RAPID DETECTION OF NOVEL CORONAVIRUS SARS-COV-2 BY SOLID-STATE NANOPORE

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ABSTRACT

The COVID-19 pandemic spreads rapidly and globally. To quell the pandemic propagation, rapid and accurate detection of SARS-CoV-2 is urgently needed. Here, we present a nanopore coupled RT-LAMP method for SARS-CoV-2 detection. After comparing all information from the nanopore experiment, we develop a method to use the event rate change of the amplicons translocation event to measure the amplification. As a result, our platform can distinguish positive from negative samples in 15 min with around 65 copies/reaction limit of detection and 100% specificity. We believe that the nanopore coupled RT-LAMP platform would provide a sensitive and specific detection for SARS-COV-2.

KEYWORDS:

SARS-CoV-2, RT-LAMP, Solid State Nanopore

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in late 2019, and it was rapidly announced as a Public Health Emergency of International Concern by the World Health Organization (WHO). These RNA viruses have high mutation rates, and many cases are asymptomatic[1]. Therefore, an accurate, reliable, rapid, and inexpensive diagnosis on a regular basis is urgently needed to quell the pandemic propagation. Currently, reverse transcription-polymerase chain reaction (RT-PCR) is the gold-standard technique for SARS-CoV-2 virus detection. However, laboratory-based RT-PCR tests require complex facilities and elaborately trained operators, limiting testing capacity and delayed results[2]. Loop-mediated isothermal amplification (LAMP) seems to be a great alternative for COVID-19 rapid detection. The reaction can be carried out at a constant temperature and has wide pH and temperature range tolerance. To date, the readout for LAMP results mainly focuses on optical-based methods to detect the change of turbidity, color, or the most commonly used fluorescence[3]. Alternative sensing technologies which do not require complex optical systems have been developed but not yet applied to COVID-19 detection. Our previous work demonstrated the possibility of using electronic-based nanopore as a readout for LAMP results[4]. In this work, we further investigated all the information (dwell time, current drop, and event rate) obtained from the nanopore experiment and developed a scheme using event rate as a readout for SARS-CoV-2 rapid detection. In conclusion, our platform can distinguish positive saliva spiked RNA samples from negative samples in 15 min with around 100 copies/ reaction limit of detection and 100% specificity. We believe the nanopore coupled RT-LAMP platform has the capability to rapidly identify the presence of SARS-CoV-2 RNA with high reliability.

THEORY

Figure 1 provides an overview of the COVID-19 detection by nanopore sensor. First, the saliva/nasal swab sample will be collected and extracted. Next, the RNA sequence will be transcript into complementary DNA and further used for LAMP amplification **(Figure 1c)**. Therefore, in the presence of the target RNA, the cDNA can be recognized and amplified, and the concentration of the amplicons increases exponentially. In the absence of the target RNA, the cDNA cannot be identified. As a result, no amplicons will be generated. Next, a nanopore experiment would be carried out to determine the amplicons concentration through the single molecule counting. Finally, based on the sharp contrast of the

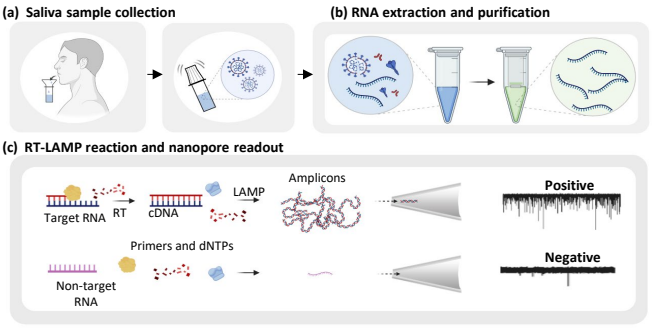

Figure 1: *RT-LAMP coupled nanopore method for SARS-CoV-2 detection workflow.*

translocation rate in positive and negative samples, we can confirm the existence or the absence of the target RNAs in the system.

RESULTS AND DISCUSSION

To detect SARS-CoV-2 RNA with RT-LAMP, we first validated the RT-LAMP assay. We ran a serial dilution heat-inactivated SARS-CoV-2 RNA sample using a real-time instrument. The obtained threshold time values ranged from 7 to 10 min, with the heat inactivated RNA concentration from 10^5 to sub hundred copies per reaction. The estimated LoD with 95% confidence was 65 copies((**Figure 2).**

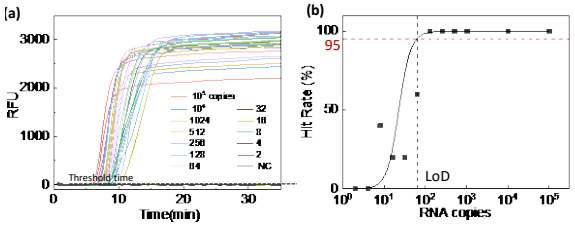

Figure 2: *RT-LAMP assay validation by fluorescence method using benchtop real-time PCR machine.*

Next, we set out to find the best criterion for capturing the positive samples by nanopore. We performed RT-LAMP assays with heat-inactivated RNA samples for different reaction times. First, all the reactions were confirmed by gel electrophoresis (**Figure 3a).** Next, we extracted two different kinds of information from the nanopore experiment as our readout method: molecular size and concentration. **Figure 3b** shows the current dip and dwell time information at each LAMP reaction time. Expectedly, the dwell time and current drop of the events increase as the reaction time increases. To evaluate these data quantitatively, the distribution of the current dip, dwell time, and event charge deficit (ECD) is presented in **Figure 3c**. As we can see, the average value of current dip, dwell time, and ECD starts to capture the amplification after 9 min reaction and increase 4.2, 1.3, and 5.7 times from 0 min to 16 min, respectively. Afterward, we analyzed the relationship between amplicon concentration and LAMP reaction time **(Figure 3d).** The event rate increases 8096 times as the reaction time goes from 0 min to 16 min. The concentration changes are much more sensitive than molecule size changes regarding the pick-up time and the magnitude of change.

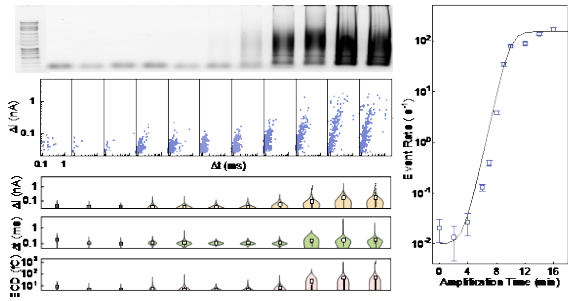

Figure 3*: Nanopore counting to probing the RT-LAMP reaction dynamics.*

To evaluate the sensitivity and specificity of the system, we performed reactions with saliva spiked RNA samples at different concentrations and three human coronaviruses (229E, NL63, and OC43). As shown in **Figure 4**, the analytical sensitivity and specificity of platform for SARS-CoV-2 are 100 copies/reaction and 100%.

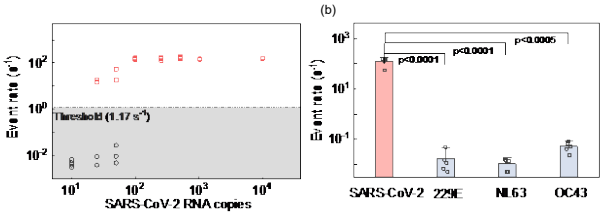

Figure 4*: The analytical sensitivity and specificity tes with saliva spiked RNA sample.*

CONCLUSION

In summary, we developed and evaluated nanopores-coupled RT-LAMP for SARS-CoV-2 detection. Our platform can distinguish positive samples from negative samples in 15 min with around 64 copies/ reaction limit of detection and 100% specificity. We believe that the nanopore coupled RT-LAMP platform would provide a sensitive and specific detection for SARS-COV-2.

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