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Development of Sensitive, Specific, Isothermal One-pot Fluorescence RNA Detection Assay Based on Cell-free Synthetic Biology

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PURPOSE OF THE ABSTRACT

The COVID-19 pandemic has highlighted the crucial necessity for efficient and accurate diagnostic tools to identify infectious diseases caused by RNA viruses. The epidemic has reinforced the significance of rapid and sensitive detection of viral RNA in order to detect and control outbreaks. Consequently, there is an urgent need for the advancement and implementation of new RNA detection methods.

Current gold-standard RNA detection technique as known as reverse transcription-polymerase chain reaction (RT-PCR) has played a critical role in COVID-19 diagnosis. However, it necessitates specialized equipment and highly skilled technicians, posing challenges to its usage in resource-constrained settings. Furthermore, RT-PCR technique often entails lengthy sample preparation, amplification, and analysis, leading to delays in patient diagnosis and treatment.

To address these issues, we developed a cell-free, one-step assay for detecting pathogen-derived RNAs based on fluorescence. The assay uses a ligation-dependent one-pot isothermal reaction cascade. It consists of two simple enzymatic reactions: single-stranded DNA ligation reaction by SplintR ligase and subsequent transcription reaction by T7 RNA polymerase. The ligation of the adjacent single-stranded DNA probes, namely the promoter probe and the reporter probe, occurs upon hybridization with a complementary RNA, which is used as a splint. Following the ligation process, the resulting product contains a T7 promoter sequence. It is recognized by T7 RNA polymerase, leading to the transcription reaction. The transcript forms an RNA aptamer that binds a cognate fluorogenic dye and emits fluorescence only when target RNA exists in a sample.

Our assay was able to detect methicillin-resistant Staphylococcus aureus (MRSA) RNA in 30 min with a limit of detection of 0.1 aM (6 copies RNA per reaction). Merely by altering the hybridization regions of the probes, we were able to employ this assay to detect a diverse array of pathogens, comprising Vibrio vulnificus, Escherichia coli O157:H7, Middle East respiratory syndrome-related coronavirus (MERS-CoV), Influenza A viruses and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2).

Our research indicates that our assay has the potential for detecting various pathogen RNA markers by simply redesigning the probe sequences. This can be easily achieved using readily available web-based software such as primer-blast and NUPACK, requiring minimal computational effort.

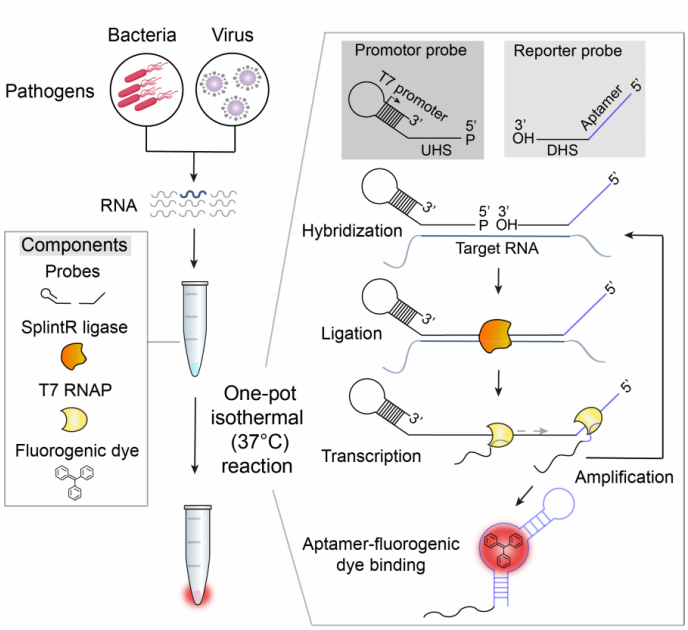
Through a clever use of the straightforward probe design, we pushed the boundaries of our assay's capacity to facilitate the detection of two separate target RNAs simultaneously within a one-pot, single reaction. This is crucial in accurately mitigating the risks of false positives and false negatives, improving diagnostic precision. By leveraging the high specificity of our probes and unique spectral properties of light-up RNA aptamers, we substantiated the effectiveness of dual detection of multiple regions within the RdRp gene of SARS-CoV-2.

Finally, we culminated in the successful validation of our assay as a reliable tool for detecting SARS-CoV-2 virus from clinical samples, underscoring its potential as a valuable asset in the arsenal of diagnostics assay. We were able to display a remarkable diagnostic performance with 95% of sensitivity and 100% specificity achieved within a

mere hour without the requirement of RNA extraction.

In conclusion, the presented diagnostic assay has the potential to revolutionize pathogen detection. The future automation of probe design will be essential for the rapid development of assays for emerging pathogens. Additionally, expanding its utility into portable, field-deployable devices that offer isothermal incubation and fluorescence measurement could be a significant step toward creating simple and transportable paper-based or lateral-flow-type tests.

FIGURES



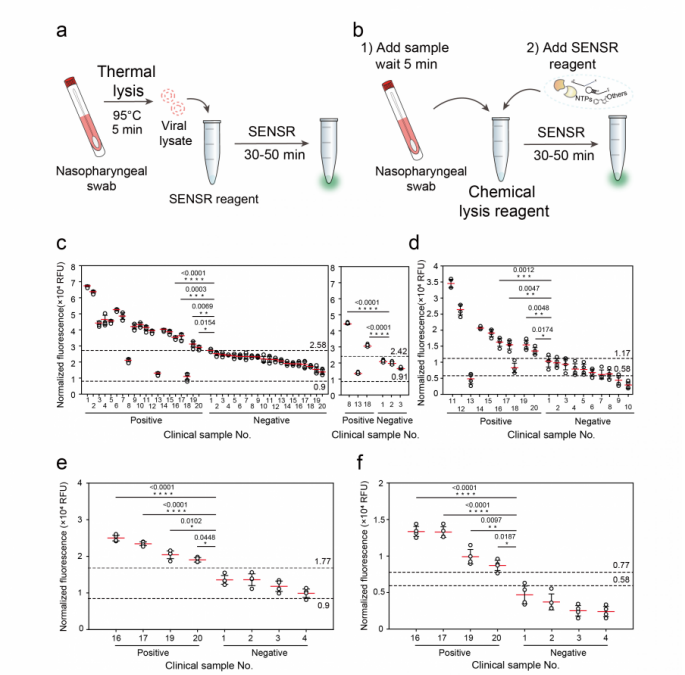


FIGURE 1

Graphical scheme of SENSR

SENSR, a one-pot isothermal reaction cascade for the RNA detection.

FIGURE 2

Schematics of SARS-CoV-2 detection from clinical samples via SENSR

Clinical samples in UTM were treated by either thermal (a, c and e) or chemical lysis (b, d and f) and mixed directly with the SENSR mixture. c,d, SARS-CoV-2 detection from clinical samples. e,f, Detection of SARS-CoV-2 by 30-min SENSR reaction.

KEYWORDS

Cell-free synthetic biology | Molecular Diagnostics | Isothermal, One-pot RNA detection | Point-Of-Care test

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