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Research Paper

Ultrafast and absolute quantification of SARS-CoV-2 on food using hydrogel RT-LAMP without pre-lysis

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HIGHLIGHTS

- Ultrafast quantification for SARS-CoV-2 is achieved in 15 min without pre-lysis.
- The detection limit is down to single virus.
- The mechanism for ultrafast detection is the isolation of single virus in hydrogel.
- SARS-CoV-2 can be tested on site by integrated system directly in cold chain foods.

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ABSTRACT

With rapid growing of environmental contact infection, more and more attentions are focused on the precise and absolute quantification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus on cold chain foods via point-of-care test (POCT). In this work, we propose a hydrogel-mediated reverse transcription loop-mediated isothermal amplification (RT-LAMP) for ultrafast and absolute quantification of SARS-CoV-2. Cross-linked hydrogel offers opportunities for digital single molecule amplification in nanoconfined spaces, facilitating the virus lysis, RNA reverse transcription and amplification process, which is about 3.4-fold faster than conventional bulk RT-LAMP. Ultrafast quantification of SARS-CoV-2 is accomplished in 15 min without virus pre-lysis and RNA extraction. The sensitivity can accurately quantify SARS-CoV-2 down to 0.5 copy/μL. Furthermore, the integrated system has an excellent specificity, reproducibility and storage stability, which can be also used to test SARS-CoV-2 on various cold chain fruits. The developed ultrafast and simple hydrogel RT-LAMP will be an enormous potential for surveillance of virus or other hazardous microbes in environmental, agricultural and food industry.
1. Introduction

A worldwide epidemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has started as the coronavirus disease 2019 (COVID-19) spread in December 2019 (Nasrollahzadeh et al., 2020; Soufi et al., 2020; Zhang et al., 2022). Recently, considerable attention has been focused on the cold chain foods, since in a time of crisis they can be carriers for the long-range transport of SARS-CoV-2. (Laborde et al., 2020; Lacombe et al., 2021; Olaimat et al., 2020). Food transmission evidence has been disclosed in China (Xinfadi agricultural produce wholesale market, Beijing) early July 2020 by the detection of SARS-CoV-2 on frozen foods, with confirmed re-emergent outbreaks (Han et al., 2021). Other countries, such as Australia, Canada, Brazil, Germany, and Iceland, have also been reported outbreaks of COVID-19 infections among workers and contaminated food sources (Hu et al., 2021). Besides, there are numerate reports that many overseas imported cold-chain fruits have been contaminated by SARS-CoV-2, which caused a lot of economic losses and showed a great demand for rapid test of SARS-CoV-2 on food.

Here, we propose an ultrafast hydrogel-mediated RT-LAMP POCT system for absolute quantification of SARS-CoV-2. All steps were integrated in a single hydrogel RT-LAMP, improving the amplification efficiency and greatly reducing the testing time. This study aimed to fulfill the knowledge gap of ultrafast and precise quantification of SARS-CoV-2 with POCT on food. Both SARS-CoV-2 RNA and SARS-CoV-2 virus were amplified within 10 min, and the cost per reaction is only $1.11. Notably, no lysis operation, RNA extraction, or purification were required. The underlying mechanism of ultrafast detection in gel RT-LAMP was investigated. Moreover, the tolerance of gel RT-LAMP to inhibitors from actual cold chain fruits was explored. This simple hydrogel RT-LAMP system revealed ultrafast and digital quantification of SARS-CoV-2, providing a competitive platform for POCT of other viruses with low viral load in various fields.

2. Experimental

2.1. Materials

RT-LAMP reagents (dNTP, buffer, Mg\(^2+\), Bst 2.0 WarmStart polymerase, reverse transcriptase) were obtained from New England Biolabs (Ipswich, MA). Primers, RT-PCR probe and SARS-CoV-2 RNA positive quality control containing nucleoprotein (N) gene (Fig. S1) were synthesized by Sangon Biotechnology (Shanghai, China). Armored SARS-CoV-2 virus, containing MS2 bacteriophage coat protein, were purchased from Xiamen Zeesan Biotech (Xiamen, China). Inactivated Influenza A virus, Norovirus and SARS-CoV-2 virus containing whole genome were obtained from BDS Biological Technology (Guangzhou, China). Altermaria alternata was separated from naturally occurring jujube, which was cultured in potato dextrose agar (PDA) and then genome was extract by commercial DNA extraction kit (Demeter Biotech, Beijing, China). Listeria monocytogenes CICC 21633 was cultured in Brain Heart Infusion broth. Salmonella typhi CICC 10871 and Methicillin-resistance Staphylococcus aureus ATCC 43300 were cultured in Luria-Bertani broth. These bacterial genomes were obtained by Extraction kit (ThermoFisher Scientific, San Jose, CA). Double stranded DNA (dsDNA) for SARS-CoV-2 N gene was obtained from SinoGenoMax (Beijing, China). Single stranded DNA (ssDNA) for SARS-CoV-2 N gene was obtained from Tszingke Biotechnology (Nanjing, China). 4 Arm-polyethylene glycol-acrylate (4 arm-PEG-ACRL) (average molecular weight (AMW) 10,000) and thiol-polyethylene glycol-thiol (SH-PEG-SH) (AMW 3400) were purchased from Laysan Bio (Arab, Alabama). The lyophilized powders of 2 hydrogen monomers at -20°C store were measured and dissolved in sterile water for direct use. Frame-seal (9 × 9 mm in width, 270 μm in depth) was obtained from Bio-Rad (California, USA). TaqMan® fast virus 1-step master mix and SYBR Green I were purchased from ThermoFisher Scientific (San Jose, CA).

2.2. Protocol for gel RT-LAMP

Each gel RT-LAMP system was in a 25 μL reaction mix containing 1.4 mM of dNTP, 1 × isothermal buffer, 1.6 μM of each FIB and BIP, 0.2 μM of each F3 and B3, 0.4 μM of loop primer, 6 mM of total MgSO\(_4\), 1 × SYBR Green I, 16 U of Bst 2.0 WarmStart polymerase, 7.5 U of reverse transcriptase, 1.6 mg 4 arm-PEG-ACRL, 1.1 mg SH-PEG-SH, and 2 μL of template (10\(^{-10}\)-10\(^{-6}\) copies/μL) or ddH\(_2\)O (negative control). SARS-CoV-2 N gene was chosen as the amplification target, and 4 groups of primers were used to detect the different areas in N gene (Fig. S1). The primer sequences of RT-LAMP were shown in Table S1. The primer with the highest amplification efficiency was chosen according to the amplification rate and hydrogel counts using same template concentration.

As shown in Scheme 1a, frame-seal was pasted on a glass slide to form a microchamber that was used to load the prepared reaction mix. The chamber was sealed and incubated at 65 °C on the heat block (MJ Research PTC-100, Watertown, MA) to conduct the gel RT-LAMP. Under the excitation of blue light, the positive reactions generated bright...
amplicon dots. The final fluorescence images were captured using an inverted fluorescence microscope (Leica DMi8, Leica Biosystems, Germany) or the digital camera module of smartphone (ELE-AL00, Huawei HarmonyOS 2.0.0, China). These images were wirelessly transferred to the Image J (NIH, MD) software for the following analysis. The images were processed firstly through the binary system and amplicon dots were selected. Then, the watershed function and analyze particles module were applied to auto-count individual points. The data were recorded as digital documents for subsequent analysis. Every test was performed in triplicate and contained the non-template control.

2.3. Real-time RT-LAMP

To observe the real-time amplification process, the reaction mix was added to 8 strip real-time PCR tubes and incubated at 65 °C in an Applied Biosystems™ real-time PCR apparatus (ThermoFisher Scientific). The reaction mix was the same as the hydrogel RT-LAMP section. The fluorescence intensity of the system was recorded every minute, and the data was saved as digital documents for subsequent analysis using the OriginPro (version 9.0, OriginLab) software.

2.4. Commercial digital RT-PCR

In order to compare the quantitative performance of the developed hydrogel RT-LAMP assay, the commercial QuantStudio 3D digital PCR program (ThermoFisher) was used to implement the chip-based digital RT-PCR for SARS-CoV-2 quantification. Briefly, the RT-PCR reaction mix (20 μL) composed of the following composition: 1 × TaqMan® fast virus 1-step master mix, 625 nM each of forward and backward primers, 250 nM of probe, and 2 μL of template. The prepared reaction mix was loaded into commercial digital chip through the sample injector (ThermoFisher Scientific). Oil-seal was employed to prevent evaporation of liquid in the digital chip, and incubated on the heat block. The RT-PCR reaction conditions were 50 °C for 5 min, 95 °C for 20 s, 40 cycle of 95 °C for 3 s, and 60 °C for 30 s. The sequences of primer and probe for RT-PCR are shown in Table S1.

2.5. Detection of SARS-CoV-2 virus

Similarly, SARS-CoV-2 virus was amplified at 66 °C for 10 min using the same gel RT-LAMP system without any sample pretreatment. The end-point fluorescence images were recorded through the fluorescence microscope for digital counting of single virus.

2.6. Characterization

The TEM image of SARS-CoV-2 virus was obtained using a transmission electron microscope (H-7650, Hitachi High-Technologies Corporation, Tokyo, Japan). The UV–visible absorption spectrum was measured by the microplate reader (200 PRO, Tecan, Grödig, Austria). SEM and EDS images of hydrogel were measured through a field emission scanning electron microscope (S-4000, Hitachi High-Technologies Corporation, Tokyo, Japan). The complex modulus of hydrogel was tested using a rheometer (DC-2006A, Anton Paar Trading Co., LTD, Shanghai, China). The agarose gel electrophoresis images were obtained through the electrophoresis apparatus (ELITE 300, WEALTEC, NV, USA) and gel imaging system (SH-510, SHST, Hangzhou, China).

2.7. Simulation

ChemDraw (version 20.0) software and Materials Studio (version 2020) were applied for molecular drawing and simulation between PEG hydrogel monomer and SYBR Green. According to their respective total energy, the binding energy of PEG hydrogel and SYBR Green was calculated.

2.8. Application in actual cold chain food detection

Commercially available cold chain fruits were purchased from the local supermarket, which were chosen because they have been tested as a carrier for SARS-CoV-2 in diverse reported incidents. The fruits were taken after grinding or surface scrub. Their surfaces were tested by the real-time RT-PCR to prove the absence of SARS-CoV-2 according to the criteria by the China Quality Inspection Association (T/CAQI 159-2020). For SARS-CoV-2 virus detection in juices, the gel RT-LAMP system containing various juices after grinding was investigated, and the same target concentration (10^2 copies/μL) was tested without adding juices as a control group to check the interference of fruit matrices. For SARS-CoV-2 virus detection on the fruit surface, three different concentrations (10^1-10^3 copies/μL) of SARS-CoV-2 virus were prepared to spike fruits surface through simulating real pollution and sampled using the disposable long-handle sampling swab according to the criteria by the China Quality Inspection Association (T/CAQI 159-2020). The fruits surfaces and surface gaps were vertically and repeatedly sampled for 5 times through sampling swab soaked in sterile normal saline. The hand-contacting part was cut off and place it into a sampling tube filled with sampling liquid. Same amounts of virus were spiked in the water directly for analysis, and designed as “control group”. The comparison of the results in fruits and control group showed the interference of fruit matrices. Both the artificially contaminated cold chain fruits samples and control group were simultaneously incubated at 66 °C for 10 min. These processes were performed using the gel RT-LAMP assay in three replications. The fluorescence dots were counted for subsequent comparison and analysis.

3. Results and discussion

3.1. Workflow in gel RT-LAMP

Scheme 1 illustrates the integrated POCT system for testing SARS-CoV-2 through the hydrogel RT-LAMP. Only two procedures, loading sample and heating, are required to complete the quantification, which are more suitable for POCT. The reaction mix containing samples, hydrogel monomers, and RT-LAMP reagents was loaded into a chip for the crosslinking and gelation (~1 min). The two hydrogel monomers are displayed in Fig. S2a–b, which can crosslink to form porous hydrogel network through the Michael addition reaction (Fig. S2c). After crosslinking, the single RNA molecule or single virus can be randomly

![Scheme 1. (a) Workflow of hydrogel RT-LAMP for the detection of SARS-CoV-2. (b) Schematic diagram of hydrogel RT-LAMP for the detection of SARS-CoV-2.](image-url)
confined in the hydrogel network, whereas reaction reagents diffuse freely via the nano-porous channel (Lin et al., 2022). Subsequently, the hydrogel chip was heated on a heating block for the digital isothermal amplification. In this phase, RNA conversion to RNA-cDNA hybrid could be achieved through reverse transcriptase, which could be further amplified by the strand displacement and extension of Bst 2.0 polymerase. Amplified products were accumulated in the nanconfinement spaces (Luo et al., 2022), resulting in a series of fluorescence dots for digital quantification of SARS-CoV-2. The number of positive dots reflected the precise number of viruses in the sample. Compared to other methods using sophisticated microfluidic device (Ganguli et al., 2020), this system is pretty simple and more available for non-specialized people to conduct point-of-care SARS-CoV-2 quantification.

3.2. Ultrafast gel RT-LAMP for SARS-CoV-2 RNA quantification

The encapsulation of nucleic acid inside the hydrogel network was demonstrated. The size of hydrogel nanopores is calculated using shear modulus through the following classical theory (Li and Mooney, 2016).

\[ d_{\text{mesh}} = \left( \frac{6RT}{\pi N_{\text{Av}} G} \right)^{1/3} \times 10^9 \quad (\text{nm}) \]

where \( R \) is the gas constant, \( T \) the absolute temperature (298 K), \( N_{\text{Av}} \) the Avogadro’s number, and \( G \) shear modulus (166.14 Pa) that are tested through the rheometer. Thus, the hydrogel nanopore is measured to be 36.17 nm, which is similar to the SEM results (Fig. S3).

The diameters of nucleic acids molecular are obtained by the following equation (Kalwarczyk et al., 2012).

\[ d = 0.048M_w^{0.57} \quad (\text{nm}) \]

where \( M_w \) is the molecular weight of nucleic acid. Here, the fragment of single RNA molecular is 1260 nt, the maximum primer is 43 nt, and the minimum DNA amplicon is 181 bp. Thus, the sizes of nucleic acids were calculated to be 77.87 nm, 11.17 nm, and 37.61 nm for single RNA molecular, primer and minimum DNA amplicon, respectively. Therefore, primers can freely mobilize in hydrogel system due to their small size compared to hydrogel nanopores, which can be consumed for single molecule amplification. RNA template and amplicons, however, are nicely confined within nanopore due to the greater molecular size. As

Fig. 1. Preparation of gel RT-LAMP system. (a) Amplicons size at different hydrogel concentrations. (b) Complex modulus of hydrogel at different heating time. (c) Hydrogel area at different temperature and heating time. The stable of hydrogel volume during heating indicate hydrogel matrix is not changed at reaction temperature. (d) Real-time RT-LAMP curve of SARS-CoV-2 (10^2 copies/μL) using 4 sets of primers. (e) Hydrogel counts at different amplification time. (f) Real-time amplification curve of gel RT-LAMP and aqueous RT-LAMP with same amount of template. (g) End-point fluorescence images at different amplification time.
the hydrogel concentration increases, amplicons were further confined and the dots size was gradually decreased (Fig. 1a). The confinement of each amplicon and random distribution of single RNA molecule in space were also demonstrated through three-dimensional imaging (Fig. S4). Complex modulus results indicated that cross-linked gelation was maintained at 65 °C (Fig. 1b) and hydrogel matrix was stable even at 80 °C (Fig. 1c).

The detection time is a crucial factor for the prevention and control of COVID-19 epidemic. Four sets of primers targeting various regions of SARS-CoV-2 N gene were used to explore the ultrafast amplification of gel RT-LAMP system. Real-time amplification curves showed that fluorescence intensity began to increase within 8 min in all primers (Fig. 1d). Primer 2 had a highest amplification efficiency, which was selected for subsequent experiments. As shown in Fig. 1e, the hydrogel counts toward SARS-CoV-2 reached maximum within 10 min, which was consistent with gel amplification curve (Fig. 1f). As the reaction time prolonged, the fluorescence dots number didn’t further increase, only the amplicons size became bigger (Fig. 1g). Hence, 10 min was the most appropriate reaction time to monitor SARS-CoV-2 quantitatively in gel system. This is an ultrafast amplification for the SARS-CoV-2 testing compared with other methods (Broughton et al., 2020; Jiang et al., 2021; Manzanas et al., 2021; Zhu et al., 2020). Similarly, we selected different concentrations of reagents (total Mg²⁺, loop primer, and reverse transcriptase) and temperature gradients to investigate the highest single-molecule amplification efficiency within 10 min (see Figs. S5–S8). Based on the end-point fluorescence counts and intensity, the optimal recipe for gel RT-LAMP system was 6 mM of total Mg²⁺, 4 μM of loop primer, 7.5 U of reverse transcriptase, and 65 °C reaction temperature.

The real-time RT-LAMP amplification curve of SARS-CoV-2 RNA in hydrogel and aqueous solution was compared in Fig. 1f. Interestingly, the same RT-LAMP amplification in the hydrogel was much faster than in the aqueous solutions with identical template concentration. The time threshold (Tt), an indicator for isothermal amplification when the fluorescence intensity began to increase along with time, was 5 min in gel RT-LAMP, which is about 3.4-fold shorter than in routine bulk RT-LAMP (Tt = 17 min). This revealed that the ultrafast detection of SARS-CoV-2 could be achieved using gel RT-LAMP. In previous reports, amplification time in aqueous RT-LAMP required at least 25–30 min (Broughton et al., 2020; Viet Loan Dao et al., 2020; W. Zhang et al., 2021), which was consistent with our results in bulk solutions. The shortening in amplification time has a vital significance to improve the screening performance and prevent the spread of the disease.

3.3. Mechanism for ultrafast SARS-CoV-2 detection

Compared to the aqueous RT-LAMP, the gel RT-LAMP could achieve an ultrafast detection of SARS-CoV-2 RNA, and the reason for the enhanced amplification was explored. For RT-LAMP process, RNA should undergo a reverse transcript first to become the RNA-cDNA hybrid which is analogous to dsDNA structure (Sun et al., 2013). Therefore, the real-time amplification curves of dsDNA and ssDNA fragments, with complementary sequence to SARS-CoV-2 N gene, were investigated. For dsDNA, a similar phenomenon was observed that LAMP process in the hydrogel was ultrafast while that in aqueous solution was suppressed (Fig. 2a). The Tt value was 5 min in gel system as compared to aqueous system (20 min). On the contrary, little difference was found for ssDNA amplification in gel and in aqueous system (Fig. 2b). The suppress of dsDNA or RNA amplification in the aqueous solution may be caused by the certain inhibitor, such as fluorescence dye (Fig. 2c). It is well known that SYBR Green is an intercalating dye, which will likely bind with dsDNA and RNA-cDNA hybrid, while its affinity to ssDNA is low. Thus, the intercalating of SYBR Green dye will affect their amplification process of dsDNA and RNA, while ssDNA is less affected.

In order to verify the reasons affecting the RNA amplification in solution and hydrogel, the more detailed studies were conducted to explore role of SYBR Green dye and function of hydrogel. When different concentrations of SYBR Green were added into the reaction, the aqueous RT-LAMP assay showed increased Tt values (8–34 min), while the Tt values remain same in hydrogel (~4 min) (Fig. 2d). These results confirm the inhibitory effect of dye in conventional aqueous RT-LAMP. These intercalating dyes were typically positively charged, while PEG hydrogel was negatively charged (Xu et al., 2016). Thus, the hydrogel matrix could protect the RT-LAMP reaction against interferences, due to the adsorption of SYBR Green onto the hydrogel surface (Lin et al., 2022). The adsorption could be confirmed by the UV–visible absorption spectrum (Fig. 2e and Fig. S9) and EDS mapping (Fig. S10). Furthermore, the theoretical simulation also confirmed the adsorption of hydrogel and SYBR Green dye using Materials Studio software. As shown in Fig. 2g,i, the binding energy of PEG hydrogel and SYBR Green was calculated as −337.00977 kJ/mol (< 0), confirming the auto-adsorption between dye and PEG hydrogel.

Moreover, even without SYBR Green included, the RT-LAMP amplification in the nanofloned hydrogel was still enhanced compared to that in solution, as shown in Fig. 2f. A low template concentration (10 copies/μL of SARS-CoV-2 RNA was tested here, and the RT-LAMP in the hydrogel is successfully performed with clear bands, while that in aqueous solution was completely failed even without dye included (Fig. 2f). This enhancement has been reported previously that attributed to the crowding effect. The crowded environment could increase the activity of polymerase for isothermal nucleic acid amplification (Jiang et al., 2022; Ozay and McCalla, 2021; Zimmermann and Harrison, 1987). Thus, the sensitivity would be enhanced in the hydrogel through cross-linked crowding environment.

In more detail, an underlying mechanism of ultrafast detection in gel RT-LAMP is proposed as follows (Fig. 2j). The fluorescent dye can combine with RNA-cDNA hybrid in bulk system, which significantly reduce amplification efficiency, and postpone Tt values. Whereas the amplification in gel RT-LAMP is not affected due to the nanofloned effect of hydrogel and strong binding force between hydrogel network and the dye. The strand displacement of outer primer, formation of looped structure, and cycling amplification are all maintained under the nanofloned surroundings of hydrogel. The plentiful products are stabilized and accumulated in hydrogel RT-LAMP, and the amplification time is greatly reduced compared to bulk RT-LAMP.

3.4. Ultrafast quantification of SARS-CoV-2 virus without pre-lysis

In addition to ultrafast RT-LAMP process, virus lysis and RNA purification was also typically required in the SARS-CoV-2 routine test, because the presence of outer protein shell would affect the RNA reverse transcription and amplification process. However, lysis and purification processes would significantly increase detection time and operation complexity. Direct quantification of SARS-CoV-2 virus without pre-lysis could be achieved using the developed hydrogel RT-LAMP system. The TEM image of SARS-CoV-2 virus was displayed in Fig. 3a, and the intact protein coat was apparent. As shown in Fig. 3b, the ultrafast amplification of SARS-CoV-2 virus could be maintained in gel RT-LAMP. However, the amplification curve for the virus in aqueous RT-LAMP was much more inhibited as the Tt values increased from 17 min to 32 min compared to the detection of their extracted RNA. Thus, the protein coat would be another aspect affecting the amplification efficiency. The single virus amplification efficiency of SARS-CoV-2 in commercial solution-based droplet digital RT-LAMP system was reduced due to the presence of protein shells, whereas the amplification efficiency in gel RT-LAMP was not affected (Fig. 3c). Thus, the nanoconfinement effect in hydrogel was favorable for direct quantification of SARS-CoV-2 virus without pre-treatment. It has been reported that the structure of SARS-CoV-2 virus was easily damaged by high temperature, and protein coat was released (see TEM image in Fig. S11). Without purification, these released proteins would still affect the LAMP process. However, in the hydrogel, these released proteins would be adsorbed.
Fig. 2. Mechanism for ultrafast SARS-CoV-2 detection. (a-b) The real-time amplification curves of dsDNA (a) and ssDNA (b) with complementary sequence to SARS-CoV-2 N gene. (c) Gel electrophoresis of aqueous RT-LAMP products for SARS-CoV-2 RNA at the high template concentration ($10^3$ copies/μL) without SYBR Green. N: negative group, P: positive group, M: 2000 bp DNA marker. (d) Time threshold of hydrogel RT-LAMP and aqueous RT-LAMP with different SYBR Green concentrations. (e) UV-visible absorption spectrum of dye after incubation with hydrogel. The concentration of SYBR Green in the assay was decreased after incubation. (f) Gel electrophoresis at the low template concentration (10 copies/μL) without adding dye. Line 1–2: Negative group of bulk RT-LAMP and hydrogel RT-LAMP. Line 3–4: Positive group of bulk RT-LAMP and hydrogel RT-LAMP. M: 2000 bp DNA marker. (g-i) Simulated free energy of PEG hydrogel (g), SYBR Green (h), and their mixture (i). (j) Mechanism diagram of ultrafast detection for SARS-CoV-2 RNA.
Fig. 3. Ultrafast detection of SARS-CoV-2 virus in gel RT-LAMP. (a) The TEM morphology of SARS-CoV-2 virus. (b) Real-time RT-LAMP curve of SARS-CoV-2 RNA and virus in hydrogel and aqueous solutions. (c) Comparison of amplification efficiency using hydrogel and commercial droplet digital chip. (d) Comparison of gel RT-LAMP counts to the expected values. (e) End-point fluorescence images of hydrogel with a concentration series of SARS-CoV-2 virus.
onto the surface of hydrogel (as confirmed by EDS results in Fig. S12), similar to function of SYBR Green as we discussed above. Thus, the RNA of SARS-CoV-2 virus was purified in the hydrogel, which lead to the ultrafast quantification of SARS-CoV-2 virus without pre-lysis. In order to further prove this, we also tested the A260/A280 values to characterize the purity of nucleic acid after incubation of SARS-CoV-2 virus in hydrogel. The A260/A280 values of SARS-CoV-2 virus were changed from 1.51 ± 0.29–1.95 ± 0.14 by incubating at 66 °C for 10 min in the hydrogel (Table S2). A higher A260/A280 value demonstrates that a higher purity of nucleic acids was obtained without proteins contamination, which lead to the ultrafast quantification.

The hydrogel RT-LAMP system was evaluated using a concentration series of SARS-CoV-2 virus to explore the quantitative performance. For comparison, the accurate SARS-CoV-2 concentration in the sample was obtained by standard digital PCR assay. As indicated in Fig. 3d and e, fluorescence dots could be observed clearly as low as 0.5 copy/μL. Progressively more positive dots were counted with increasing concentration of SARS-CoV-2 virus. With final concentrations ranging from 0.5 to 271 copies/μL, five groups were all accurately observed without the background interference. No fluorescence was detected in the negative control as the end-point fluorescence image shows. A high correlation (R² =0.9999) was acquired between the end-point fluorescence counts and the expected values. Thus, the quantification of SARS-CoV-2 virus can be ultrafast, precise and absolute through the developed hydrogel RT-LAMP. The sensitivity level can accurately quantify 0.5 copy/μL of SARS-CoV-2 virus, which is excellent than most work reported to date.

Moreover, the developed hydrogel RT-LAMP was compared with other reported techniques for COVID-19 detection. As shown in Table 1, the gel RT-LAMP demonstrates an ultrafast amplification time (10 min) for the nucleic acid tests of SARS-CoV-2. In previous work, an efficient lysis was necessary before the amplification to extract and purify the nucleic acid (Lin et al., 2018), whereas the pretreatment of SARS-CoV-2 could be eliminated in the developed hydrogel RT-LAMP. The amplification time in routine bulk RT-LAMP required at least 30 min. The amplification efficiency can be enhanced in conventional digital format through facilitating the binding of polymerase and primer (Ozay and McCalla, 2021). Nonetheless, even after RNA extraction, the amplification time using digital droplets assay required 25 min (W. Zhang et al., 2021), which was much larger than 10 min in hydrogel RT-LAMP. The absolute quantification, a growing trend in the rapid POCT field (Lin et al., 2019; Liu and Lei, 2021), could also be achieved through the developed assay. Thus, the integration of multiple advantages was achieved in gel RT-LAMP, in particular ultrafast amplification time, merging the reverse transcription into amplification, no RNA extraction or pre-lysis, and absolute quantification for single molecule. These combined performances were beyond the capacity of other detection methods for nucleic acid testing of SARS-CoV-2. Moreover, the gel RT-LAMP has the potential as a simple assay without specific instruments and rigorous conditions for on-site diagnosis of SARS-CoV-2 in resource-limited settings.

### 3.5. Performance evaluation of hydrogel RT-LAMP

SARS-CoV-2 N gene is a high conservation region and is a necessary fragment for diagnosis. As shown in Fig. 4a and Fig. S13, only SARS-CoV-2 N gene region can be detected. So, the gel RT-LAMP has an excellent specificity towards SARS-CoV-2 N gene compared with other SARS-CoV-2 E gene and ORF8 gene, or other virus (Influenza A virus and Norovirus), bacteria, and plant-pathogenic fungi genome. Moreover, if other specific primers were applied, the developed hydrogel digital amplification possessed a high applicability for detecting various microbes using their respective primers (Table S3 and Fig. S14), including plant-pathogenic fungi (Alternaria alternata), gram positive bacteria (Listeria monocytogenes and Methicillin-resistant Staphylococcus aureus), and gram-negative bacteria (Salmonella typhi). The premixed assay could be stored stably for at least 7 months (Fig. 4b), which offered an outstanding potential for the commercial kit and POCT. Reproducibility was also reliable between 8 groups of parallel (Fig. S15) with 4.33% of relative standard deviation (RSD).

### 3.6. Real samples analysis

The nucleic acid amplification can be inhibited due to the presence of various inhibitors from fruits, such as proteins, polysaccharides, and polyphenols (Schrader et al., 2012). The surface and matrices of cold chain fruits with artificially spiked SARS-CoV-2 were tested. The photographs of actual fruits and juice are displayed in Fig. 5a–b. To evaluate the performance of gel RT-LAMP assay in tolerating inhibitors from juice matrices, we spiked longan, kiwifruit, pitaya and cherry juices into system, respectively. The end-point fluorescence images and amplification curves are shown in Fig. S16a–d and Fig. S16a–b. The fluorescence dots were clearly observed in all juice groups, as well as the control group. In the traditional real-time quantification method, there was a delay in Ts values of cherry and kiwifruit juices, which probably derived from the acidic components in the two fruits. Thus, the quantification ability of real-time nucleic acid amplification was questioned in the POCT analysis for real sample matrices. However, for end-point digital count in the gel system, the number of positive dots was not affected. Thus, the gel RT-LAMP was robust to tolerate inhibitors from multiple cold chain fruits matrices.

SARS-CoV-2 have been detected on various cold chain fruits surface from a number of reported cases in China, causing numerous economic losses. We applied the SARS-CoV-2 virus as model to artificially spike surface with five kinds of cold chain fruits which were proclaimed to be tested positive with SARS-CoV-2 in China. Coating samples were collected from the surface of these fruits using disposable sampling tool, and they were examined by the gel RT-LAMP. The quantification of SARS-CoV-2 on fruits surface was shown in Table 2. All artificially spiked cold chain fruits could be detected at three different SARS-CoV-2

| Technique | Amplification time (min) | RNA extraction or pre-lysis | Absolute quantification | Reference |
|-----------|--------------------------|----------------------------|-------------------------|-----------|
| Magnetic  |                          |                            |                         |           |
| RT-LAMP   |                          |                            |                         |           |
| Two-color RT-LAMP and sequencing | 30 | Yes | No | (Viet Loan Dao et al., 2020) |
| Colorimetric RT-LAMP | 30 | Yes | No | (Rabe and Cepko, 2020) |
| Microfluidic cartridge-based RT-LAMP | 30 | Yes | No | (Ganguli et al., 2020) |
| Single-tube nested qRT-PCR | –79 | Yes | No | (Wang et al., 2020) |
| RT-LAMP-based lateral flow strip | 40 | Yes | No | (Zhu et al., 2020) |
| CRISPR-Cas12-based lateral flow strip | 30–40 | Yes | No | (Broughton et al., 2020) |
| Paper-based device | 50 | Yes | No | (Manzanares et al., 2021) |
| Pipette tips analyzer | 25 | Yes | Yes | (W. Zhang, 2021) |
| Hydrogel RT-LAMP | 10 | No | Yes | This work |
gradients \(10^3-10^5\) copies/\(\mu\)L). Thus, the developed gel RT-LAMP had a good quantitative performance for SARS-CoV-2 detection on surface of different cold chain fruits, and the ultrafast amplification could be achieved within 10–15 min for different samples.

4. Conclusion

In this study, we reported a hydrogel-mediated RT-LAMP for ultrafast and absolute quantification of SARS-CoV-2 on the cold chain fruits. The hydrogel matrix has the strong adsorption for dye and cell debris, and has a unique advantage in isolating SARS-CoV-2 RNA from fluorescent dye and viral coat protein. Ultrafast detection of SARS-CoV-2 virus could be achieved through the developed hydrogel assay without RNA extraction, which is about 3.4-fold faster than that in aqueous RT-LAMP. The developed assay has a high specificity, reproducibility and storage stability. 0.5 copy/\(\mu\)L of SARS-CoV-2 can be accurately quantified within 15 min. Moreover, SARS-CoV-2 could be monitored in actual cold chain fruits, including longan, kiwifruit, pitaya, cherry, and banana. In the near future, this gel RT-LAMP could be a promising tool for point-of-care detection of SARS-CoV-2 or other pathogens in order to ensure clinical, environmental and food safety.
Table 2
Recovery of SARS-CoV-2 by hydrogel RT-LAMP on spiked cold chain fruits.

| Samples     | Spiked (copies/reaction) | Detected (copies/reaction) | Recovery (%) | RSD (%) |
|-------------|--------------------------|----------------------------|--------------|---------|
| Cherry      | 2 × 10^4                | 1.5 × 10^3                 | 74           | 16      |
|             | 2 × 10^5                | 1.5 × 10^4                 | 72           | 7       |
|             | 1.2 × 10^4              | 8.3 × 10^3                 | 68           | 3       |
| Pitaya      | 2 × 10^4                | 1.5 × 10^3                 | 63           | 15      |
|             | 2 × 10^5                | 1.5 × 10^4                 | 73           | 7       |
|             | 1.2 × 10^3              | 7.4 × 10^2                 | 61           | 2       |
| Longan      | 2 × 10^4                | 2.1 × 10^3                 | 103          | 21      |
|             | 2 × 10^5                | 2.2 × 10^4                 | 107          | 4       |
| Kiwifruit   | 2 × 10^4                | 1.2 × 10^3                 | 98           | 2       |
| Banana      | 2 × 10^4                | 1.7 × 10^3                 | 83           | 12      |
|             | 2 × 10^5                | 1.5 × 10^4                 | 77           | 8       |
|             | 1.2 × 10^3              | 8.4 × 10^2                 | 70           | 3       |

Environmental implication

With rapid growing of environmental contact infection, more and more attentions are focused on absolute quantification of SARS-CoV-2 on cold chain foods. Several environmental elements can contaminate cold chain foods, resulting in a cross-spread of SARS-CoV-2 across different environmental areas. We propose an ultrafast hydrogel-mediated RT-LAMP point-of-care test (POCT) system for absolute quantification of SARS-CoV-2 virus in 15 min without pre-lysis. This is the first study of ultrafast and absolute quantification of SARS-CoV-2 on cold chain foods. The integrated system has an enormous potential for SARS-CoV-2 or other hazardous materials POCT in environmental, agriculture and food industry.

CRediT authorship contribution statement

Tao Yang: Investigation, Conceptualization, Data curation, Software, Writing – original draft. Dong Li: Conceptualization, Software. Yuhua Yan: Investigation, Conceptualization. Fatima-ezzahra Ettoumi: Conceptualization, Writing – review & editing. Ricardo A. Wu: Writing – review & editing. Zisheng Luo: Resources, Software. Hanry Yu: Writing – review & editing. Xingyu Lin: Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130050.
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