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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

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.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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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	Multiplex loop-mediated isothermal amplification (mLAMP) is important for molecular diagnostics, with the ability to detect multiple targets in a single sample. Previous studies have reported mLAMP detection methods through agarose gel, amplicon annealing temperature, pyrosequencing or real-time and target-specific fluorescence signal analysis. The comparative analysis should be done with those reported methods. There aren't enough data presented inside the manuscript to support conclusion. mLAMP is not simple superposition of two or more single LAMP. To develop a rapid and sensitive mLAMP assay for multiplex detection, primers screening, sensitivity and specificity analysis should be done first comparing to single LAMP. Gradient dilution of the sample and other virus nucleic acid samples should be analyzed with the mLAMP. When clinical sera being detected by mLAMP, reverse transcription LAMP for RNA from virus HIV and HCV must be carried out. The number of clinical samples was not enough for the application analysis, so the result is short of conviction. There is no relevance between mLAMP detection of different virus and different mice gene in the manuscript. And mLAMP can not be qualified for quantitative gene expression analysis. English language should be strongly revised.	Because we did not have a serum sample infected with HIV and HCV, we were not able to test it. In discussion, we wrote that "Although this study represents the first step toward using target mixed LAMP in diagnostic testing the limited number of clinical samples prevented a more thorough analysis, and we aim to increase the number of clinical samples in the future. " In discussion, we e wrote that "Due to the variability of gene expression in a normal cell, we question the suitability of this method for quantitative gene expression analysis, although further testing is necessary."



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<u>Minor</u> REVISION comments	HBV DNA amplified using HBVF3 and HBVB3 was less than 300bp, why the PCR reaction was performed using Expand long template kit in the manuscript ? To corroborate the results reported, biological samples other than blood should be tested in order to evaluate the presence of inhibitors in some type of samples.	Expand long template kit is composed of the enzyme mix containing Taq and Pwo DNA polymerase. This polymerase mixture is designated to give a high yield of PCR products from template DNA. Therefore we used this enzyme. We want to check it in future when we test biological sample.
<u>Optional/General</u> comments	The manuscript “Multi-detection by target mixed loop-mediated isothermal amplification” involves a mLAMP method, with the ability to detect multiple targets in a single sample, which is important for molecular diagnostics. However, it has no comparative analysis with others reported mLAMP methods. The design of the experiments was not rational, and the data presented in this manuscript can not support the conclusion that the established mLAMP can effectively detect HBV, HCV and HIV simultaneously. English language should be strongly revised.	There may be little number of the samples, but this method could detect HBV, HCV and HIV simultaneously by dot blot hybridization. We employed an English-language editing service to polish our wording.