does not exclude dengue and the molecular detection of viral genome is still the preferable method for confirmation of acute dengue diagnosis in complementary to dengue-IgM ELISA especially in the dengue endemic regions. Therefore, in our study, we did not include the rapid format immunochromatographic NS1 test as it is not being used or currently considered for implementation in our hospital.

Reference:

Comment 2
p6: RNA extraction: precise volume subjected to extraction and elution volume so that the volume of samples corresponding to the 5 ul of extract can be deduced.

Reply: The precise volume of samples used has been added. Please see page 6, line 138.

Comment 3
p7, line 168: replace "non-fluoresce" by either "non-fluorescent" or "does not fluoresce".

Reply: The "non-fluoresce" has been replaced by "non-fluorescent". Please see page 7, line 170.
Comment 4

Table 1: could the authors display the primers in alignment with the serotype consensus sequences?

Reply: A new supplementary figure displaying the map of primers has been added (please see page 10, line 236-240, and please refer to Additional File 1: Figure S1). A figure legend has also been added accordingly, please see page 23.

Comment 5

Figure 1 and 2, please put labels on the figure in addition to the legend, to make reading of the figures easier.

Reply: The Figure 1 and 2 have been edited as suggested. Please refer to Figure 1 and 2.

Comment 6

p8, line 186: what means "genesig": typo?

Reply: "genesig" is not a typo, it is a product name for the molecular detection kits by PrimerDesign company.

Comment 7

p11, line 275: clarify: 50 copies per reaction or per ml?

Reply: The sentence has been revised. Please see page 12, line 284-285.
Comment 1:
"In addition to the reviewers’ remarks, the authors should edit Figure 5, providing a clearer definition of positive samples (a combination of positive tests)"

Reply: Figure 5 has been edited as suggested, please see Figure 5. In our study, the acute DENV infection was confirmed by the positive detection of anti-DENV IgM by ELISA and/or the presence of DENV RNA detected by qRT-PCR assay. Several paragraphs have been revised to further explain the definition of positive samples, please see page 9, line 213-216; page 11, line 273-277.

Comment 2:
"Tabulating in a table the diagnostic performance of each assay (sensitivity, specificity, PPV and NPV)"

Reply: Table 3 has been added to summarize the diagnostic performance of RT-LAMP in comparison to the qRT-PCR assay. Please see Table 3.

Editorial Requirement:
--> Please clarify within your methods section if all clinical samples were taken as part of standard patient care.

Reply: Yes! All clinical samples were taken as part of standard patient care. The method section has been revised as suggested, please see page 6, line 132-135; page 9, line 218-222.