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Rapid and sensitive detection of Zika virus by reverse transcription loop-mediated isothermal amplification

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A B S T R A C T

Background: Zika virus (ZIKV) is an arbovirus that recently emerged and has expanded worldwide, causing a global threat and raising international concerns. Current molecular diagnostics, *e.g.*, real-time PCR and reverse transcription PCR (RT-PCR), are time consuming, expensive, and can only be deployed in a laboratory instead of for field diagnostics.
Objectives: This study aimed to develop a one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) platform showing sensitivity, specificity, and more convenience than previous methods, being easily distributed and implemented.
Methods: Specific primers were designed and screened to target the entire ZIKV genome. The analytical sensitivity and specificity of the assay were evaluated and compared with traditional PCR and quantitative real-time PCR. Three different simulated clinical sample quick preparation protocols were evaluated to establish a rapid and straightforward treatment procedure for clinical specimens in open field detection.
Results: The RT-LAMP assay for detection of ZIKV demonstrated superior specificity and sensitivity compared to traditional PCR at the optimum reaction temperature. For the ZIKV RNA standard, the limit of detection was 20 copies/test. For the simulated ZIKV clinical samples, the limit of detection was 0.02 pfu/test, which was one order of magnitude higher than RT-PCR and similar to real-time PCR. The detection limit of simulated ZIKV specimens prepared using a protease quick processing method was consistent with that of samples prepared using commercial nucleic acid extraction kits, indicating that our ZIKV detection method could be used in point-of-care testing.
Conclusions: The RT-LAMP assay had excellent sensitivity and specificity for detecting ZIKV and can be deployed together with a rapid specimen processing method, offering the possibility for ZIKV diagnosis outside of the laboratory.

1. Introduction

Zika virus (ZIKV) is an arbovirus member of the *Flaviviridae family*, genus *Flavivirus*, which is related to Dengue fever, Yellow fever, West Nile fever, Japanese encephalitis, and Chikungunya viruses (Petersen et al., 2016), and it has two lineages (Asian and African) (Haddow et al., 2012). ZIKV is a single-stranded, positive-sense, RNA virus (Fleming et al., 2016) that causes common symptoms (*e.g.*, fever, rash, and joint pain) similar to those of many other diseases. Thus, it has not received much attention, and most ZIKV infections in humans are actually asymptomatic (Duffy, 2009). Recently, however, the emerging ZIKV outbreak in the Americas has attracted global attention.

ZIKV is primarily transmitted to people through the bite of infected *Aedes* species mosquitoes, but it can also be transmitted through blood, perinatally, and sexually (Haddow et al., 2012; Ibrahim, 2016). ZIKV was first identified and isolated from a

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rhesus monkey in Uganda in 1947 (Haddow et al., 2012), and the first isolation of ZIKV from humans was in Nigeria in 1954. Subsequently, sporadic human ZIKV infections occurred until a major epidemic on Yap Island, Micronesia in 2007 (Duffy, 2009; Hayes, 2009; Lanciotti et al., 2008). In 2015, ZIKV was identified in Brazil, and then spread rapidly to the Neotropics and other continents around the world, causing characteristic birth defects associated with microcephaly and Guillain-Barré syndrome related to neurological disorders (Calvet et al., 2016; Johansson et al., 2016; Rasmussen et al., 2016; Smith and Mackenzie, 2016; Victora et al., 2016). As of April 2016, a total of 4800 laboratory-confirmed, natural ZIKV infections have been reported in over 46 countries, and 13 ZIKV cases are known in China since the first patient was reported on February 5, 2016 (Zhang et al., 2016b). The genetic diversity of ZIKV may have gradually increased since late 2013 with its geographic expansion (Shi et al., 2016). On Feb 1, 2016, the World Health Organization (WHO) declared ZIKV as a public health emergency of international concern (Heymann et al., 2016; WHO, 2016).

Considering the above, ZIKV displays the potential to be introduced into new areas and cause an epidemic in the future. Thus, there is a critical need for a rapid, specific, and reliable diagnostic method to detect the virus. However, current diagnostic tests for ZIKV are limited (Pardee et al., 2016). The traditional diagnosis of ZIKV is based on serology, which has low specificity due to cross-reaction with other flaviviruses circulating in the region (Zammarchi et al., 2015). Viruses can also be directly isolated from humans, animals, and mosquitoes, but this is time consuming (Ibrahim, 2016). RT-PCR and real-time PCR are more sensitive and specific molecular diagnosis assays that have also been applied to confirm ZIKV infection (Faye et al., 2013, 2008; Lanciotti et al., 2008), but they must be deployed in a laboratory, not in the resource-limited field. Recently, a paper-based ZIKV molecular detection platform combining isothermal RNA amplification was developed that can be used in low-resource locations (Pardee et al., 2016). This detection platform resolves practical limitations to the deployment of diagnostics in the field, but the detection time is still too lengthy. In view of this, rapid and accurate molecular diagnostics outside of research laboratories are critically needed to monitor the ongoing ZIKV outbreak.

Loop-mediated isothermal amplification (LAMP) is a promising tool that is simple, efficient, and robust. The assay is generally conducted under isothermal conditions (60–65 °C) within 60 min, and its results can be analyzed by the naked eye through a color change of the reaction mixture (Nemoto et al., 2010). Further, LAMP assays can be performed with impure templates, decreasing sample processing times. Such assays have been widely used for the detection of various pathogens due to their low cost, simplicity, convenience, speed, sensitivity, and specificity (Liu et al., 2014, 2012; Livingstone et al., 2016).

In this study, we developed a one-step reverse-transcription LAMP (RT-LAMP) platform capable of detecting ZIKV specimens in the field and coupled it with a rapid and simple method for pretreatment of clinical samples. The detection platform can perform all sample processing and testing steps, utilizing a portable battery-powered metal bath within 1 h, and the results can be analyzed by the naked eye. This detection platform makes the ZIKV test procedure easier, faster, and more convenient for sample processing, the actual reaction step, and interpretation of the results. The test specifically detected ZIKV with no cross-reactivity to other arboviruses or influenza virus. These advantages suggest that our diagnostic platform is useful for identifying ZIKV cases in the laboratory or for point-of-care testing in endemic rural areas. The objective of this study was to develop a novel, portable, rapid, low-cost, and accurate ZIKV RT-LAMP detection platform, including a rapid nucleic acid release technique for clinical specimens.

2. Materials and methods

2.1. Viruses and simulated clinical specimens

The ZIKV strain (SZ_SMGC-1) used in this work was isolated from a serum sample of an imported case returning to China from Fiji and Samoa at the Shenzhen Port (Wang et al., 2016). The entire viral genome was sequenced (GenBank: KX266255). The plaque forming units (pfu) of the ZIKV stock solutions were titrated on Vero cells (African Green Monkey Kidney). The simulated clinical specimens were prepared by spiking ZIKV stocks into normal human saliva, urine, or serum (1%) at a final concentration of 2×10^4 pfu/mL. The saliva, urine, and serum samples were collected from healthy donors. Four different serotypes of dengue virus (DENV-1, DENV-2, DENV-3, and DENV-4) and influenza A virus strain PR8 were propagated in C6/36 and MDCK cells, respectively, and used to identify the specificity of the RT-LAMP assay because they cause similar clinical symptoms to ZIKV (Ibrahim, 2016). Viral RNAs were extracted using a QIAamp Viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol.

2.2. Preparation of the reference RNA template

To prepare the ZIKV RNA standard, the NS1 gene fragment of ZIKV was synthesized (GenScript, Nanjing, China) and cloned into the *Nco*I and *Bam*HI sites of the pET15a vector. Then, the recombinant plasmid was linearized with *Eco*RI (New England Biolabs). RNA transcripts were generated using T7 RNA polymerase (Promega) according to the manufacturer's instructions. The transcripts were further treated with RNase-free DNase I (Promega) to remove any template plasmid DNA. After purification using an RNeasy clean up kit (Qiagen), the copy numbers of RNA transcripts were calculated based on the concentrations determined by a Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific). Serial dilutions of RNA were prepared and frozen at −80 °C.

2.3. Primer deign

Nucleic acid sequence alignments of ZIKV and other Flavivirus strains were performed using ClustalX. According to the sequence of ZIKV in GenBank (accession number: KU740184.2), primers specific to a highly conserved ZIKV region were designed using the online open source Primer ExploerV.4 software (Eiken Chemical, Japan, http://primerexploer.Jp/e/). They include an outer pair (F3, B3), an inner pair (FIP, BIP), and an addition loop pair (LF, LB).

2.4. Specimen processing

To determine the most effective technique for quick sample processing, we tested three different strategies: boiling lysis, proteolysis, and alkaline lysis. For thermal lysis processing, simulated clinical specimens were directly heated to 95 °C for 5 min. For proteolytic processing, proteinase K was added to the specimens to a final concentration of 20 μg/mL, incubated at 65 °C for 10 min, and inactivated at 95 °C for 2 min. The simulated serum specimens needed to be pre-diluted 10 times in water, but the other two types did not. For alkaline lysis processing, 180 μL of 50 mM NaOH was added to 20 μL of simulated specimen, mixed by vortexing, incubated at 95 °C for 10 min, and then 20 μL 1 M Tris-HCl (pH 8.0) was added to the mixture. The resulting lysates were directly used to initiate RT-LAMP reactions.

2.5. RT-LAMP

The RT-LAMP reactions were performed using a Loopamp RNA Amplification Kit (Eiken Chemical Co., Ltd., Japan) with 1.6 μM each

of the FIP and BIP primers, 0.2 μM each of F3 and B3, 0.8 μM each of LF and LB, and 5 μL template RNA solution in a final reaction volume of 25 μL. A small amount of paraffin wax was added into the reaction tubes after all of the reagents were added to prevent the formation of aerosols. The mixture was incubated at 65 °C for 50 min.

2.6. Detection of RT-LAMP products

The RT-LAMP products were detected using two different methods: monitoring turbidity and direct visual inspection. In the first method, real-time amplification reactions were monitored using a Loopamp real-time turbidimeter (LA-500; Eiken Chemical Co., Ltd., Japan) that recorded and analyzed the optical density at 650 nm every 6 s. In the second method, the amplification products were observed by the naked eye under natural light or UV irradiation by adding 1 μL calcein fluorescent reagent (Eiken Chemical Co., Ltd., Japan) to 25 μL RT-LAMP reaction before amplification. A color change from orange to green indicated a positive reaction, while a negative reaction remained orange.

2.7. Rt-pcr

RT-PCR was performed using a TransScript One-Step RT-PCR SuperMix kit (Transgen Co., Ltd., China). The 20-μL reaction mixtures contained 10 μL 2 × one-step reaction mix, 0.4 μL each forward and reverse primers (10 μM) (Table 1), 0.4 μL enzyme mix, the same amount of RNA template used in the RT-LAMP reaction, and RNase-free water. The reaction program was 50 °C for 20 min, 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 20 s, and a final extension at 72 °C for 10 min. The products were analyzed by 2% agarose gel electrophoresis.

3. Results

3.1. The most appropriate RT-LAMP primers for detection of ZIKV

We designed seven primer sets to detect ZIKV RNA. Under the same reaction conditions, three turbidity curves were observed after reaction for 14 min, and the primers in the ID-6 set, ID-79 set, and ID-24 set, which amplified the target gene in a shorter time than the other primer sets, were candidates for the most optimal reaction primers (Fig. 1). Next, the specificity of these three primer sets was tested, and we found that primer set ID-79 was the most optimal (Fig. 2). The ID-79 primer set was thus chosen for ZIKV detection in the RT-LAMP assay (Table 1).

3.2. Specificity of the RT-LAMP assay

The specificity of the RT-LAMP primers for ZIKV was validated by testing 10 samples, including ZIKV isolate RNA, four different serotypes of dengue virus RNA (11.3 ng/μL DENV-1, 15.8 ng/μL DENV-2, 8.9 ng/μL DENV-3, and 4.1 ng/μL DENV-4), H1N1 influenza virus RNA (24.6 ng/μL), a plasmid containing the NS1 gene of Spondweni virus, and healthy human saliva, urine, and serum samples. Although all of the primers screened for ZIKV genomic sequences were mostly distinct from those of related viruses, the ZIKV sequences do share substantial similarity with their Dengue virus counterparts. Therefore, only one primer set demonstrated good specificity (Pardee et al., 2016). For the ID-79 primer set, we found that turbidity increased only when ZIKV RNA was used as the template and not with other virus samples and healthy human specimen controls (Fig. 2A), demonstrating the high level of RT-LAMP specificity. In contrast, for the ID-6 and ID-24 primer sets, dengue virus template resulted in similar amplification to ZIKV (Fig. 2B, C).

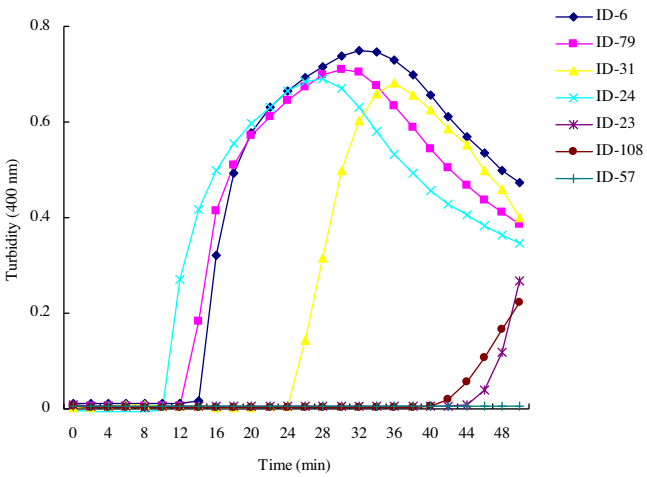

Fig. 1. ZIKV RNA amplification by seven primer sets under the same conditions.

3.3. Reaction temperature of the RT-LAMP assay

To optimize the reaction conditions of the RT-LAMP reaction, the assay was performed at temperatures between 61 and 65 °C. As shown in Fig. 3, 64 °C appeared to yield the fastest reaction, but the turbidity reading at 64 °C was lower than at 65 °C.However, 65 °C gave the highest maximum turbidity reading and quickest reaction, indicating that the target gene was amplified with high efficiency. Thus, we achieved our goal to develop a visual technique for point-of-care diagnosis. These results showed that 65 °C was the most suitable temperature for the RT-LAMP assay.

3.4. Sensitivity of the RT-LAMP assay

To evaluate the sensitivity of the assay, RT-LAMP and RT-PCR were performed using a series of 10-fold dilutions of ZIKV RNA standard (prepared by *in vitro* transcription) or RNA extracted from ZIKV specimens. The detection limit of the RT-LAMP assay for the RNA standard was 20 copies/test (4000 copies/mL) (Fig. 4A), and the estimated sensitivity of the assay for ZIKV reached 0.02 pfu/test (4 pfu/mL) (Fig. 4C). For RT-PCR, the sensitivity reached 0.2 pfu/test (40 pfu/mL) (Fig. 4E).

Table 1
Nucleotide sequences of the primers used for RT-LAMP and RT-PCR.

Primer	Type	Primer sequence (5′-3′)
ID79-F3	Forward outer	TGGAGGGACAGGTACAAGT
ID79-B3	Backward outer	TGTGGACCTCTCCACATGG
ID79-FIP	Forward inner	CGCAGATACCATCTTCCCAGGCATCCTGACTCCCCCCGTA
ID79-BIF	Backward inner	AAGGGGAGCTCAACGCAATCCGATCCCACAACGACCGTC
ID79-LF	Loop forward	TGCTTGACTGCTGCTGCC
PCRF	Forward primer	ATGCGGAAGTCACTGGAAACA
PCRR	Reversed primer	GCAGGCACATCCCATAGAGC

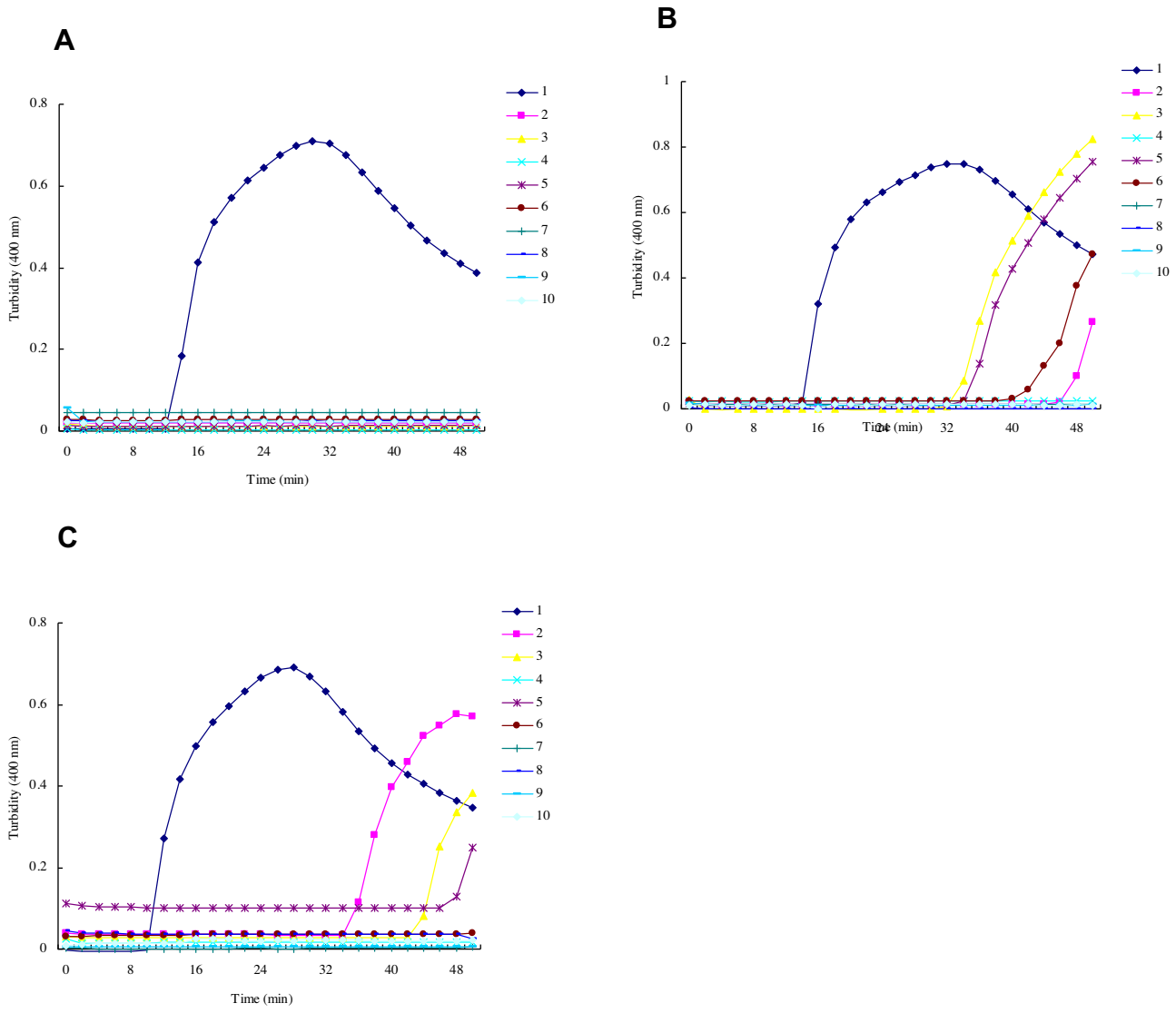

Fig. 2. Specificity of the three sets of primers for the RT-LAMP assay. A. Specificity of primer set ID-79. B. Specificity of primer set ID-6. C. Specificity of primer set ID-24. Sample 1, ZIKV; 2, DENV-1; 3, DENV-2; 4, DENV-3; 5, DENV-4; 6, Spondweni; 7, influenza; 8, negative serum sample; 9, negative urine sample; and 10, negative sputum sample.

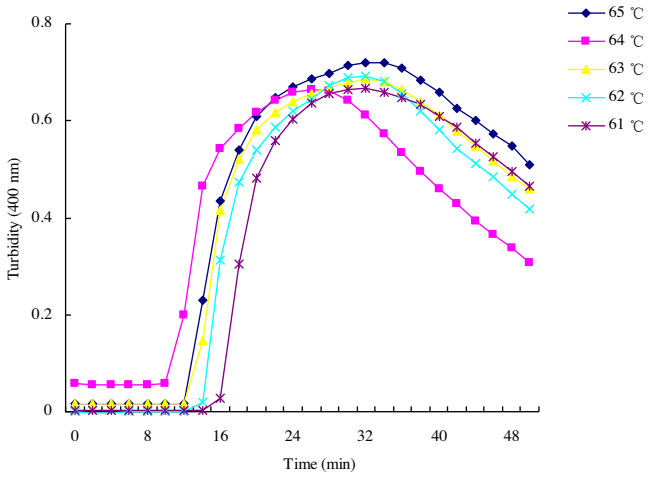

Fig. 3. Effect of different RT-LAMP reaction temperatures in ZIKV detection.

Moreover, we monitored the results of RT-LAMP by the direct visual method. Before the RT-LAMP reaction, 1 μL calcein was added to the 25-μL reaction mixture. When the reaction was complete, all positive reactions turned from orange to green, while the negative reactions did not change color (Fig. 4B, D). We concluded that the turbidity detection and visual inspection methods had the same sensitivity.

3.5. Rapid and field-ready sample treatment scheme

Most of the previously reported LAMP assays used to detect viruses require nucleic acid extraction under laboratory conditions. Thus, a rapid and simple pretreatment procedure that does not require specialized laboratory equipment is needed for ZIKV point-of-care diagnostic testing. We evaluated three different specimen processing techniques (boiling lysis, proteolysis, and alkaline lysis) to release RNA from the Zika viral capsids using simple methodologies for RT-LAMP detection assays. Three simulated clinical specimens were prepared by spiking titrated ZIKV into serum, urine, or sputum. The simulated sputum samples were first 10-fold serially diluted with sputum and then processed by the three rapid

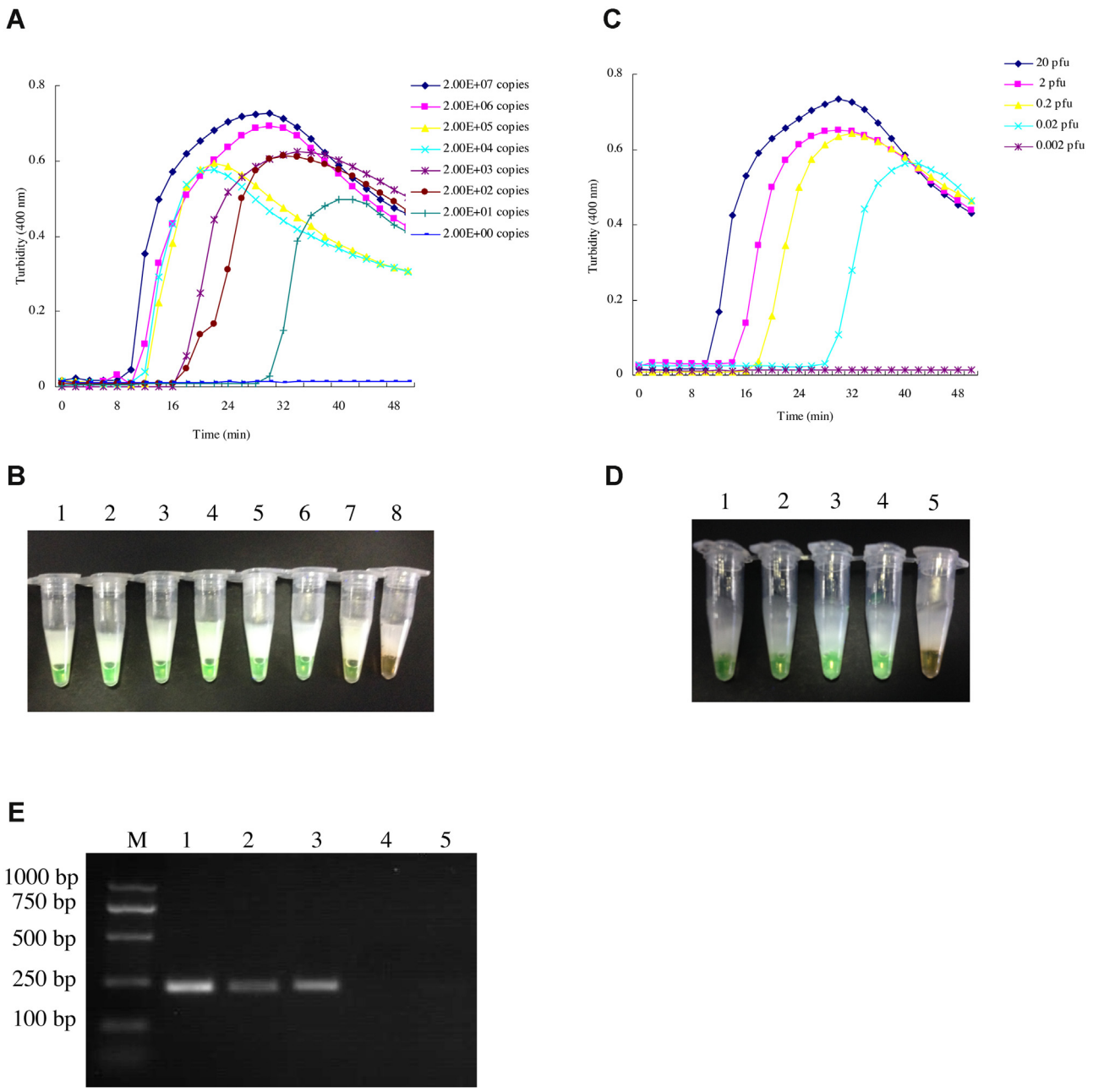

Fig. 4. Comparison of the sensitivity between RT-LAMP and RT-PCR for detection of ZIKV. The ZIKV RNA standard and extracted RNA from simulated ZIKV sputum samples were 10-fold serially diluted. Both RT-LAMP reactions (A, B, C, and D) and RT-PCR (E) were performed for each dilution. A and B. The detection limit of RT-LAMP for transcripts was observed by turbidity monitoring and visual color change of the products. Tubes: 1–8, 2.00E+07 to 2.00E+00 copies. C and D. The detection limit of RT-LAMP for ZIKV specimens was observed by turbidity monitoring and visual color change of the products. Tubes: 1–5, 20-0.002 pfu/test. E. PCR products were analyzed by agarose gel electrophoresis. M. DNA standard, Lane 1–5, 20-0.002 pfu/test.

processing methods. The sensitivity of RT-LAMP using samples prepared by all three methods was evaluated. The detection limit of the RT-LAMP assay for simulated sputum samples respectively treated with proteinase K, alkaline lysis, or boiling lysis was 0.02 pfu/test (4 pfu/mL), 0.2 pfu/test (40 pfu/mL), or 20 pfu/test (400 pfu/mL) (Fig. 5A–C). We found that the sensitivity of the RT-LAMP assay performed on simulated samples treated with proteinase K was equal to that of samples extracted using a QIAamp viral RNA mini kit, while the sensitivity of samples prepared by alkaline lysis and boiling lysis were worse.

Next, we attempted to process simulated serum and urine samples using the proteolysis treatment. The detection limit of the RT-LAMP assay for simulated serum and urine samples treated with proteinase K was 0.2 pfu/test (40 pfu/mL) and 0.02 pfu/test (4 pfu/mL), respectively (Fig. 5D, E). However, because the serum sample contained more protein, heating led to a large volume of solid phase. Therefore, the sample needed to be diluted 10-fold to smoothly perform the next operation, which reduced the detection sensitivity accordingly. Regardless, the detection limit of the serum samples was still 0.2 pfu/test (40 pfu/mL). Zika viral loads have been documented as high as 202×10^6 copies/mL, 3×10^6 copies/mL, and 7.2×10^5 copies/mL in urine, saliva, and serum, respectively (Barzon et al., 2016; Gourinat et al., 2015; Lanciotti et al., 2008).

3.6. Versatility of the RT-LAMP assay

It is beneficial for a diagnostic platform to be able to tolerate genetic mutations within a particular nucleic acid sequence

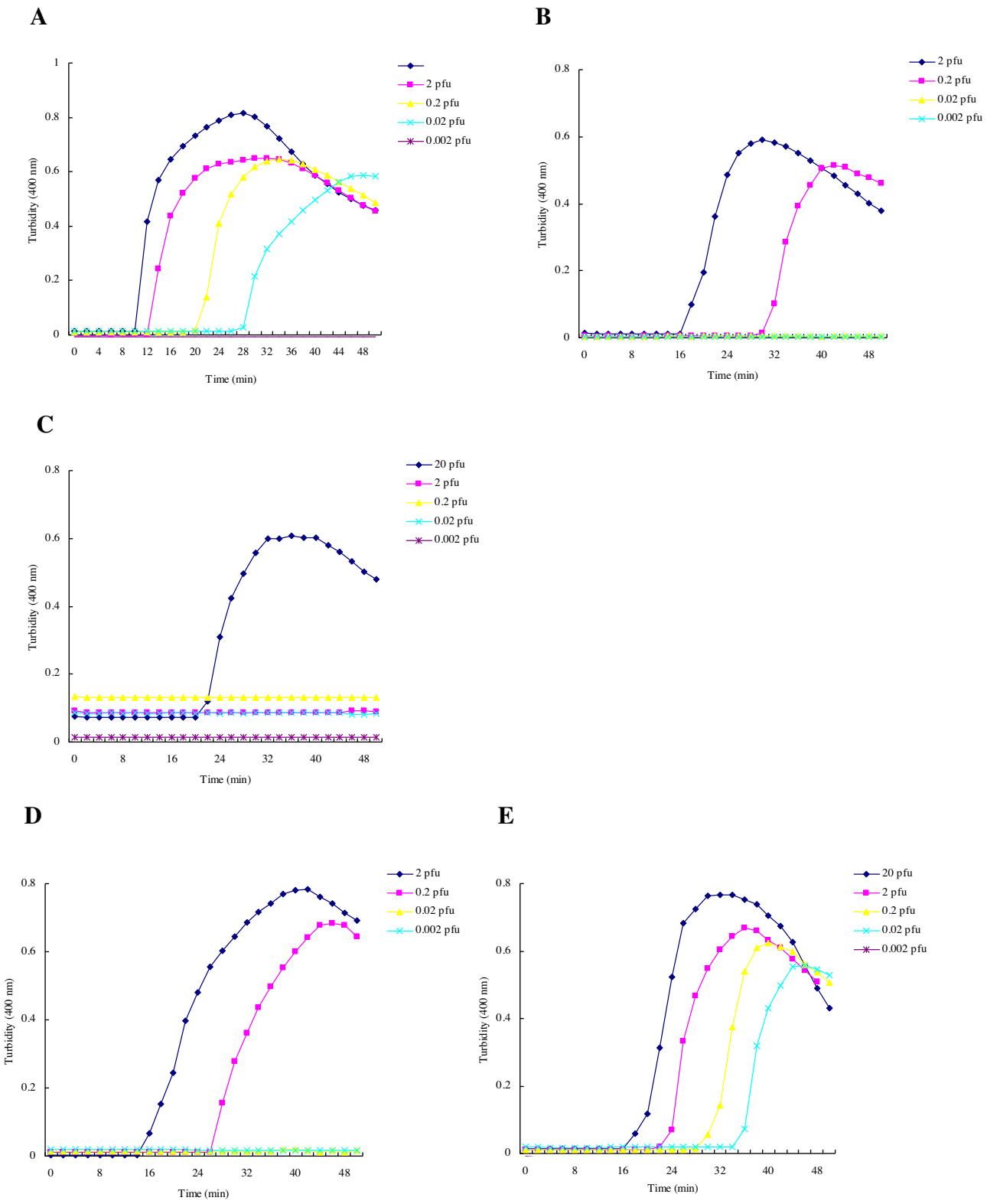

Fig. 5. Detection of ZIKV in simulated sputum, urine, and serum samples after different rapid treatment methods. The simulated sputum samples were respectively treated by proteolysis (A), alkaline lysis (B), or heating lysis (C). The simulated serum and urine samples were treated by proteolysis (D, E). The RT-LAMP reactions were performed on a series of diluted samples. The results were observed by turbidity monitoring.

because evolutionary drift is an unavoidable feature of the ongoing ZIKV outbreak. To assess the utility of our RT-LAMP assay for diagnosis of diverse strains of ZIKV, four plasmids that contained the NS1 gene of different previously reported ZIKV strains (MR 766, ARB7701, ArD7117, and ArD128000) (Berthet et al., 2014; Faye et al., 2014; Grard et al., 2010) were synthesized and transcribed *in vitro*. Then, the RNA transcripts were detected using the RT-LAMP assay developed in this study. The four different NS1 RNA transcripts could all be detected by the RT-LAMP assay (Fig. 6A). Moreover, we monitored the results of RT-LAMP by the direct visual

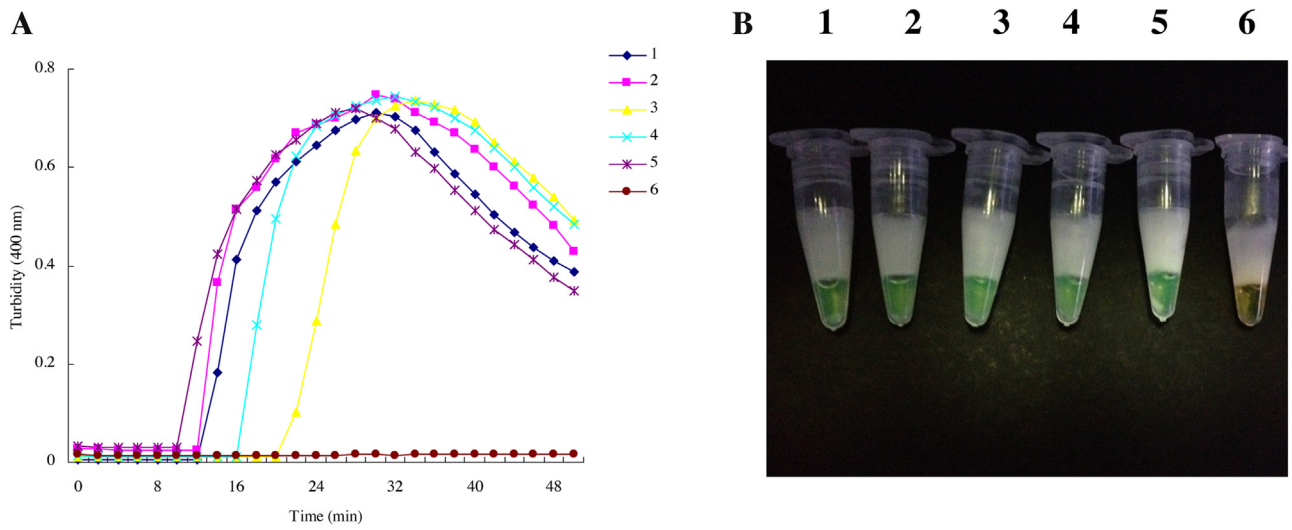

Fig. 6. Detection of diverse strains of ZIKV by the RT-LAMP assay. Four ZIKV strain NS1 gene transcripts were detected using the RT-LAMP assay, and positive results were obtained. Tubes 1–4 are the various transcripts; tubes 5 and 6 are the positive and negative controls. A: RT-LAMP for transcripts was observed by turbidity monitoring. B RT-LAMP for transcripts was observed by visual color change of the products.

method, which yielded the same results (Fig. 6B). The detection limits of the RT-LAMP assay for the four different ZIKV strains were 20 copies/test (4000 copies/mL). Although there were 12–14 mismatched positions in the primers for the NS1 region against the different viral sequences, we still successfully amplified the RNA transcripts, indicating that the assay developed here has the capacity to tolerate the expected genetic variation found in nature.

4. Discussion

The rapid spread of the emerging ZIKV epidemic in the Americas has raised concerns of the potential for large-scale outbreaks in equatorial regions and the northern hemisphere, where ~80% of the human population resides (Shi et al., 2016). Diagnosis of ZIKV infection based on clinical manifestations, especially in the early stages of infection, is very difficult because its symptoms are similar to those of other common diseases, including influenza A virus and dengue virus infection. Traditional diagnostic methods based on serology and virus isolation have low specificity and are time consuming. Real-time PCR is a sensitive, specific, and reliable molecular diagnostic technique for detecting ZIKV that is widely used to identify cases of ZIKV infection during outbreaks. However, real-time PCR requires expensive equipment and a stable power supply. Moreover, it must be deployed in a laboratory and requires technical expertise to operate and interpret results. More rapid and sensitive field diagnostic techniques are urgently required for early identification of ZIKV-infected patients and suspected cases to stem the transmission chain of ZIKV.

LAMP is an isothermal process that relies on the *Bacillus stearothermophilus* (Bst) DNA polymerase I and does not require temperature cycling. Therefore, a portable metal bath is able to meet the detection requirements. Therefore, unlike PCR, it offers an opportunity for field detection because of its low technological requirements. In the LAMP assay, the primers recognize six to eight target sequences on a target gene, guaranteeing its strong specificity compared to PCR. Further, the sensitivity is typically 10–100 times higher than conventional PCR, commensurate with the sensitivity of fluorescence quantitative PCR (Faye et al., 2008).

In this study, we established a RT-LAMP assay for the rapid detection of ZIKV with outstanding analytical sensitivity and specificity. We designed seven sets of primers specific to the entire ZIKV genome using Primer ExploerV.4, and the primers in set ID-79 targeting the NS1 gene of ZIKV were chosen as the final primers for ZIKV detection. We also found that confirmation of a positive RT-LAMP reaction is consistent between spectrophotometry and visual detection. Visual detection is simple, inexpensive (requiring no specialized equipment), and makes the RT-LAMP assay possible for field applications. Our RT-LAMP had a limit of detection of 20 copies/test (4000 copies/mL) for a ZIKV RNA standard and a limit of detection of 0.02 pfu/test (4 pfu/mL) for simulated ZIKV clinical specimens. We found that the RT-LAMP method is more sensitive than RT-PCR in this study and displayed high concordance with previously reported quantitative real-time PCR detection of ZIKV (Faye et al., 2013). In addition, this technique required less time (within 1 h) for confirmation of results compared to 2–3 h for quantitative real-time PCR.

LAMP is a rapid and robust molecular diagnostic method that can be performed with impure templates because the *Bst* DNA polymerase demonstrates robust performance even in high concentrations of amplification inhibitors. Song et al. (2016) established a point-of-care diagnostic system for ZIKV by using the characteristics of LAMP (Song et al., 2016). Thus, we attempted to amplify ZIKV specimens from crude lysates. ZIKV could be detected in several bodily fluids, including urine, serum, and saliva, and testing results suggest that urine might be the preferred specimen type to identify ZIKV infection (Barzon et al., 2016; Bingham et al., 2016; de et al., 2016; Musso et al., 2015; Zhang et al., 2016a). Therefore, we prepared simulated urine, serum, and saliva clinical samples and performed testing with our diagnostic platform. We compared three rapid specimen treatment procedures (heating lysis, proteolysis, and alkaline lysis) to process the simulated samples to release nucleic acid from viral capsids. The detection limit of the RT-LAMP assay coupled to the rapid specimen processing was 0.02-0.2 pfu/test (4–40 pfu/mL), and we found that the efficacy of proteolysis of simulated samples was superior to the other two methods. In short, the rapid proteolysis treatment was able to extract sufficient quantities of RNA from sputum, serum, and urine samples for ZIKV detection. The use of nucleic acid release treatment together with the rapid amplification time indicates that the RT-LAMP assay is suitable for point-of-care testing in the laboratory or for field detection of ZIKV.

Furthermore, to determine the diagnostic versatility of this platform, we conducted an evaluation study using RNA transcripts selected from different ZIKV strains. We found that all of the tested ZIKV strains were detected, showing the same detection limit, even when there were 12–14 mismatched positions between

primer sets and viral target sequences. LAMP may be rather tolerant to mismatches in the primer recognition sequences, as DNA amplified from the primer that incompletely hybridizes to the target sequence can be complemented by other primers (Kurosaki et al., 2016). Our ZIKV diagnostic platform resolves several practical limitations to the deployment of nucleic acid-based molecular diagnostics in response to the ongoing ZIKV outbreak.

5. Conclusion

In this study, we first solved the false positive problem of nucleic acid molecule diagnostics. Second, we established an efficient method for fast processing of clinical samples. Third, we demonstrated a sensitive, specific, and universal ZIKV detection assay based on RT-LAMP. Finally, we developed a ZIKV diagnostic platform coupled with rapid specimen processing. Clinical sample processing and molecular diagnostics can be completed in 1 h using a portable metal bath, and the results can be visually determined by a color change. The ZIKV diagnostic platform has potential use for early clinical testing and field screening of ZIKV infections in settings with insufficient infrastructure.

Funding

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Competing interests

None declared.

Ethical approval

Ethics approval was provided by the donors to use their saliva, urine, and serum samples for this research.

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