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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

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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	This manuscript describes a study of the development of Multi-detection by target mixed loop-mediated isothermal amplification (LAMP). LAMP is a powerful technique to rapid amplifies specific nucleic acid for diagnosis application. So far, very few LAMP methods can be applied to detect multiple DNA a target in one reaction, because of there is no way to distinguish the typical DNA ladder-like pattern produced by one LAMP reaction from other LAMP reactions. Even through, authors used a real-time LAMP to monitor the amplifying signal, it still cannot figure out this problem. Special Comments is placed on the following points: (1) The authors indicate that the established LAMP method herein is able to amplify more than one target simultaneously, namely mixed LAMP. In the cases of figure 1 & figure 2, authors cannot provide evidence that to describe the mixed LAMP can be performed at same time in terms of a single curve of figure 1B and 2B. Thus, authors should make a designation by using different kind of fluorescent for labelling the individual LAMP product within a target mixed LAMP reaction during monitoring the reaction. (2) As to above problem, in the cases of the figure 1A and 1B, authors provides a result for demonstrating the entire single LAMP can be performed for detect individual DNA target. In these figures, various LAMP reactions showed	(1), (2) 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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	different sensitivities in terms of three single curves (figure 1A and 1B). If all of LAMP reactions (mixed LAMP) with different sensitivity of reaction, therefore, the result of mixed LAMP should not be demonstrate a perfect and single reaction curve (in the figure 1B & 2B). Authors should explain it and provide a detail data as to the sensitivity of LAMP when various LAMP primers were used. (3) In this manuscript, author newly designed several set of LAMP primers to amplify the specific target DNA. However, there is no any figure to show their specificity of LAMP. Why? (4) In this work, 5'-DIG-label primer was used to detect the amplified target DNA. However, the signal sensitivity of DIG is high, how authors can avoid the interference of background DNA (Temple DNA) when DIG primer was probed. (5) When mixed LAMP was performed, their reactions are very complex. Authors should design an experiment to show their mixed LAMP is really stable. All LAMP reaction can be performed simultaneously without any interference result from other LAMP reaction. (6) Both of these issues needed to be addressed for the manuscript to be acceptable.	(3) To confirm the specificity of target mixed LAMP, we performed dot blot hybridization. Because the gene is not amplified with negative control DNA, we believe that the specific target could be amplified by LAMP. (4) The signal of dot blot hybridization was detected using the DIG Detection kit (Roche, Mannheim, Germany) according to the manufacturer's protocol (5) In discussion, we wrote that "In order to succeed target mixed LAMP, it is necessary to be careful about interaction of primers, such as primer dimer, when primer sets are constructed.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		