-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.