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Carrying out pseudo dual nucleic acid detection from sample to visual result in a polypropylene bag with CRISPR/Cas12a

Hui Wu [a,1], Yanju Chen [a,1], Ya Shi [b], Liu Wang [c,***], Mengyao Zhang [a], Jian Wu [a,e,*], Huan Chen [b,d,**]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China
[b] Key Laboratory of Microbiol Technology and Bioinformatics of Zhejiang Province, Zhejiang Institute of Microbiology, Hangzhou, 310012, China
[c] State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Key Laboratory of Information Traceability for Agricultural Products, Ministry of Agriculture and Rural Affairs, Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, China
[d] Hangzhou Digital-Micro Biotech Co., Ltd., Hangzhou, 311215, China
[e] Key Laboratory of on Site Processing Equipment for Agricultural Products, Ministry of Agriculture, Hangzhou, 310058, China

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ABSTRACT

Amplification-based nucleic acid detection is widely employed in food safety, medical diagnosis and environment monitoring. However, conventional nucleic acid analysis has to be carried out in laboratories because of requiring expensive instruments and trained personnel. If people could do nucleic acid detection at home by themselves, the application of nucleic acid detection would be greatly accelerated. We herein reported a polypropylene (PP) bag-based method for convenient detection of nucleic acids in the oil-sealed space. The PP bag has three chambers which are responsible for lysis, washing and amplification/detection, respectively. After adding sample, nucleic acids are adsorbed on magnetic particles (MPs) and moved into these three chambers successively through immiscible oil channel by an external magnet. Combined with isothermal amplification, the PP bag can be incubated in a water bath or milk warmer and acted as a reaction tube. With highly specific CRISPR technology, *Salmonella typhimurium (St)* and SARS-CoV-2 can be visually detected in these PP bags within 1 h, indicating its potential household application. To further improve the reliability of nucleic acid testing at home, a logic decision method is introduced by detecting both target and endogenous reference gene. Positive/negative/invalid detection result can be obtained by chronologically adding the CRISPR reagents of target and endogenous reference gene. We anticipate that this PP bag can provide a novel toolkit for nucleic acid detection in people's daily life.

1. Introduction

Polymerase chain reaction (PCR) and isothermal amplification such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) have been widely employed in food-borne pathogen detection (Zhang et al., 2020) and diagnosis (Chen et al., 2020a). The outbreaks of COVID-19 illustrate the importance of nucleic acid detection again (Lee et al., 2020). If people could carry out nucleic acid testing at home by themselves, the control of such pandemic would be faster. However, current nucleic acid detection methods require complex liquid transfer steps, which limits its application mainly in laboratories by trained personnel. Researchers have tried to integrate such complicated steps in microfluidic device, paper-based sensors or bulky instrument. Microfluidic technologies need to transfer liquid by the pump or centrifuge. Some researchers also developed paper-based integrated nucleic acids detection devices (Chen et al., 2019; Tang et al., 2017; Trinh et al., 2019). However, due to the complexity and diversity of the samples, paper-based nucleic acid extraction method

* Corresponding author. College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China.
** Corresponding author. Key Laboratory of Microbiol Technology and Bioinformatics of Zhejiang Province, Zhejiang Institute of Microbiology, Hangzhou, 310012, China.
*** Corresponding author.
E-mail addresses: wangliually@126.com (L. Wang), wujian69@zju.edu.cn (J. Wu), chenhuan7809@gmail.com (H. Chen).
[1] These authors contribute equally to the article.

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needed to be further optimized.

In order to achieve the goal of on-site detection, integrated devices such as Abbott ID NOW (Mitchell and George 2020), Cobas Liat (Tanriverdi et al., 2010), BioFire FilmArray (Poritz et al., 2011) and Cephid GeneXpert (Smithgall et al., 2020) have been developed. Nevertheless, such kind of devices still need dedicated instruments to fulfill complicated nucleic acid detection steps in a closed space, which are normally too complicated and expensive to be installed at home.

Magnetic particles (MPs) can adsorb nucleic acids and move under magnetic fields without pipetting. In 2010, Sur et al. proposed a structure based on immiscible interface between water and oil to facilitate automatic nucleic acid purification with MPs (Sur et al., 2010). Later on, Haselton et al. reported that nucleic acid purification could be realized through water/air immiscible interface in a thin tube (Adams et al., 2013; Bordelon et al., 2011). There are also some works on the integration of nucleic acid extraction and amplification/detection using immiscible interface assisted structures. Yin et al. connected a PCR chip to a thin tube in which an immiscible interface for nucleic acid extraction was established (Yin et al., 2019). Shin et al. designed an immiscible interface-based cartridge to carry out LAMP reaction by using MPs to carry nucleic acids from lysis buffer to amplification solution (Shin et al., 2017). However, in those systems, nucleic acid eluent was usually flowed into the amplification solution by a pump, and the end-point amplification results were observed by accessary instruments, which increased the cost and complexity of detection.

CRISPR/Cas system can indiscriminately cleave single-stranded DNA probes (5′ -Fluorophore/3′-Quencher) and produce fluorescent signal once it recognizes target nucleic acid sequence with the help of guide RNA (gRNA) (Chen et al., 2018). This provides a possibility of highly sensitive endpoint fluorescent visual detection. Broughton et al. (2020) and Joung et al. (2020) applied CRISPR/Cas technique to detect SARS-CoV-2 by lateral flow-based strips. However, the nucleic acid extraction, amplification and detection are not totally integrated in a system yet. To carry out nucleic acid endpoint detection at home, an integrated, closed and low-cost device is essential.

Herein, for the purpose of household utilization, we present a polypropylene (PP) bag-based method for conveniently and visually detecting nucleic acids. The PP bag has three chambers that can integrate the whole process of nucleic acids detection in a closed space. After adding sample into the lysis chamber, nucleic acids are adsorbed on MPs and moved from lysis chamber to wash chamber and amplification/detection chamber through water/oil immiscible interface by an external magnet. In this way, liquid transfer is avoided in the whole process. All those steps are carried out in the closed space of PP bag so that possible aerosol contamination can be avoided. Reaction solution can be thoroughly mixed by pressing chambers of PP bag with fingers. The cost of such a flexible PP bag is less than 0.2 US dollars. Considering that the detection is going to be done at home, isothermal amplification such as LAMP rather than PCR is employed. Combined with highly specific CRISPR technology, the detection results can be read out by the naked eye with the help of a portable ultraviolet (UV) lamp. The testing results can be observed within 1 h without any expensive instruments. Furthermore, to enhance the reliability of nucleic acid detection at home, target gene and endogenous reference gene can be simultaneously tested through chronologically adding the CRISPR reagents. We anticipate that this PP bag could facilitate the nucleic acid detection at home, which may help accelerate the application of nucleic acid detection in people's daily life.

2. Materials and methods

2.1. Materials

The inactivated bacterial culture of *Salmonella typhimurium* (*St*) (2×10^7 CFU/ml) were provided by Zhejiang Institute of Microbiology (Hangzhou, China). Pseudovirus of SARS-CoV-2 were synthesized by Genewiz Co., Ltd (Suzhou, China). Pseudoviruses was formed by lentivirus protein and coated target nucleic acid. Magnetic bacteria/virus extraction kits (including MPs, lysis buffer and wash buffer) were purchased from Shanghai Lingjun Biotechnology Co., Ltd (Shanghai, China). The primers were synthesized by Sangon Co., Ltd (Shanghai, China). gRNA was synthesized by SunYa Biotechnology Co., Ltd (Hangzhou, China). The primers and gRNA sequence of SARS-CoV-2 were from the previous paper (Chen et al., 2020b). The detailed sequence information is listed in supplementary information.

2.2. LAMP reaction systems

For LAMP reaction of *St*, the mixture contained 1 × Thermopol Reaction buffer (New England Biolabs Inc., Ipswich, MA, USA), 2 mM $MgSO_4$ (New England Biolabs Inc., Ipswich, MA, USA), 1.4 mM dNTPs (New England Biolabs Inc., Ipswich, MA, USA), 0.64 U/μL *Bst* 2.0 DNA polymerase (New England Biolabs Inc., Ipswich, MA, USA), 1.6 μM FIP primer, 1.6 μM BIP primer, 0.2 μM F3 primer, 0.2 μM B3 primer, 0.4 μM LF primer, 0.4 μM LB primer. The real-time LAMP reaction contained 2 μM of SYTO 9 dyes (Thermo Fisher Scientific Inc., Waltham, MA, USA). For reverse transcription LAMP (RT-LAMP) of SARS-CoV-2, the concentrations of $MgSO_4$ and *Bst* 2.0 DNA polymerase were 8 mM and 0.32 U/μL respectively. 0.3 U/μL WarmStart® RTx Reverse transcriptase (New England Biolabs Inc., Ipswich, MA, USA) was also contained. For dual RT-LAMP, the concentrations of all components were consistent with RT-LAMP. LAMP reaction was performed at 65 °C for 30 min.

2.3. CRISPR/Cas12a detection system

CRISPR/Cas12a mixtures contained 1 × NEB buffer 2.1 (New England Biolabs Inc., Ipswich, MA, USA), 2.5 μM single-stranded DNA probe (5′6-FAM-TTATT-3′BHQ1, Sangon, Shanghai, China), 200 nM Cas12a protein (New England Biolabs Inc., Ipswich, MA, USA), 600 nM gRNA (SunYa Biotechnology Co., Ltd, Hangzhou, China), 0.5 U/μL RNase inhibitor (Takara Biotechnology Co., Ltd., Dalian, China). CRISPR reaction was performed at 37 °C for 10 min.

2.4. The fabrication of PP bag

The PP plastic bag was carved from double layers of PP film with thickness of 0.06 mm (Lingchuang Novel Material Co., Ltd, Zhuhai, China) by a laser engraver (WER 4060, Vollerun Laser Technology Co., Ltd, Liaocheng, China). During laser ablation, the instant high temperature would melt PP film and in this way, the upper and lower layer of PP film would be fused together, which formed the edges of plastic bag. The 3D printed acrylonitrile butadiene styrene (ABS) (Dianchuangsanwei Technology Co., Ltd, Xuchang, China) hard plate lid was connected to the plastic bag using AB glue (EX-507, Chandor Adhesive Science & Technology Co., Ltd, Fujian, China). The plastic bag was nested on a foam pad to form the final device.

2.5. Nucleic acid detection in the PP bag

For nucleic acid detection in PP bags, 700 μL lysis buffer, 800 μL wash buffer and 50 μL LAMP solution were respectively added into three chambers. 50 μg MP were added into lysis chamber. After MPs transferring from lysis buffer to wash buffer and LAMP solution by a magnet, the PP bag was put into a milk warmer (SUPOR, Zhejiang, China) at 65 °C for LAMP reaction. For further CRISPR detection, 20 μL CRISPR solution was added into amplification chamber and the PP bag was put into milk warmer at 37 °C. To visualize the nucleic acid detection results in the PP bag, a portable UV lamp (Tianmai Henghui Light Source Electric Co., Ltd, Beijing, China) was used. For positive sample, after CRISPR reaction, the single strand DNA probes (5′6-FAM-TTATT-3′BHQ1) were cleaved so 6-FAM fluorophore was separated from the BHQ1 quencher. 6-FAM molecules would absorb light under the UV

wavelength and subsequently emit light of longer wavelength. The wavelength of the emission light was in the visible range, which could be seen by the naked eyes. The observation process was carried out in the dark room.

2.6. Nucleic acid detection with MPs in the tube

For LAMP detection in the tube, the lysis and wash steps were carried out according to the directions provided by the magnetic extraction kit. After washing steps, 50 μL LAMP solution was added into the tube without elution steps. For normal single LAMP, MPs and LAMP amplification solution were thoroughly mixed and put into QuantStudio™3 Real Time PCR system (Thermo Fisher Scientific Inc., Waltham, MA, USA) to collect fluorescent signals. In the verification assay of dual detection, the fluorescence in the tube was observed by the previously developed 3D printing instrument (Chen et al., 2020b).

3. Results and discussions

3.1. PP bags for nucleic acid analysis

In order to prevent possible aerosol contamination, it is better to carry out all steps of nucleic acid detection in a closed space. However, it is difficult to transfer microliter liquid in closed space without any driving force. MPs can move between different liquid phases by water/oil immiscible interface without pipetting. Therefore, we proposed a structure shown as Fig. 1A. The device comprised three chambers, namely lysis chamber, wash chamber, and amplification/detection chamber. These chambers were filled with different aqueous solutions. On the top of the aqueous solution was covered with an oil layer, which could provide a closed environment for nucleic acid detection. During nucleic acid testing, nucleic acids from sample were adsorbed on the surface of MPs. Then through moving MPs with an external magnet, the adsorbed nucleic acid would move from lysis chamber to wash and amplification/detection chambers successively, without any pipetting.

For the household utilization, the cost of the device was an issue. Therefore, we chose common polymer materials to fabricate it. Initially, the device was made of polymethyl methacrylate (PMMA), a common material adopted in microfluidics. To promote liquid mixing in such hard material as PMMA, syringe structure was included in the chamber. This could solve the mixing issue in the hard PMMA device but increased the complexity of the whole device (Fig. S1) (Video S1). Therefore, we decided to use flexible materials to facilitate mixing. Considering that PCR tube was made of PP for its good chemical inertness, hydrophobicity, and low price (Sun et al., 2019), we tried to use thin PP film to fabricate the plastic bag with designed structure (Fig. S2).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bios.2021.113001

The designed PP plastic bag was made by laser engraving (Fig. S3). The schematic diagram of the bag is shown in Fig. 1B. An ABS plate with three holes was designed to cover PP bag to provide connections to the outside. To make the device float on the water during isothermal amplification, a thin layer of foam was stuck under the ABS plate. PP bag was flexible so MPs and the reaction solution could be fully mixed by pressing with fingers (Fig. 1C). The operational details of mixing with fingers can be seen in Video S2. Household appliances, such as electric toothbrush, could also be used to promote mixing (Video S3).

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MPs could move smoothly throughout the water/oil immiscible interface (Video S4). Even if the aqueous solution was not covered with oil, MPs could pass through water/air interface (Video S5), which might be ascribed to the hydrophobic surface of PP film. However, in order to prevent possible amplicons aerosol contamination, oil layer was still employed in our bag to seal reaction solutions.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bios.2021.113001

3.2. St detection in PP bag

To explore whether the nucleic acid analysis could be carried out in PP bags, we chose a common foodborne pathogen *St* to test. The culture media of *St* and MPs were added into the lysis chamber. Nucleic acids released from *St* were adsorbed by MPs. Then, together with MPs, under the effect of external magnet, nucleic acids would be transferred into wash chamber for removing the impurities and amplification/detection chamber for amplification. CRISPR/Cas system can specifically recognize target gene and indiscriminately slash single-stranded DNA (5′-FAM/3′-BHQ1 labeled) probes to produce fluorescent signals. Our group have done some work on end-point fluorescent visual method using CRISPR/Cas system for detecting pathogen and genetically modified (GM) food (Qian et al., 2019; Wu et al., 2020a, 2020b; Zhang et al., 2020). Here, to visualize the amplification result in PP bags, CRISPR reagents of *St* target sequence were added into the amplification chamber after LAMP reaction. Fig. 2 shows the entire process of *St* detection in the PP bag. The time consumed for lysis, amplification and

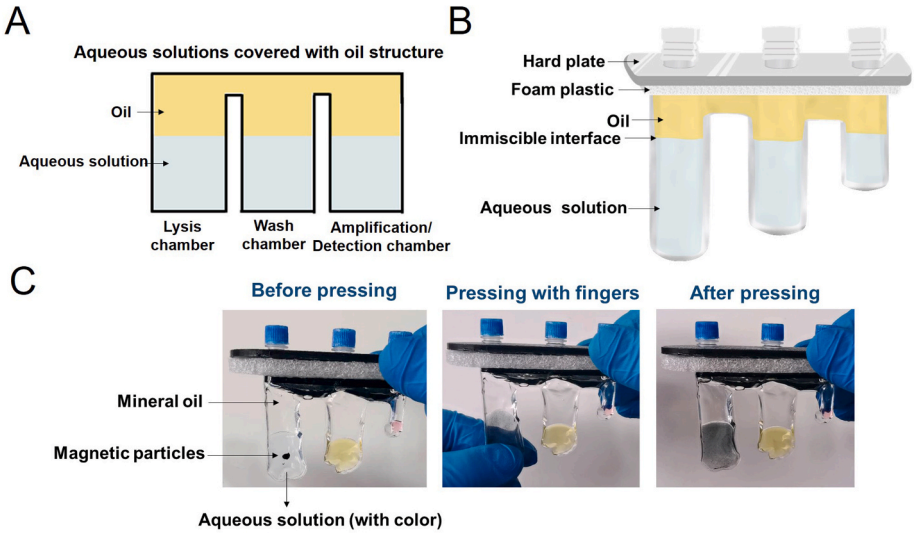


Fig. 1. PP bag for nucleic acid analysis. (A) The structure of water solutions covered with an oil layer on top, forming a closed environment. (B) The overall schematic diagram of the plastic PP bag. (C) The MPs and solution mixing with fingers.

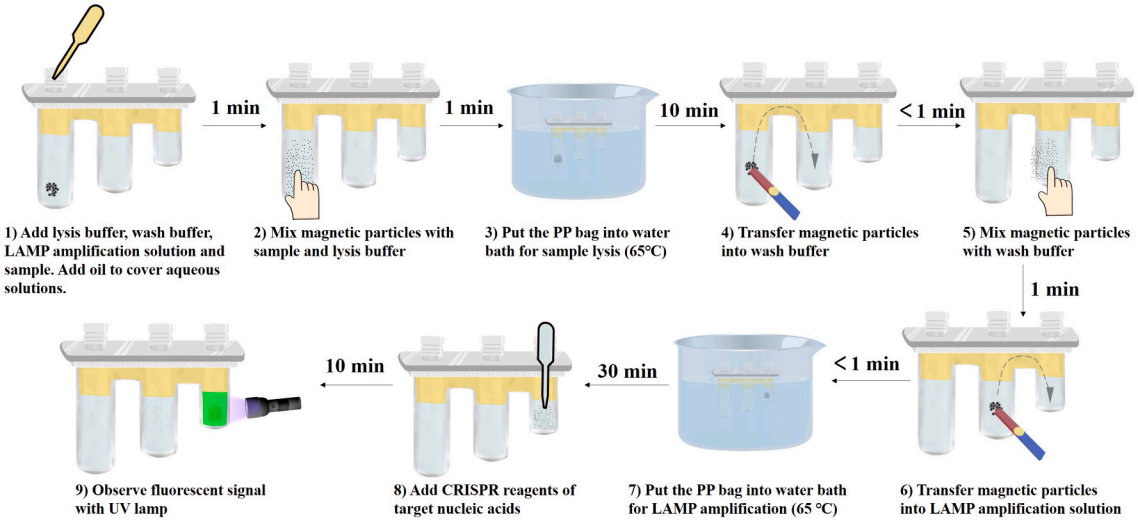


Fig. 2. The overall workflow of nucleic acid detection in the PP bag. 1) Lysis buffer, wash buffer and LAMP amplification solutions were added into lysis chamber, wash chamber and amplification/detection chamber respectively. MPs and sample were added into lysis chamber. Oil was covered onto the liquid to form the closed environment. Solutions were added by plastic dropper. 2) MPs were mixed with lysis buffer and sample with fingers pressing on the soft PP bag. 3) The PP bag was put into the water bath for lysis at 65 °C for 10 min. 4) MPs were transferred into wash chamber with an external magnet. 5) MPs were fully mixed with wash buffer with fingers pressing on the soft PP bag. 6) MPs were transferred into amplification chamber. 7) The PP bag was put into the water bath for LAMP amplification at 65 °C for 30 min. 8) CRISPR reagents of target were added into amplification/detection chamber with plastic dropper inserting under the water-oil interface. 9) Portable UV lamp was used to observe fluorescence after 10 min.

CRISPR detection was 10 min, 30 min, 10 min respectively.

Fig. 3A shows the reaction in every chamber. Compared with conventional magnetic extraction and amplification process, there was no nucleic acid elution step in our approach. Nucleic acids adsorbed on the surface of MPs were amplified directly with MPs. Therefore, firstly, we investigated the effect of different amounts of MPs (25 μg, 50 μg, 75 μg, 100 μg) on LAMP reaction in normal PCR tubes in the QuantStudio™3 Real Time PCR system. The results showed that MPs had little impact on LAMP reaction (Fig. S4). For the convenience of operation, 50 μg MP were used in all following experiments.

In order to facilitate nucleic acid test at home, a household milk warmer was used as water bath for LAMP reaction in PP bag. To demonstrate the feasibility of LAMP amplification in PP bag by a milk warmer, SYTO 9 dyes was added in the amplification system to test the melting curves after LAMP amplification at 65 °C for 30 min. The melting curves suggested that the nucleic acid testing from sample extraction to amplification was successfully realized in PP bags for *St* (Fig. 3B).

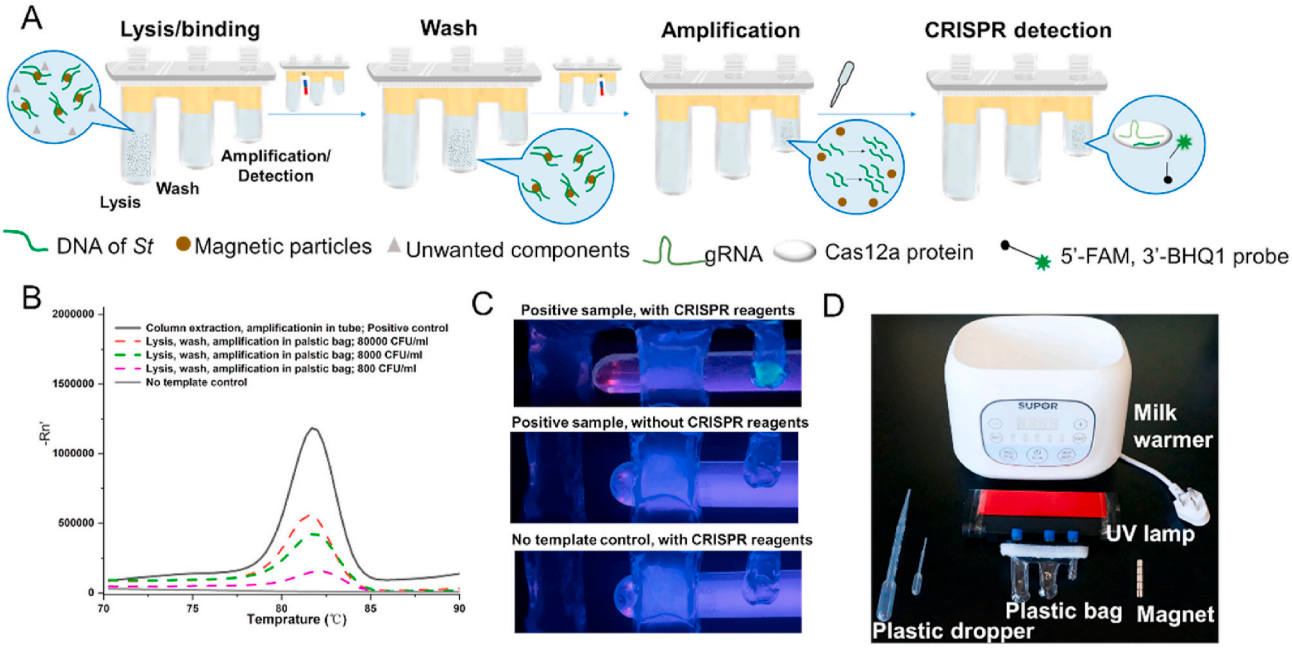


Fig. 3. The detection of *St* in PP bags. (A) The water/oil immiscible interface based integrated *St* lysis, wash, amplification and detection. (B) For only verifying of LAMP amplification in the PP bag in the milk warmer, LAMP amplicons was taken out from amplification chamber of PP bag to conduct melting curves of *St* LAMP amplicons in QuantStudio™3 Real Time PCR system. (C) For positive sample, without/with CRISPR reagents, the fluorescent signal observed by naked eyes with a portable UV lamp in the dark room. (D) All the apparatus used in *St* visual detection in the PP bag, including milk warmer, UV lamp, magnet and plastic dropper.

Having demonstrated that feasibility of using a milk warmer to hold MP-assisted LAMP amplification in the PP bag, we next integrated the complete process, from lysis, wash, amplification to CRISPR detection in the PP bag. For the complete detection process, SYTO 9 was not contained. After adding CRISPR reagents for 10 min, as shown in Fig. 3C, there was bright fluorescent signal under the UV lamp for positive sample detection. The limit of detection (LOD) of *St* in bags was 800 CFU/ml. The sensitivity of this method was comparable to or better than the reported method (Azizi et al., 2019; Zhuang et al., 2020). It was noteworthy that our method had higher specificity by combining with CRISPR. Furthermore, the entire process was integrated in the PP bag and no complicated instruments were required, which can effectively avoid amplicon formed aerosol contamination. Moreover, after washing step in the PP bag, the MPs were also taken out into the tube and amplified by LAMP reaction with specialized instrument. The LOD of *St* detection in the tube by specialized instrument was comparable to that in the PP bag by milk warmer, which could further illustrate the feasibility of this PP bag-based method (Fig. S5).

Video S6 shows the entire nucleic acid detection process. For LAMP amplification and CRISPR detection in the PP bag, a milk warmer and a portable UV lamp are needed. They are cheap and available at home. With this approach, the whole test process can be accomplished within 1 h. In the entire process, as shown in Fig. 3D, only plastic droppers, a portable UV lamp, a magnet and a milk warmer were needed, which totally took less than 30 US dollars.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bios.2021.113001

3.3. Dual detection of SARS-CoV-2 in the tube

Having demonstrated DNA detection with *St*, then we tried to detect RNA and took SARS-CoV-2 as an example. Considering biological safety, SARS-CoV-2 pseudovirus samples were used for testing. Before using the PP bag, we first carried out the LAMP reaction with a tube using a QuantStudio™3 Real Time PCR system to optimize the experimental parameters and conditions. We selected E gene (coding envelope protein), N gene (coding nucleocapsid protein), ORF gene (open reading frames) of SARS-CoV-2 as target gene respectively for LAMP reaction and found that detection of ORF gene was the most sensitive (Figs. S6, S7, S8). Therefore, we selected ORF gene as target gene to carry out further LAMP detection.

To improve the reliability of detection, target gene and endogenous reference gene are usually detected simultaneously. For example, human RNase P gene extracted from throat swab was used as endogenous reference gene for SARS-CoV-2 detection. qPCR reaction can easily realize real-time dual nucleic acid detection with specific TaqMan

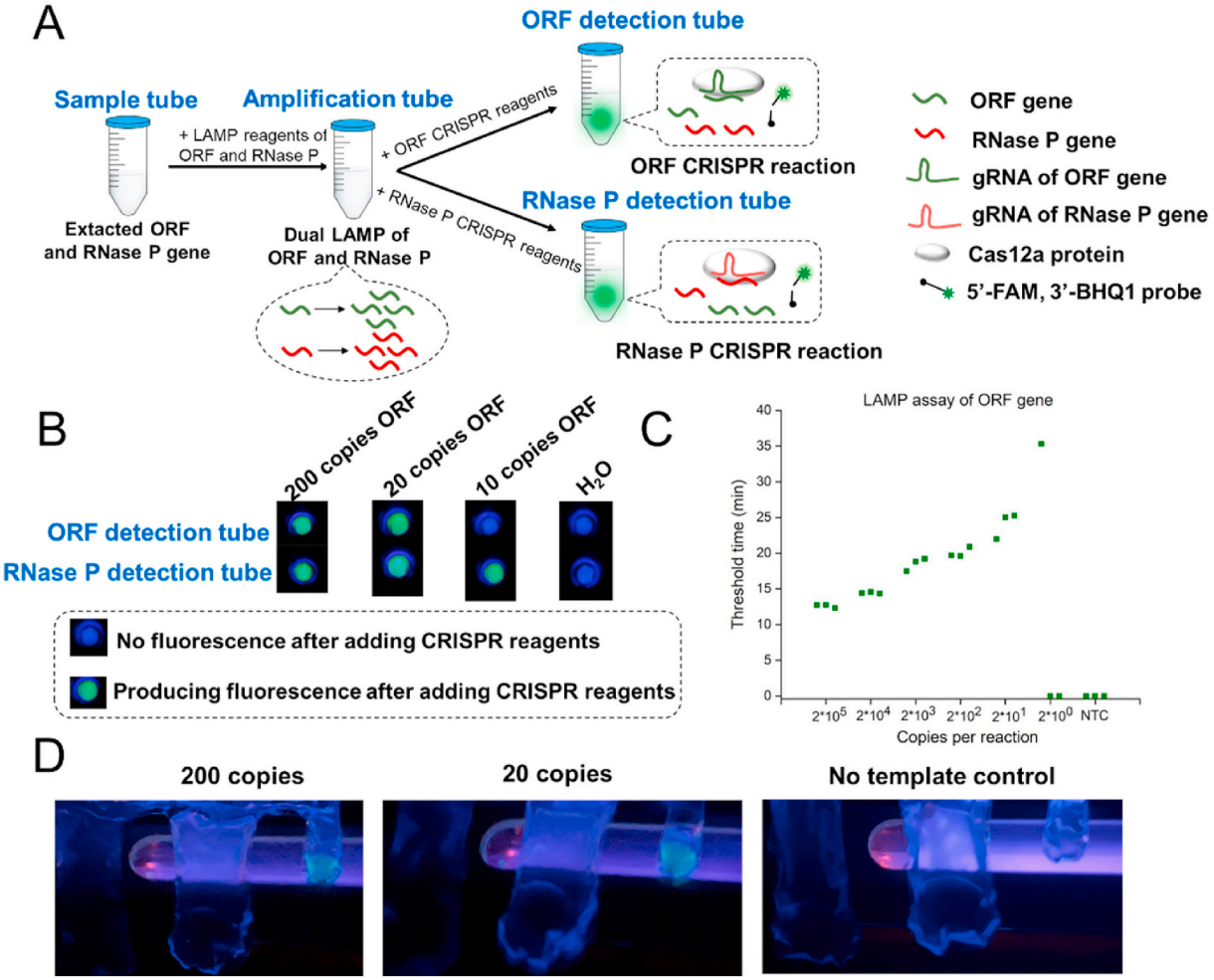


Fig. 4. Detection of dual LAMP amplicons with CRISPR/Cas. (A) The verification principle of dual LAMP reaction and CRISPR/Cas detection in the tube. After dual LAMP reaction, part of the amplified solution was respectively added into two tubes containing CRISPR reagents of ORF gene and CRISPR reagents of RNase P gene. (B) After dual LAMP of ORF and RNase P gene, the CRISPR/Cas detection results for ORF and RNase P respectively in the tube. The fluorescence was observed in the 3D printing instrument designed to see the fluorescence in the tube (Chen et al., 2020b). (C) The threshold time vs. target copy number of normal single LAMP reaction of ORF gene data measured in QuantStudio™3 Real Time PCR system. (D) Detection of different concentrations of SARS-CoV-2 pseudovirus mixed with throat swab in the PP bag.

probes. However, it was difficult to achieve real-time dual detection of LAMP due to lacking related isothermal polymerase with 5′ to 3’ exonuclease activity. CRISPR/Cas technology was a highly specific end-point nucleic acid detection tool, but it was hard to detect two genes in one reaction pot because Cas protein indiscriminately cleaved single strand signal probes once it recognized one of the target genes.

To realize dual detection in the PP bag, firstly, we explored dual LAMP reaction of ORF gene and RNase P gene in a normal PP tube. Different concentrations of SARS-CoV-2 pseudovirus mixed with throat swab were extracted and then added into the tube containing LAMP reagents for both ORF and RNase P gene. After dual LAMP reaction, part of the amplified solution was added into two different tubes containing CRISPR reagents of ORF gene and RNase P gene respectively (Fig. 4A). The fluorescent signals in the tubes were observed by a previously developed 3D printing instrument (Fig. S9). It was worth emphasizing that 3D printing instrument was used to observe the fluorescence in the tube rather than in the PP bag. As was shown in Fig. 4B, the dual LAMP LOD of ORF gene was 20 copies per reaction, the same as single normal real-time LAMP detection of ORF gene (Fig. 4C). The calibration curve was shown in Fig. S10. Also, the SARS-CoV-2 pseudovirus mixed with throat swab was detected in the PP bag and the LOD was 20 copies (Fig. 4D). The performance comparison between our method and recent reported SARS-CoV-2 detection method was shown in Table S1. The assay demonstrated the feasibility of dual LAMP in one pot. Based on it, we further explored dual detection in the PP bag.

3.4. Pseudo dual detection of SARS-CoV-2 in the PP bag

Having demonstrated the feasibility of dual LAMP amplification in one pot, we proposed a novel protocol to fulfill pseudo dual nucleic acid detection protocol in PP bag. As illustrated in Fig. 5A, the sample containing both target gene and endogenous reference gene was added into the PP bag for detection. After dual LAMP reaction, CRISPR reagents of target gene were firstly added into the amplification/detection chamber and mixed with dual LAMP amplicons. If there was fluorescent signal, it meant that Cas12a protein could recognize target gene amplicons and indiscriminately cleave single strand DNA probes under the guidance of gRNA of target, which implied the sample was positive. If there was no fluorescent signal after adding CRISPR reagents of target gene, it meant the sample was possible negative. For further confirmation the possible negative result, CRISPR reagents of endogenous reference gene were also added into the amplification/detection chamber and mixed with dual LAMP amplicons. If there was fluorescent signal, it meant that Cas12a protein could recognize endogenous reference gene amplicons and cleave single strand DNA probes under the guidance of gRNA of endogenous reference gene, which confirmed the sample was negative. If there was no fluorescence, which means that even the endogenous reference genes were not amplified, the total detection was actually invalid. Compared with other common methods such as lateral flow-based strips with control line/test line (only positive or negative result for one strip), a logic decision method was established by the pseudo dual detection. Therefore, no extra time would be taken if the sample was positive. If the sample was negative, extra 10 min would be taken to exclude false-negative results caused by possible errors. The positive/negative/invalid detection system established by chronologically adding CRISPR reagents would enhance the reliability of the nucleic acid detection at home.

In the Spring 2020, there was a qualification test to evaluate the capacity of different laboratories on nucleic acid detection of SARS-CoV-2 samples, which was organized by Shanghai Center for Clinical Laboratory, China. We signed up for this test and were provided with inactivated samples. Those samples were tested both in PP bags and with standard qPCR approach. The results showed that our pseudo dual testing approach were 100% positive agreement and 100% negative agreement with the qPCR results (The Ct values of positive samples were 32.9, 29.1, 31.4, 34.2), indicating the potential ability of the PP bag for

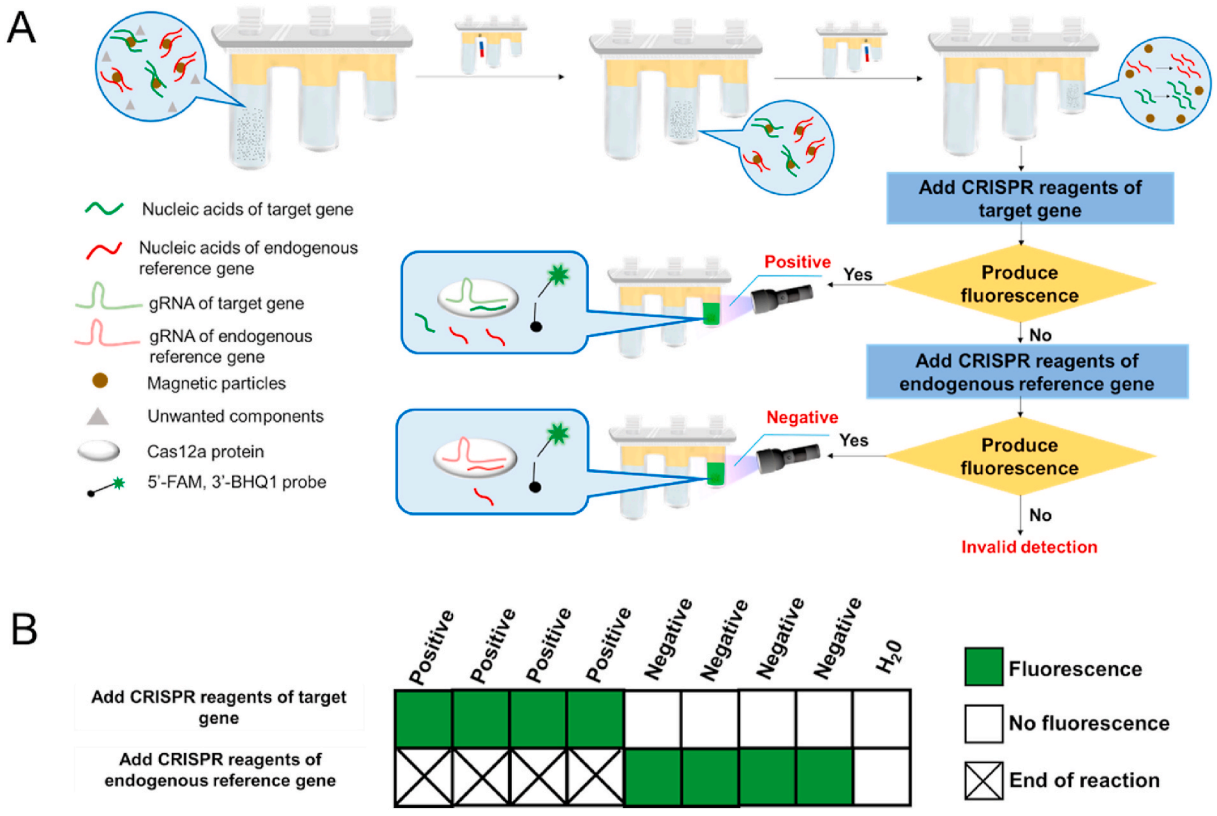


Fig. 5. The pseudo dual nucleic acid testing in PP bags. (A) The schematic diagram of pseudo dual nucleic acid detection protocol. (B) The pseudo dual detection results of SARS-CoV-2 samples.

SARS-CoV-2 detection at home (Fig. 5B).

The toughness and storage is important for nucleic acid detection in the PP bag. The tensile strength of the PP bag was tested by stretching the reaction chamber of PP bag. The PP bag could withstand more than 16 N stress (Fig. S11), which was enough strong for the operation. To avoid buffer contamination in different chambers, different reaction liquids could be stored in different tubes for transportation. Users could transfer the reaction liquids into corresponding chambers by the disposable plastic dropper. For stability of the reagents, lysis buffer and wash buffer could store for approximately a year at room temperature according to the magnetic extraction kit. We also tried to prepare lyophilized CRISPR reagents (Fig. S12). The lyophilized reagents could smoothly enter into the amplification chamber, which might simplify the detection procedure and help store reagents (Video S7). The detailed steps of lyophilization could be found in our published work (Qian et al., 2020).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bios.2021.113001

4. Conclusions

In summary, we developed a MPs-assisted nucleic acid detection method in a closed environment with the PP bag. The reaction solution in the flexible bag could be thoroughly mixed with fingers. Using this PP bag, *St* and SARS-CoV-2 could be detected within 1 h, demonstrating its potential application for the home testing. The pseudo dual test could be realized by chronologically adding CRISPR reagents of target gene and endogenous reference gene, which could effectively avoid false negative result. The PP bag only costs 0.2 US, which is quite cheap. Some household tools, such as a milk warmer and a portable UV lamp, can meet all the demands of PP bag-based detection. This contamination-free and low-cost method can promote the application of nucleic acid detection from labs into people's daily life.

CRediT authorship contribution statement

Hui Wu: Investigation, Methodology, Validation. **Yanju Chen:** Writing - original draft. **Ya Shi:** Formal analysis. **Liu Wang:** Conceptualization, Supervision, Writing - review & editing. **Mengyao Zhang:** Conceptualization, Supervision. **Jian Wu:** Conceptualization, Supervision, Methodology, Funding acquisition, Writing - review & editing. **Huan Chen:** Conceptualization, Project administration, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113001.

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