-ABSTRACT

the capacity to identify an infectious agent in a widespread outbreak is critical to the effectiveness of quarantine attempts. Using loop-mediated isothermal amplification (LAMP) and a visual, colorimetric detection tool, we were able to distinguish SARS-CoV-2 (COVID-19) virus RNA from purified RNA or cell lysis. The test was also validated using RNA samples purified from COVID-19 patients' respiratory swabs in Wuhan, China, which performed similarly to a commercial RT-qPCR test while involving only heating and visual examination. This basic and responsive approach allows virus identification in the field to be facilitated without the need for sophisticated diagnostic infrastructure.

-INTRO

The diagnostics industry reacted quickly, with the US Centers for Disease Control and Prevention (CDC) granting Emergency Use Authorization for PCR-based tests. Many other companies, including the CDC Seegene in Korea, and BGI in China are launching reagents, primers Since many of the symptoms are similar to those of a common cold or influenza, a precise molecular result is important for final diagnosis.Metagenomics sequencing mNGS and RT-qPCR are two of these molecular methods, both of which are excellent and adaptive techniques with drawbacks .Throughput, turnaround time, high cost, and a high level of technical competence are all constraints for mNGS.RT-qPCR is the most commonly used tool of molecular diagnostics, but it includes costly laboratory equipment and is difficult to use outside of well-equipped facilities. A high proportion of patients were diagnosed as false negatives due to the various patient samples containing varying numbers of virus.

Using loop-mediated isothermal amplification (LAMP) and simple visual amplification detection, we identify a molecular diagnostic method for SARS-CoV-2 RNA detection that could be used in rapid field applications. LAMP was created as a quick and dependable way to amplify a small amount of target sequence at a single reaction temperature, .A number of advances in detection technology have helped develop LAMP as a standard method for simple isothermal diagnostics since its initial description.These methods have enabled identification by visual inspection without the use of instruments, using dyes that make use of by-products of comprehensive DNA synthesis. Malachite green, calcein and hydroxynaphthol, blue are some examples . We recently created a pH-sensitive dye-based system for visual detection of LAMP amplification that takes advantage of the pH transition caused by proton accumulation due to dNTP incorporation.This approach has been used in a large-scale field study of Wolbachia-containing mosquitos, testing urine samples for Zika virus, and even amplification detection on the International Space Station .This research used short (300bp) RNA fragments made with in vitro transcription and RNA samples from patients to test and validate five sets of LAMP primers targeting two fragments of the SARS-CoV-2 genome. We also show compatibility with clinical swab samples obtained from COVID-19 patients, as well as easy approaches to RNA purification to simplify the detection process and prevent complicated RNA extraction.

-Related work

Since many of the signs are similar to those of the common cold and influenza, a reliable molecular result is important for final diagnosis. Metagenomics sequencing mNGS and RT-qPCR are two of these molecular technologies, all of which are excellent and adaptive tools, but all have drawbacks. Throughput, turnaround time, high cost, and the need for high technological competence limit mNGS. RT-qPCR is the most commonly used tool of molecular diagnostics, but it includes costly laboratory equipment and is difficult to use outside of well-equipped laboratories. A high percentage of patients were diagnosed as false positives as a result of the multiple patient samples having varying amounts of virus. We present a molecular diagnostic technique for detecting SARS-CoV-2 RNA using loop-mediated

isothermal amplification (LAMP) and basic visual amplification detection for use in rapid field applications. LAMP was created as a quick and accurate way to amplify a limited amount of target sequence at a single reaction temperature, without the use of expensive thermal cycling equipment. A variety of advances in detection technologies have helped define LAMP as a basic tool for simple isothermal diagnostics since its initial description. These methods have enabled identification by visual inspection without the use of instrumentation, using dyes that use inherent by-products of extensive DNA synthesis, such as malachite green, calcein, and hydroxynaphthol blue. We recently created a system for visually detecting LAMP amplification using pH-sensitive dyes, which takes advantage of the pH transition caused by proton accumulation due to dNTP incorporation. This technique has been used to diagnose amplification on the International Space Station, as well as a large-scale field survey of Wolbachia-containing mosquitos, Grapevine red blotch virus without DNA extraction, checking urine samples for Zika virus. The wide range of applications demonstrates how visual detection approaches can be used to have a benefit in terms of flexibility and portability for allowing modern, rapid diagnostics.

- MATRIALS AND METHODS

WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (M1800) was used in LAMP reactions, which were supplemented with 1 mM SYTO®-9 double-stranded DNA binding dye (Thermo Fisher S34854) and incubated on a real-time qPCR machine (BioRad CFX96) for 120 cycles at 15 seconds each (total of 40 minutes). An office flatbed scanner was used to record the colour of the completed responses.Hela cells were spiked with synthetic RNAs before being diluted and lysed with the Luna® Cell Ready Lysis Module (E3032). Each lysate was then diluted 10 times with 0.1x TE +0.01% Tween 20 before being put to standard colorimetric LAMP assays in 1 litre. RNA was seeded into 200 litres of whole human blood (Quadrant Health Strategies) to ensure compatibility with blood recovery, and the whole blood RNA was subsequently isolated using Monarch® Total RNA Miniprep Kit .Sampling and processing of samples Following the criteria for identifying SARS-CoV-2 nucleic acid in clinical samples, all samples were handled and deactivated first in a biosafety level 2 laboratory with personal protective equipment for biosafety level 3.

COVID-19 Patients' RNA Samples in LAMP Assays .The test was carried out in a 20-liter reaction mixture including 2 litres of 10x primer mix including 16 mM of Forward Inner Primer (FIP) and Backward Inner Primer (BIP), 2 mM of F3 and B3 primers, 4 mM of Forward Loop (LF) and Backward Loop (LB) primers, 10 litres of WarmStart Colorimetric Lamp 2X Master Mix (M1800), 5 litres of DNAse,3 l of RNA template and RNAase-free water (Beyotime Biotech, China). On a dry bath, the reaction mixture was heated to 65°C for 30 minutes.

-RESULTS AND DISCUSSION

Synthetic RNAs were serially diluted in LAMP reactions from 120 million copies down to 120 copies (per 25 L reaction) at 10-fold intervals to assess detection sensitivity.All five primer sets had similar detection sensitivity, detecting as little as a few hundred copies on a regular basis and as little as 120 copies (or 4.8 copies/L) on rare occasions. The colorimetric detection findings were 100 percent in accord with the real-time detection findings.On a real-time LAMP signal, we compared synthetic RNA with equally diluted gBlock dsDNA to assess relative efficiency utilising RNA or DNA templates. The slower amplification and detection using RNA template was seen in one of the two primer sets we examined,

whereas the other appeared to be slightly quicker.showing that the reverse transcriptase (WarmStart RTx) successfully converts RNA to cDNA and that the DNA-dependent DNA polymerase amplifies the cDNA through LAMP (Bst 2.0 WarmStart).Purified RNA is employed in the input of modern diagnostic procedures, such as RT-qPCR. We looked at whether detection might be done without the need for RNA purification by utilising crude cell lysate. The findings showed that all four primer sets identified 480 copies, which is comparable to the detection sensitivity of synthetic RNA alone , with no interference from the lysate in terms of amplification efficiency or visual colour change.We also used a fake experiment to see if we could recover the synthetic RNA injected into the biological sample during total RNA purification. The entire blood RNA was isolated after varying amounts of synthetic RNA were introduced into whole human blood.We were able to recover and identify the spiked RNA, showing that there was no interference with the total RNA during purification or detection.While the column-based technique is less compatible with colorimetric LAMP's easy field detection, it is a common laboratory workflow that can be utilised with basic isothermal amplification in the same way as more expensive and complicated qPCR detection techniques.The highest performing sets (ORF1a-A, GeneN-A) were shared and manufactured in Wuhan for testing with actual COVID-19 samples based on this early evaluation of prospective LAMP primer sets. RNA was extracted from swabs using conventional laboratory procedures, and the RNA was analysed in a colorimetric RT-LAMP assay alongside a commercial RT-qPCR assay.A total of 7 patient RNA samples were analysed, 6 of which were found to be positive by RT-qPCR using ORF1a primers (Cq 25–36.5,) and 4 of which were found to be positive by Gene N primers. Both RT-qPCR primer sets were negative for one sample.All six RT-qPCR positive samples displayed obvious colour change in the colorimetric LAMP assay, suggesting positive amplification, whereas the solitary RT-qPCR negative sample remained pink and was deemed negative. Over a wide range of Cq values, the colorimetric LAMP test exhibited 100 percent agreement with the RT-qPCR data.Despite the small number of samples studied, the colorimetric LAMP assay allows for accurate SARS-CoV-2 identification in the field and in point-of-care settings, matching the performance of RT-qPCR.Finally, colorimetric LAMP provides a simple and quick approach for detecting SARSCoV-2 RNA.