QUANTITATIVE PATHOGEN DETECTION BASED ON DIGITAL LOOP-MEDIATED ISOTHERMAL AMPLIFICATION USING CROSS-INTERFACE EMULSIFICATION TECHNOLOGY

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A number of attempts have been made for performing digital PCR[1] or isothermal amplifications[2] for the absolute quantification of nucleic acids using microfluidic technologies. Here, we describe a portable and easy-to-use digital loop-mediated isothermal amplification (dLAMP) system built on our recently reported cross-interface emulsification (XiE) technology[3].

XiE is a simple method which generates monodisperse droplets using vibrating capillaries, which can be easily set up and is user-friendly to those who lack microfabrication facilities. A schematic view of the droplet generation by cross-interface emulsification (XiE) is illustrated in Figure 1. The XiE method can generate size-tunable droplet arrays independent of device design. Thousands of nanoliter droplets can be generated in each well of a 96-well plate, and used for digital nucleic acids quantification based on dLAMP. Moreover, droplet arrays with various sizes can further expend the dynamic range of detection based on multivolume digital analysis.

We applied this XiE-based dLAMP system in rapid detection of an important infectious pathogens, *Mycobacterium bovis* (*M. bovis*), a pathogen which causes bovine tuberculosis (bTB) and human infections. We constructed the mpb70-T1 plasmid which contains unique mpb70 gene of *M. bovis*[4] and used it as the standard to investigate the detection limit of dLAMP (Fig. 2). A good linearity between input templates and measured concentration were obtained. Next, we tested the robustness of dLAMP in direct detection *M. bovis* in real samples. The plasmids with two concentrations (13fg and 130fg) were added in fetal bovine serum (Fig. 3) with or without dilution, and used as samples to perform dLAMP without further treatment. Our results showed that dLAMP is very robust and succeeded in the amplification of mpb70 gene in fetal bovine serum with 2x dilution, and the results were consistent with water control. This result validated that dLAMP can be directly used to analyze complex samples to avoid loss of nucleic acid targets during purification and further reduce the delay in diagnosis.

In summary, our results shows that the XiE-based dLAMP is highly specific and displays comparable sensitivity to real-time PCR (qPCR) and digital PCR (dPCR)[5], with reduced detection time of 30 min to 1 hour. Moreover, dLAMP can be used directly for rapid detection of diluted real samples. Therefore, our dLAMP system is especially suitable for environmental and clinical samples with hard-to remove contaminants, and can be widely applied in quantitative and timely diagnosis of infectious diseases.

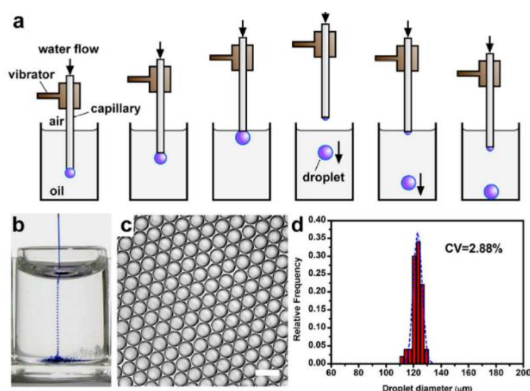

Fig 1. Droplet generation by cross-interface emulsification (XiE). (a) Schematic of a droplet generation cycle by the XiE method. (b) A picture of generating food dye droplets in an oil-filled microwell. The capillary was vibrated at 50 Hz at the air/oil interface, as food dye solution was injected at a flow rate of 50 nL/s. (c) Monodisperse water droplets formed a planar monolayer droplet array (PMDA) at the bottom of the microwell. Scale bar=200 µm. (d) The diameter distribution of droplets in (c).

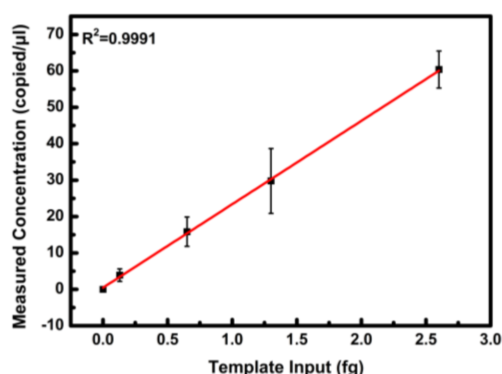

Fig 2. The linear correlation (red solid line) between the input and the measured concentration of mpb70-T1 plasmid templates based on dLAMP.

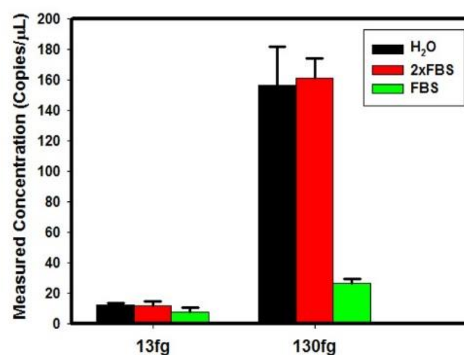

Fig. 3 Absolute quantification of 13fg and 130fg mpb70-T1 plasmid using dLAMP by XiE in the absent of fetal bovine serum and in the present of fetal bovine serum with two-fold dilution or not.

References:

1. A. C. Hatch, J. S. Fisher, A. R. Tovar, A. T. Hsieh, R. Lin, S. L. Pentoney, D. L. Yang and A. P. Lee, *LAB CHIP*, 2011, **11**, 3838.

2. B. Sun, F. Shen, S. E. McCalla, J. E. Kreutz, M. A. Karymov and R. F. Ismagilov, *ANAL CHEM*, 2013, **85**, 1540-1546.

3. P. Xu, X. Zheng, Y. Tao and W. Du, *ANAL CHEM*, 2016, **88**, 3171-3177.

4. H. Zhang, Z. Wang, X. Cao, Z. Wang, J. Sheng, Y. Wang, J. Zhang, Z. Li, X. Gu and C. Chen, *ARCH MICROBIOL*, 2016, **198**, 905-911.

5. Y. Hu, P. Xu, J. Luo, H. He and W. Du, *ANAL CHEM*, 2017, **89**, 745-750.