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Real-time isothermal nucleic acid amplification detection in resource-limited settings: a description of an open-source miniature fluorimeter

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Abstract

Background The detection and quantification of nucleic acids traditionally relies on PCR, which requires the use of expensive thermocyclers with integrated fluorescence detection of amplicons. While isothermal nucleic acid amplification technologies eliminate the need for thermocycling, fluorescence-based detection of products is still required for real-time, quantitative readout. Several commercially available isothermal heaters with integrated fluorescence detection exist, but the cost of these devices remains a significant barrier to the use of isothermal nucleic acid amplification in low-resource settings. A low-cost device for nucleic acid amplification detection could be used to contain the spread of the SARS-CoV-2 virus during the current pandemic as well as other disease targets in the future. Increasing COVID-19 testing capacity is particularly relevant when variations of the virus that appear to be more infectious are spreading rapidly and efforts to scale up delivery of COVID-19 vaccines are in the early stages. Here, we describe a modular, low-cost fluorimeter for use in resource-limited settings that lack access to and infrastructure for traditional testing platforms.

Methods We present a fluorimeter that is constructed from off-the-shelf components enclosed in a compact 3D printed housing that removes the need for specialised optical alignment. The fluorimeter is designed to be placed atop a commercially available heat block holding a PCR-tube and was optimised to detect fluorescein (FITC) dye. The modular design allows it to be easily adapted for use with other dyes commonly used as reporters in nucleic acid amplification reactions.

Findings Clinical applicability of the system was demonstrated using the system to measure amplification with two different isothermal amplification technologies with significantly different temperature, volume, and fluorescence requirements: recombinase polymerase amplification (RPA) and reverse transcription loop-mediated isothermal amplification (RT-LAMP). RPA detection was demonstrated with a positive control DNA provided in a commercial kit while RT-LAMP detection of clinically meaningful levels of SARS-CoV-2 RNA was demonstrated in a custom RT-LAMP assay. In a preliminary analysis of nasopharyngeal swab samples from 74 individuals, the custom RT-LAMP assay showed a positive agreement of 92·3% and a negative agreement of 91·4% with the SARS-CoV-2 RT-qPCR test developed by the US Centers for Disease Control and Prevention.

Interpretation This open-source system was designed to improve accessibility to nucleic acid testing with isothermal amplification methods. The system can be easily produced using only a low-end 3D printer. Moreover, the use of single-board computers obviates the need to produce printed circuit boards. At a time when global supply chains are at their most fragile, open-source diagnostic equipment has the potential to reduce pandemic-related health inequities.

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Declaration of interests
We declare no competing interests.