Reviewer's report

Title: Development and Evaluation of Reverse Transcription-Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Enterovirus 71

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Reviewer: Patchara Phuektes

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The manuscript described development and evaluation of Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) for detection of human enterovirus 71 (EV71). The RT-LAMP assay can be very useful for rapid and sensitive detection of EV71 from patients with HFMD. However, the manuscript still contains a number of badly worded/constructed sentences. The authors will have to check and refine the language carefully.

Minor Revisions

Background
-Ref 15 should be placed after the sentence “Recently, reverse transcription-PCR (RT-PCR) and real-time PCR assays have been used for EV71 detection”. Additionally, more references on real time PCR assays should be added.

Materials and methods
-“pharynx swab” should be “pharyngeal swab”
- A section on “virus” may be added to provide information on virus strains and/or genotype used for specificity and sensitivity tests. How many EV71 strains were tested in this study?
- Please provide manufacturers for “betaine” and “Bst DNA polymerase”
- Please provide information on “preservation solution”

Results
- Fig 2A: DNA marker should be included.
- Fig 3: please check figure legend; lane 3 is positive??
- Fig 4: lane 1-4 should be specified as RT-LAMP products of EV71
- Fig 5: the sensitivity seems to be at 10 PFU/ml rather than 1 PFU/ml, the picture of GoldView staining of each dilution reaction should be included as figure 5B. Please check figure legend; 10-3 PFU/ml.
- Fig 6: each line may be labeled as 105, 104 PFU/ml...........10 PFU/ml.

Discussion
- Line 1: “enterovirus” should be changed to “human enterovirus”. This sentence should be modified.
-Line 17: the sentence “In this study, a one step……..” need to be rephrased.

-Line 24-27: “In the present study, the amplified product….non-specific products could not be detected in LAMP assay due to without digestion” this sentence is rather confusing and need to be clarified.

-In the result section “Diagnosis of 123 clinical specimens” : the sentences “Two positive samples detected by RT-LAMP assay were then inoculated into RD cells and the positive culture was identified by indirect immunofluorescence test: however, only one sample was verified as positive EV71 infection by PCR fluorescence probing assay. This result is different from the explanation in the discussion section on line 33-36. Please clarify and explain the result.

**Level of interest:** An article of importance in its field  

**Quality of written English:** Needs some language corrections before being published  

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.