



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2013_IJBcRR_7747
Title of the Manuscript:	Multi-detection by target mixed loop-mediated isothermal amplification
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Line 32: What is SDA and NASBA? The author should make abbreviations clear to the readers, by providing the full names the first time they are mentioned in the paper. Results – Section 3.1 “Normalization of the primer to the LAMP reaction” and Table 1, should be part of the methods instead of the results. What do the authors mean by normalization of the primers? The authors should provide details of how the normalization of the primers was carried out. This would enable other researchers to reproduce the results. Lines 134-136: “In target mixed LAMP, the signal detection was 24 min using mixed primers. The specificity of the products was confirmed by dot blot hybridization, resulting in that all cDNA was amplified. However, the amplification level of each cDNA was unknown because target mixed LAMP products cannot be shown separately”. This presupposes that using target mixed LAMP for diagnosis comes with the additional need for confirmation using dot blot, since the target mixed LAMP products cannot be shown separately. While LAMP on its own can be carried out in less than 1 hour, hybridization takes time and requires technical knowhow and equipment. The authors should discuss this as a possible drawback to their target	We wrote the full names of SDA and NASBA. We shifted result 3.1 to Materials and Methods section. In Materials and Methods section, we wrote that “We normalized the primer concentration for each set of primers so that the reaction time for each set was the same for a set quantity of template DNA.” To confirm the specificity of target mixed LAMP, we performed dot blot hybridization. In clinical field and so on, we intend to examine the endpoint detection using a fluorescent metal indicator such as calcein. In conclusion, we wrote that “Although we have successfully demonstrated the effectiveness of target mixed LAMP by real-time detection and dot blot hybridization, we intend to further develop the amplification detection methods to be used clinically, with particular emphasis on utilizing turbidity and calcein detections.”



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	mixed LAMP method. There are instances in the paper, where the authors have made reference to results (data not shown). If these are not important for providing clarity to the paper, then I would suggest that these be removed. However, in this case they seem to be important in the discussion of the paper. The authors should provide these in the results/ or provide them as supplementary data files. The paper by H. Iseki et al. / Journal of Microbiological Methods 71 (2007) 281–287, describes the development of a multiplex LAMP method and is vital to the readers in understanding multiplexing in LAMP. The authors should provide this reference in their study and discuss it with their results.	We added the unpublished data in the discussion as a supplementary data. We added a paper (H. Iseki et al. / Journal of Microbiological Methods 71 (2007) 281–287) in reference and wrote it in discussion.
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<u>Minor</u> REVISION comments	Line 35 should read: We have found that LAMP can amplify more than one target DNA... Line 51: What is SRL, inc? Line 61: 1ul instead of one ul. Same for line 71. Line 113-114: LAMP amplification was occurred two of four sera. Please revise this sentence. Line 16: In comparison with TMA... Lines 148-154: The sentences are not clear, and need revision. Lines 167-168: Review the sentence. Lines 177-178: Review the sentence.	We employed an English-language editing service to polish our wording.
<u>Optional/General</u> comments	This paper represents the simultaneous detection of multiple infections using multiplexed LAMP. It provides important information on multiplexing LAMP assays, that can be applied in research and health facilities. The authors should revise the paper for typos and incorrect sentences.	We employed an English-language editing service to polish our wording.