REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Inaat: A Universal Open-source Mobile Device For Field Deployable Real-time Isothermal Nucleic Acid Amplification Testing Of Covid-19

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The polymerase chain reaction is the gold standard for detecting and quantifying viral infections such as Covid-19. However, costly and laborious logistics have limited their availability. Although multiple platforms have been developed to perform isothermal molecular tests, most specialized instruments involve convoluted sample pretreatment steps to extract, isolate and amplify nucleic acids. These instruments are rarely commercially available due to the requirement of significant capital investment, lengthy clinical validation studies, and complex regulatory approvals. Since biological research labs have limited access to such curated instruments, we present an open-source low-cost hand-held mobile device capable of performing multiple real-time isothermal assays for field-deployable molecular diagnosis in commercially available 8-strip PCR tubes.

The iNAAT can perform a RT-LAMP test to detect the SARS-CoV-2 viral RNA and a CRISPR Cas12a test to detect SARS-CoV-2 cDNA generated from the RT-LAMP reaction. The test uses a simple heat-treated (95°C) nasopharyngeal swab or saliva sample. This study aims to provide a universal molecular diagnostic tool that could enable quick, accurate, and easy identification of positive individuals at point-of-need settings such as primary clinics and airports to contain the spread and further evolution of the virus. The device's overall schematic is shown in Figure 1a. The required temperature is maintained by a resistor-based heater and thermistor-based negative feedback; the emitted fluorescence is monitored in real-time by eight pairs of a LED and a micro-spectrometer; a Raspberry Pi Zero coordinates between all components. The assembled iNAAT device that can be powered by a battery pack or a 9V wall adapter is shown in Figure 1b. The iNAAT can be paired with a cell phone to initiate a test, guide the user, and display and record the results. Figure 2 shows the overall workflow for performing a test. Figure 1: a) Schematic view of iNAAT device. b) Photograph of assembled iNAAT. Figure 2: The overall workflow of the iNAAT Covid-19 test.

We provide empirical results in Figure 3 using cultured viral RNA particles. We quantitatively amplified 1e4, 1e3, and 1e2 copies/rxn in a 40 µl RT-LAMP reaction (duplicates) on the iNAAT (Figure 3a) and a benchtop thermocycler (Figure 3b). Figure 3c indicates the correlation between the times to positive obtained on the iNAAT and the thermocycler, establishing that the iNAAT can correctly quantify viral RNA copies in a 40 µl reaction within 30 min. Based on these results, we propose to evaluate the analytical sensitivity and specificity using serially diluted synthetic samples and diagnostic sensitivity and specificity using clinical samples. Figure 3: Empirical RT-LAMP assay validation with 40 µl reaction.

Although SARS-CoV-2 is used as a case study, the iNAAT could easily be modified for other targets to detect infections in samples collected in remote environments. Thus, it aligns well with the DTRA's goal to develop revolutionary diagnostics to aid the warfighter. Additionally, coupled with an aerosol collection device, the iNAAT could allow air sample analysis to identify aerosolized biothreats and accelerate the development and validation of collection devices in a modular fashion.

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