

One-Pot Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) for Detecting MERS-CoV

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Due to the limitation of rapid development of specific antiviral drug or vaccine for novel emerging viruses, an accurate and rapid diagnosis is a key to manage the virus spread [1]. We developed an efficient and rapid method with high specificity for the Middle East Respiratory Syndrome coronavirus (MERS-CoV), based on one-pot reverse transcription loop-mediated isothermal amplification (one-pot RT-LAMP) [2]. A set of six LAMP primers [F3, B3, FIP, BIP, LF (Loop-F), and LB (Loop-B)] were designed using the sequence of nucleocapsid (N) gene with optimized RT-LAMP enzyme conditions: 100 U M-MLV RTase and 4 U Bst polymerase, implying that the reaction was able to detect four infectious viral genome copies of MERS-CoV within a 60 min reaction time period. Significantly, EvaGreen dye has better signal read-out properties in one-pot RT-LAMP reaction and is more compatible with DNA polymerase than SYBR green I. Isothermally amplified specific N genes were further evaluated using field-deployable microchamber devices, leading to the specific identification of as few as 0.4 infectious viral genome copies, with no cross-reaction to the other acute respiratory disease viruses, including influenza type A (H1N1 and H3N2), type B, human coronavirus 229E, and human metapneumovirus. This sensitive, specific and feasible method provides a large-scale technical support in emergencies, and is also applied as a sample-to-detection module in Point of Care Testing devices.

References

[1] Bhadra, S., Jiang, Y. S., Kumar, M. R., Johnson, R. F., Hensley, L. E., and Ellington, A. D. (2015). PLoS ONE 10:e0123126. doi: 10.1371/journal.pone.0123126

[2] Poon, L. L., Leung, C. S., Chan, K. H., Lee, J. H., Yuen, K. Y., Guan, Y., et al. (2005). J. Clin. Microbiol. 43, 427–430. doi: 10.1128/JCM.43.1.427-430. 2005

Figures

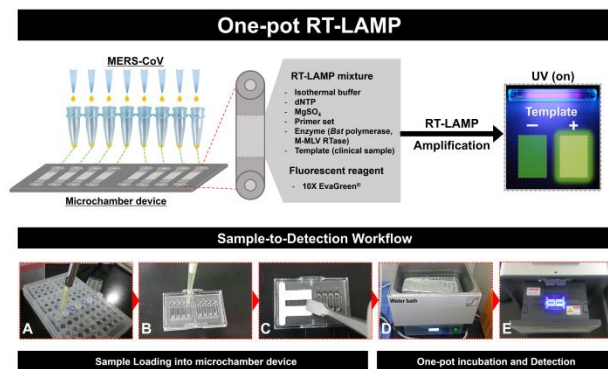


Figure 1. Schematic illustration of the entire one-pot RT-loop-mediated isothermal amplification (RT-LAMP) workflow, “Sample-to-Detection.”

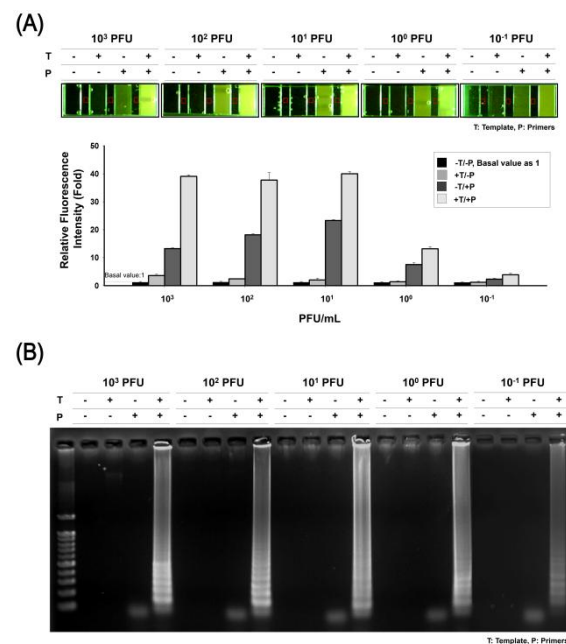


Figure 2. Sensitivity of one-pot RT-LAMP.