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Low-cost and rapid paper-based microfluidic device for wastewater surveillance at low-resource settings

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Abstract The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes respiratory illness and gastrointestinal infections, with viral material being excreted in feces (and surviving within sewage for several days). Here, we propose a low-cost and user-friendly paper-based microfluidic device incorporating reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of SARS-CoV-2 and influenza. Qualitative results were displayed by a UV torch, observed with naked eyes or recorded using a mobile phone camera. The paper-based platform could complete the concentration, extraction, amplification and detection of viruses in wastewater within 1.5 hours, with a detection limit as low as 10 copies μL-1. The device was used for on-site detection of SARS-CoV-2 and influenza virus in wastewater samples from four quarantined hotels at London Heathrow Airport, showing results comparable to those obtained using reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays. Our platform enables rapid detection of viruses without the need to send wastewater samples to centralized laboratories, providing a high-resolution data set for highly responsive measurement for the pandemic. The platform can be used as a public health early warning tool for various applications in community settings and shows great potential for rapid and on-site wastewater surveillance at low resource settings.

Keywords: SARS-CoV-2, paper microfluidic, wastewater surveillance, early warning

1. Introduction

The presence of pathogens in wastewater is one of global concerns and causes enormous health risk to humans. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) not only causes respiratory illness, but is associated with gastrointestinal infections. The Centers for Disease Control and Prevention emphasized the importance of distinguishing between infections caused by SARS-CoV-2 and influenza viruses. The viral material is excreted in feces and survives in sewage for several days. Wastewater surveillance has been shown to be capable of detecting a wide range of pathogens, providing readily available aggregated samples. We have demonstrated the application of paper-based microfluidic devices for veterinary diagnosis in India [1] and malaria testing in Uganda [2]. Here, we describe a low-cost and user-friendly approach for the detection of SARS-CoV-2 and influenza in wastewater by paper-based microfluidic device incorporating reverse transcription loop-mediated isothermal amplification (RT-LAMP). The paper-based platform is a potential public health surveillance tool in resource-limited areas.

2. Materials and Methods

2.1. Title

We fabricated a paper-based microfluidic device by CorelDRAW and wax printing, using RT-LAMP assay for the detection of SARS-CoV-2 and influenza A/B in wastewater. The method comprised a hand-held syringe-based sample preparation system to enrich pathogen samples from wastewater, enabling the extraction, purification, amplification, and detection. Results were read by illumination using a hand-held UV torch and collected by a mobile phone camera. The device was first optimized using a model surrogate organism to define channel geometries and sensing area sizes. To assess the analytical performance of the paper-based platform for pathogen detection, transcribed RNA was spiked into wastewater to construct mimic samples for laboratory testing. Subsequently, the paper-based microfluidic device was used to test 20 wastewater samples collected by Anglian Water. Finally, the paper-based platform was applied to analyze wastewater samples from four quarantine hotels based in London Heathrow Airport. These results were verified by the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method.

3. Results and Discussion

A paper-based microfluidic device was developed for wastewater surveillance in combination with RT-LAMP technology to measure nucleic acid biomarkers. Nucleic acids released into wastewater by viruses are RNA fragments whose amplification and detection include reverse transcriptase production of cDNA prior to LAMP. We demonstrated the effectiveness of the device for the detection of SARS-CoV-2 and influenza A/B in mimic wastewater samples. The paper-based platform allowed for a sample-to-answer assay in 1.5 hours, which was faster than laboratory-based RT-qPCR method (usually 4 hours). This includes concentration, extraction, amplification and detection of viruses in wastewater with detection limits of <20 copies.

The paper-based platform was used for on-site detection of the ORF1ab, S and N genes of SARS-CoV-2 in wastewater samples from four quarantined hotels near London Heathrow Airport. RT-LAMP results were in general agreement with RT-qPCR results. The analysis of genetic material in wastewater can provide an estimate of the population level of SARS-CoV-2 [3]. The N gene presented the highest detection rate in the wastewater samples, followed by the S and ORF1ab genes. The ORF1ab gene was less sensitive than the N and S genes because it has a shorter sequence and may be degraded in the wastewater.

The paper-based platform showed acceptable sensitivity and specificity and did not require expensive equipmen and skilled specialists, which allows public health officials to take action to reduce the spread of infectious diseases. Future research can be focused on integrating the concentration, extraction, detection, and analysis modules into a single device to standardize and automate wastewater surveillance to identify a variety of pathogens in resource-limited areas.

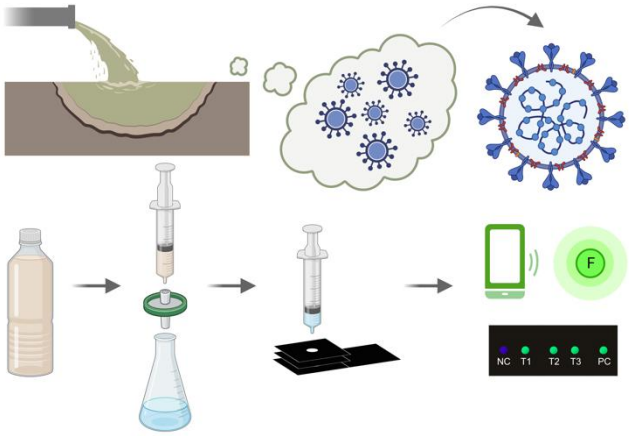

Figure 1: Schematic showing the process for virus detection in wastewater using a paper-based microfluidic device.

4. Results and Discussion

The proposed paper-based microfluidic device for pathogen detection in wastewater achieves sample-to-answer analysis in 1.5 hours with detection limits of <20 copies. This paper-based platform provides similar or higher specificity and sensitivity for pathogen detection in a cheaper and faster manner than gold standard RT-qPCR method. Overall, the paper-based platform offers great potential to be used as a public health early warning tool to reduce the burden of pandemic surveillance. It also shows great promise for rapid and on-site wastewater surveillance in low- and middle-income countries.

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