Dear Dr. Alisha DSouza:

Thank you and the referees for helping us to improve our work. We have carefully checked the comments and suggestions. Please see below for the point-to-point response. We have also modified the manuscript accordingly. We wish our manuscript is now acceptable for publication in *JoVE*. Again, thank you very much for your help.

Sincerely yours,

Sheng-ce Tao

Changes recommended by the JoVE Scientific Review Editor: **(Dark green mark in the manuscript)**

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

**We have checked it.**

• Please include at least six keywords/phrases.

**We have checked it.**

• Abstracts: Please remove the superscript citations from the long abstract, and reorder the references accordingly.

**We have omitt****ed the citations and reorder the references.**

• Introduction: Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.

**We have expanded the introduction. Line 87-92**

• Protocol Language: Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

**We have checked it.**

• Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, for steps that will not be filmed, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. For example:

1) 3.2.1: Please mention what button is clicked on in the software to do this, or which menu items need to be selected.

**We have mentioned it in line 255-269.**

• Protocol Numbering: Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

**We have checked it.**

• Protocol Highlight: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

**We have highlighted the protocol.**

• Results: Please add at least one paragraph of results text that explains your representative results in the context of the technique you describe; i.e. how do these results show the technique, suggestions about how to analyze the outcome etc. This text should be written in paragraph form under a "Representative Results" heading and should refer to all of the results figures. You may include the figure captions under this heading but the captions and figure text must be separate entities.

**We have added the representative results. line279-296.**

• Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

**We have checked and revised it.**

• Figures::

1) Please provide each figure as an individual PDF, TIFF, JPEG or PNG files.

2) Fig 3b, 4,5: Please provide scale bars, and define them in the figure legend.

**We have provided the scale bars.**

3)All figures and tables need to be referenced in your manuscript text. Please include call outs at appropriate locations.

**We have checked it.**

• Tables:: Please remove the embedded Tables from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends.

**We have removed the embedded tables and included the description of the table. line 300-306**

• Figure/Table Legends:: Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

**We have expanded the legends.**

• References: Please abbreviate all journal titles.

**We have checked and revised it.**

• Commercial Language:JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are SYBR Green, AutoCAD (Adobe), Ultra-Ever Dry Top Coat, ThermoPol, ZWB2, photoshop 7.0 software (Adobe Systems Inc., GenePix Pro 6.1, etc.

1) Please use MS Word’s find function (Ctrl+F or Command+F (on Mac)), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

**We have revised it in the manuscript. line 168, 179, 246,255,261 359.**

• Please define all abbreviations at first use.

**We have checked it.**

• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

**We have checked it.**

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**We have checked it.**

**Reply to** Reviewer #1: **(green mark in the manuscript):**

Manuscript Summary:

This paper present a capillary array device designed for visual detection of multiple nucleic acids. The main contribution of this work is reagents preloading and multiplexed detection to enhance the analysis throughput. However, the nucleic acid detection assay depends on manual operation; this may hinder acceptation of this methodology. The absence of nucleic acids extraction /purification is another limitation, which denies the device in dealing with large volume or complex samples.

Major Concerns:

It is advised that the author should add a figure showing the hydrophobic and hydrophlic area within the capillary, and the process illustration of primer immobilization, reagent preloading and sample injection.

**Answers：We thank the reviewer for pointing out this. In fact, we have showed the whole process of making capillary cassette in figure 1, including PDMS pouring, mold removing, capillary inserting & surface coating, primer fixing and cassette anchoring. And we have showed the process of sample injection in figure 2. line 308-323**

Reviewer #2: **(pink mark in the manuscript)**

Manuscript Summary:

This manuscript describes the fabrication and use of a cassette containing an array of capillaries where primers can be placed for the detection of target DNA sequences. The detection is carried out by first amplifying the DNA by LAMP and then detecting the UV absorbance of the product. As the authors state, the method appears promising for a number of environmental, industrial and health-related applications.

Major Concerns:

1) I think the English of the article should be reviewed. In its current form, I had difficulty understanding some parts, including parts of the protocol, which is key in this article.

**Answers：We thank the reviewer for pointing out this. We have carefully checked and revised the manuscript.**

2) The authors indicate that the protocol assumes that the mold with the shape for the micro-channels and the loading adapter has already been made. Although it is true that it may be too much for this protocol to explain the actual manufacturing of these parts, I think that it hinders the usefulness of the protocol. A possible solution would be for the authors to provide a working AutoCAD file of the design of the mold and the corresponding files necessary for 3D printing the adapter. They could have these files available in a repository owned by their institution or offer to provide these files to requesting researchers/developers. [Editor's Note: CAD files can be added as supplemental files in your submission]

**Answers：****We thank the reviewer for pointing out this. We have added the working Auto CAD file of the design of the mold and the adapter as supplemental files. We have also revised the manuscript accordingly, line 105-108.**

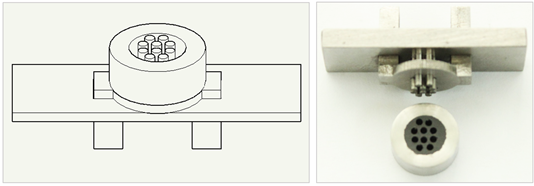
Minor Concerns:

- Step 1.2.3.) The authors indicate that the capillaries need to be shaken during the cleaning process to remove bubbles. It would be interesting here if they state whether sonication may be used for this purpose and how it compares in time and efficiency with just shaking it. They state that cleaning time may be extended when required. In this regard, it would be interesting if they state how long it may take for this cleaning process (and then, they could compare it with the time required using sonication)

**Answers：Although the capillaries need to be shaken during the cleaning process to remove bubbles, it do not need to shake all the time. It’s enough to shake the capillaries every 10 minutes. Bubble is usually produced at the beginning of adding H2O2 and as the time goes the amount of bubbles is reduced. So we haven’t try sonication. Usually 30 min is enough for cleaning of the capillaries and we haven’t observed obvious effect when extend the cleaning time.**

- Step 1.3.) Although it may be rather evident and eventually the video will show it, it would be good to state explicitly where exactly the PDMS is poured onto.

**Answers：We thank the reviewer for pointing out this. There is a cylinder of mold that will contain PDMS, when fabricating the reaction unit, we just directly pour PDMS into it. It is also showed in Figure 1. We have revised the manuscript accordingly. line 150.**



Cylinder

- The authors mention in the Discussion section that the LAMP mixture should be shaken gently instead of vortexing it because it may cause failure of the reaction. Given this consideration, the authors should discuss the convenience of having a positive control (and what they would use for that) together with the blank control they propose as a negative control.

**Answers：The positive and blank control would help us for better interpretation of the results. In this experiment, ADH1(endogenous reference gene of Maize) is set as the positive control and two capillaries without primers are set as blank controls. So, if LAMP mixture is correctly handled, the positive control would show green and blank control would keep unchanged after the test is performed. We have now discussed this in the manuscript line 368-371.**

- The authors should indicate in the table of materials and equipment a suitable supplier for the Ultra-Ever Dry Top Coat for other researchers/developers to be able to obtain it

**Answers：we have provided the supplier for the** **Ultra-Ever Dry Top Coat. Researchers can obtain it from Exiron chemistry (CHINA) CO., LTD.**