

Dear Nandita Singh,

We are very excited to submit our manuscript entitled “Visual Detection of Multiple Nucleic Acids in a Capillary Array” by Chen *et al.*, for publication in JoVE (Journal of Visualized Experiments).

Fast and low-cost methodologies for monitoring of multiple nucleic acids in a single test with simple operation is urgently desired for Genetically modified organisms (GMO) monitoring, infectious disease detection and even anti-terrorism. For example, in genetically modified organisms (GMO) monitoring, there are hundreds of known genetic modifications to be tested for a single sample. Similarly, in infectious disease diagnosis, such as pneumonia, which could be caused by a variety of pathogens. To achieve rapid diagnosis and give the patients proper treatments, doctors need to identify the causative pathogen/s or subtype/s from many possibilities as soon as possible. Importantly, the requirement of simplicity and multiplicity is even higher in point-of-care test circumstance, which is usually resource-limited.

However, traditional methodologies can hardly meet these requirements. PCR-based methodologies are usually limited by its low multiplicity, high dependency to professional equipment. Isothermal amplification methodologies also short of multiplicity.

To this end, we have described the platform called CALM (Capillary Array-based Loop-mediated isothermal amplification for Multiplex visual detection of nucleic acids) (Shao et. al., Lab chip, 2017). Herein, we described the improved fabrication and performance process. In this platform, we apply a small ready-to-use cassette assembled by capillary array for multiplex visual detection of nucleic acids. In this method, the capillary array is pre-treated into hydrophobic and hydrophilic pattern before fixing loop-mediated isothermal amplification(LAMP) primer sets in capillaries. After assembly of the loading adaptor, LAMP reaction mixture is loaded and isolated into each capillary due to capillary force by a single pipetting. The LAMP reactions are performed in parallel in the capillaries. The results are visually read out by illumination with a hand-held UV flashlight. Using this platform, we demonstrated how to monitor 8 frequently appeared elements and genes in GMO samples with high specificity and sensitivity.

To our knowledge, CALM is the most high-throughput nucleic acids detection system based on LAMP. Meanwhile, it is easy-to-use. Due to the simple operation, small size and naked eye-readout, CALM has great potential in POCT. The inherent flexibility of the capillary array format gives CALM great flexibility and expandability to be adapted to broad applications.

Reviewers who are especially well qualified to review the manuscript, along with their affiliation and contact information, are listed below:

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We claim that none of the material in this manuscript has been published or is under consideration elsewhere, including the Internet.

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