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Original article

An isothermal recombinase polymerase assay coupled with lateral flow dipstick for differentiation of pseudorabies virus wild isolates and gE-deleted vaccine strains

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Abstract

Pseudorabies virus (PRV) is one of the most important infectious diseases which leads to significant economic losses in the global swine industry. The gE-deleted vaccine is widely used to prevent susceptible pigs from PRV infection. There is no report of the differentiation of PRV wild strain and vaccine strain by recombinase polymerase amplification (RPA) coupled with a lateral flow dipstick (LFD) method. In the present study, the gD and gE gene-targeted primer-probe sets were designed. The RPA-LFD assay could discriminate between the PRV wild strain and the vaccine strain. The RPA reaction conditions were also evaluated. The optimal reaction temperature and reaction time for the RPA-LFD assay were 37°C and 20 min. The detection limit was 10 genome copies per reaction for PRV wild strain and gE-deleted vaccine strain. The assay did not have cross-reaction with other common swine viral pathogens. The effectiveness of the RPA-LFD assay for detecting the clinical samples was evaluated by testing 80 samples. The result of the assay was compared with that of the conventional PCR. The positive rate of PRV wild strain by the RPA-LFD assay was 20%, whereas the positive rate of PRV wild strain by the PCR assay was 18.8%. The assay therefore provides a novel alternative for differentiation of PRV wild strain and vaccine strain.

Keywords: detection; pseudorabies virus; recombinase polymerase amplification

Introduction

Pseudorabies (PR), which is caused by the Pseudorabies virus (PRV), leads to significant economic losses to the global pig farming industry (Zheng et al. 2022). PRV is an enveloped, double-stranded DNA virus that belongs to the subfamily *Alphaherpesvirinae*, the family *Herpesviridae* (He et al. 2019). PRV can infect many species of mammals including pigs, cattle, sheep, dogs, rats and humans. Pigs are the most susceptible to natural PRV infection, and are reservoir hosts for PRV (He et al. 2019). Although pigs of all ages can be infected with PRV, piglets and pregnant sows are more vulnerable to virulent strains. PRV infection contributes to abortion and fetal mummification in pregnant sows. The mortality rate of the infected piglets is high, and can reach 100% (Müller et al. 2011). Fattening pigs infected with PRV have shown clinical signs of mild fever, and respiratory symptoms. In late 2011, a PR outbreak occured in pig farms in China, and PRV infection has been reported in almost all the provinces in China (Liu et al. 2022). PRV is becoming one of the most serious infectious diseases of pigs and has caused major economic losses in China's pig industry. Moreover, several encephalitis cases in humans who worked on pig farms caused by PRV have been reported in different provinces in China (Yang et al. 2019, Tan et al. 2022). Humans were presumed to be infected with PRV after exposure to swine contaminants. The high risk of PRV transmission from swine to humans emphasizes the importance of PRV immunization for swineherds and the necessity for workers to implement self-protective measures during contact with swine contaminants.

Vaccination is a highly effective method to prevent PRV infection as well as morbidity and mortality in pigs. Currently, the live Bartha K-61 strain vaccine is widely used (Zhou et al. 2017, Tan et al. 2021). The glycosylation-modified membrane gE protein is a key virulence protein of PRV (Liu et al. 2020). The Bartha-K61 vaccine strain, which lacks the main virulence factor gE gene, possesses the advantages of high safety and robust immunogenicity. The differentiation of PRV wild strain and vaccine strain in swine is important in supporting measures for the elimination of PRV. However, the proportion of small-scale farms is very high in China. Lack of professional personnel and well-equipped laboratories are very common on most pig farms, and it is difficult to achieve the goal of prevention and control of PRV spread. Therefore, it is of great importance to develop detection methods suitable for livestock breeding enterprises for the scientific prevention, control, and eradication of PRV.

Polymerase chain reaction (PCR) assay is a typical type of viral diagnostic test for PRV (Sun et al. 2018). An emerging trend in nucleic acid amplification technology is the introduction of isothermal amplification. The assays for the detection of nucleic acids such as loop-mediated isothermal amplification (LAMP) are increasingly available for the diagnosis of animal infectious diseases (En et al. 2008, Zhang et al. 2010). In recent years, a novel isothermal amplification method, recombinase polymerase amplification (RPA), is the most attractive nucleic acid detection technology (Li et al. 2018). The RPA assay employs recombination protein to open the double-stranded template. The specific oligonucleotides then bind to the complementary sequence on the target template. The new DNA strand begins to synthesize by the polymerase. All the steps occurred almost simultaneously. Compared with the traditional detection methods, the RPA test has a shorter window period and a simpler operation procedure. The RPA assay takes 20-30 minutes at a constant temperature, which is the fastest test among the nucleic acid detection methods (Li et al. 2018). RPA products can be analyzed by agarose gel electrophoresis, fluorescence probe, and lateral flow dipstick (LFD) (Ma et al. 2020b, Ma et al. 2022, Tan et al. 2022). The result can be read by the color on the lateral flow dipstick (LFD), which is easy to operate in resource constrained settings.

In the last decade, a growing number of scientists have been carrying out research on the RPA assay for detecting human and animal pathogens (Boyle et al. 2013, Teoh et al. 2015, Yang et al. 2015). A real-time RPA assay for differentiation of wild-type PRV and gE-deleted vaccine strain has been established (Tu et al. 2022). Currently, there are no records of using RPA combined with the LFD technique to distinguish between the PRV wild and vaccine strains. The present study aimed to develop an isothermal RPA-LFD assay for differentiation of PRV wild strain and vaccine strain.

Materials and Methods

Preparation of standard plasmid

PRV (GenBank no. MN539749) was isolated from clinical samples and preserved in our lab (Lian et al. 2020). The virus was inoculated onto porcine kidney (PK)-15 cells (ATCC CCL-33). Following a 1-hour incubation, the cells underwent three rounds of washing and were subsequently cultured until they displayed a noticeable cytopathic effect (CPE). Subsequently, the cells were harvested, subjected to three cycles of freeze-thawing, and then centrifuged at 10000 × g for 10 minutes at 4°C. The resulting supernatant was retained. Viral RNA/DNA was isolated from the samples using a MiniBEST Viral RNA/DNA Extraction Kit

Table.1 Sequence of primers and probes for the RPA-LFD assay.

Primer name	Sequence
PRV-gD-F1	CCGCGTACCCGTACACCGAGTCGTGGCAGC
PRV-gD-R1	GCAGCCGGTCCACCTGCGGGTCGGAGATCA
PRV-gD-F2	ACTTTATCGAGTACGCCGACTGCGACCCCA
PRV-gD-R2	CCAGCTCGTCCTCCGTGGGGAACATGTAGT
PRV-gD-F3	ACGTCTACCACACGCGCCCGCTGGAGGACC
PRV-gD-R3	ATGCGGTACCAGGCCACGTGGGCGCGGTAC
PRV-gD-F4	GACTACATGTTCCCCACGGAGGACGAGCTG
PRV-gD-R4	AGCGCCACCATGAAGTCGGTGAGGATGTTC
PRV-gD-F5	GTGGACCAGCACCGCACGTACAAGTTCGGC
PRV-gD-R5	GCCGTTCTTGCGGTACCAGTAGTTCACCA
PRV-gE-F1	TCTGGGACGACCTCTCCACCGAGGCCGACG
PRV-gE-R1	AGTTGGCGCCCTCGGACACGTTCACCAGAT
PRV-gE-F2	TCGGCTTCCACTCGCAGCTCTTCTCGCCCG
PRV-gE-R2	CTCGTACACGTAGTACAGCAGGCACCGCGG
PRV-gE-F3	CGTGTCCGAGGGCGCCAACTTCACCCTCGA
PRV-gE-R3	CGGTCTCGAAGCACACCGTGGTCACCGACA
PRV-gE-F4	CCCCGGAGATGGGCATCGGCGACTACCTGC
PRV-gE-R4	CAGCGTGTAGAGGCCCGTGTCGTTGGGCGT
PRV-gE-F5	CTGGGCTCCTTCGTGATGACGTGCGTCGTC
PRV-gE-R5	CAGGCTGGTGTACACCGGAGAGAGCATGTG
PRV-gD-probe	(FAM)CGCAGGTGGACCGGCTGCTGAACGAGGCGG-THF-GGCCCACCGGCGGC (C3spacer)
PRV-gE-probe	(FAM)GAGCCGCCCATCGTCACCCCGGAGCGGTGG-THF-CGCCGCACCTGAGC (C3spacer)

(Takara, Dalian, China) following the manufacturer's instructions. The PRV gD and gE whole genes were amplified using the specific primers respectively (gD forward primer:CTGCTCGCAGCGCTATTGGC, gD reverse primer: GCTTTTAGCTCGTCGGCGTC; gE forward primer:GCCCTTTCTGCTGCGCGCCG, gE reverse primer: GGATCGCGGAACCAGACGTC). PCR was carried out using a high-fidelity Taq polymerase Kit (PrimeSTAR® Max DNA Polymerase, Takara, Dalian, China). The PCR product was purified and cloned into the vector pMD18-T (TaKaRa, Dalian, China). The product of ligation was transformed into competent TOP10 cells (Tiangen, Beijing, China). The individual bacterial colony was picked and the plasmid was determined by nucleic acid sequencing. The concentrations of the positive plasmids (PRV-gD and PRV-gE) were quantified using an ND-2000c spectrophotometer (NanoDrop, Wilmington, USA). The copy number was calculated using the previously reported formula (Ma et al. 2022). Ten-fold dilutions of the pRV-gD and pRV-gE, ranging from 10^6 to 10^0 copies/μL, were prepared using the sterile water, and aliquots of each dilution were stored at -80°C.

RPA primer and probe design

The PRA primers and probes were designed using online design software (NCBI-Primer BLAST). The parameter setting was according to the TwistDx Company design manual (www.twistdx.co.uk/en/rpa). The following key points for RPA primer design were highlighted: the primer length was about 30 bp, hairpin structure of the primer should be avoided, and target gene length was about 100-200bp. The amplification efficiency of the RPA primers should be evaluated by basic RPA assay. The best RPA primer pair was used to design the probe for the RPA-LFD assay. All the primers and probe were synthesized by Shanghai Sangon Biotech (Shanghai, China). Information on the RPA primer and probe is shown in Table 1.

Determination of the amplification efficiency of the RPA primers

The amplification reaction was carried out using the TwistAMP Basic kit (TwistDx limited, United Kingdom). The 50 μL reaction preparation consisted of the following reagents: 2.1 μL of forward primer (10μ M), 2.1 μL of reverse primer (10 μM), 29.5 μL of rehydration buffer, 1.0 μL of template DNA, 12.8 μL of distilled water and 2.5 μL of magnesium acetate

(280 mM). The magnesium acetate was added to the inner wall of the tube lid. The other reagents were added to the reaction tube containing the lyophilized enzyme preparation. The reaction tubes were incubated in a water bath at 37°C for 30min. The RPA product was purified using a PCR Clean Up Kit (beyotime, Shanghai, china) and subjected to 1% agarose gel electrophoresis.

Preparation of RPA-LFD assay

The RPA-LFD assay was performed using a TwistAMP nfo kit (TwistDx limited, United Kingdom) according to the product description. The reagents (2.1 μL of forward primer (10 μM), 2.1 μL of reverse primer (10 μM), 0.6 μl of probe (10 μM), 29.5 μL of rehydration buffer, 1 μL of template DNA, 12.2 μL of distilled water) were added to the reaction tube containing the lyophilized enzyme preparation. 2.5 μl of magnesium acetate (280 mM) was then added to the inner wall of the tube lid. The tube was closed immediately and incubated in a metal bath at 39°C for 30 min. The RPA product was diluted 1:100 with buffer and added on LF strips (BioUSTAR, Hangzhou, China). When a red color band was present on the control line, the test result was valid. Meanwhile, a red color on the test line indicated the sample was PRV-positive, and no color on the test line indicated the sample was PRV-negative. The work flow of the RPA-LFD assay is displayed in Fig.1A.

Optimization of reaction conditions

Optimizing reaction conditions for the RPA reaction is to determine suitable reaction parameters, such as reaction temperature and time. The reaction conditions were optimized with the recombinant plasmid PRV-gD as the template. Several reaction temperatures (25°C, 30°C, 35°C, 37°C, 40°C) and reaction times (10min, 15min, 20min, 25min, 30 min) were optimized for the reaction system. The reaction products were detected using the LF strips.

Sensitivity Test

The PRV-gD and PRV-gE plasmids were ten-fold diluted. Seven dilutions (10^6, 10^5, 10^4, 10^3, 10^2, 10^1, and 10^0 copies/μl) of each plasmid were used to assess the detection limit of the RPA-LFD assay. The RPA-LFD reactions were performed under the optimal reaction conditions established above.

Specificity test

To assess the specificity of the RPA-LFD assay for PRV, several common swine infectious viral pathogens were detected by the assay. The pathogens included were follows: porcine epidemic diarrhea virus (PEDV), porcine rotavirus (RV), transmissible gastroenteritis virus (TGEV), classical swine fever virus (CSFV), porcine parvovirus (PPV), pig foot-and-mouth disease virus (FMDV), porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV). All the material used above was described previously (Ma et al. 2022). The total DNA/RNA of these pathogens was extracted using a DNA/RNA Extraction Kit (vazyme, Nanjing, China). The DNA genomes were used as the template in the RPA-LFD assay. The cDNA was prepared using the RNA genomes and used as the template in the RPA-LFD assay. Distilled water was used as the no template control (NTC) and tested using the assay. PRV wild strain isolated in our lab and the live Bartha K-61 vaccine strain (Harbin Pharmaceutical Group Biological Vaccine Co., Ltd, Harbin China) were included in the test as the positive control.

Validation with clinical samples

Forty brain and forty tonsil tissues were collected from the suspected cases of PRV infection. All the clinical samples were provided by Shanghai Kaiwosha Biotechnology Co., Ltd, Shanghai, China. All the samples were collected for laboratory diagnosis, not specified for our study. Moreover, no animals were experimentally infected in our study; no Institutional Animal Care and Use Committee (IACUC) protocols apply or are available. The DNA genomes of the samples were isolated using a Punch-it™ kit according to the product manual. PRV wild strain isolated in our lab and the live Bartha K-61 vaccine strain (Harbin Pharmaceutical Group Biological Vaccine Co., Ltd, Harbin China) were included in the test as the positive control. Distilled water was used as the NTC. All the samples were tested using the RPA-LFD assay established above. The clinical samples were also detected using a conventional PCR for differentiation of wild-type PRV and gene-deleted vaccine strains (Ma et al. 2013). The detection results of the samples using RPA-LFD and PCR were compared.

Results

Assessment of the RPA primers

The amplification efficiency of the RPA primers was evaluated by basic RPA assay. As shown in Fig. 2, the RPA products amplified using the primers (gD-F2/R2 and gE-F2/R2) exhibited clear bands. Therefore, these primer pairs were selected for the development of the RPA-LFD detection method. The 5'end of both reverse primers was labeled with

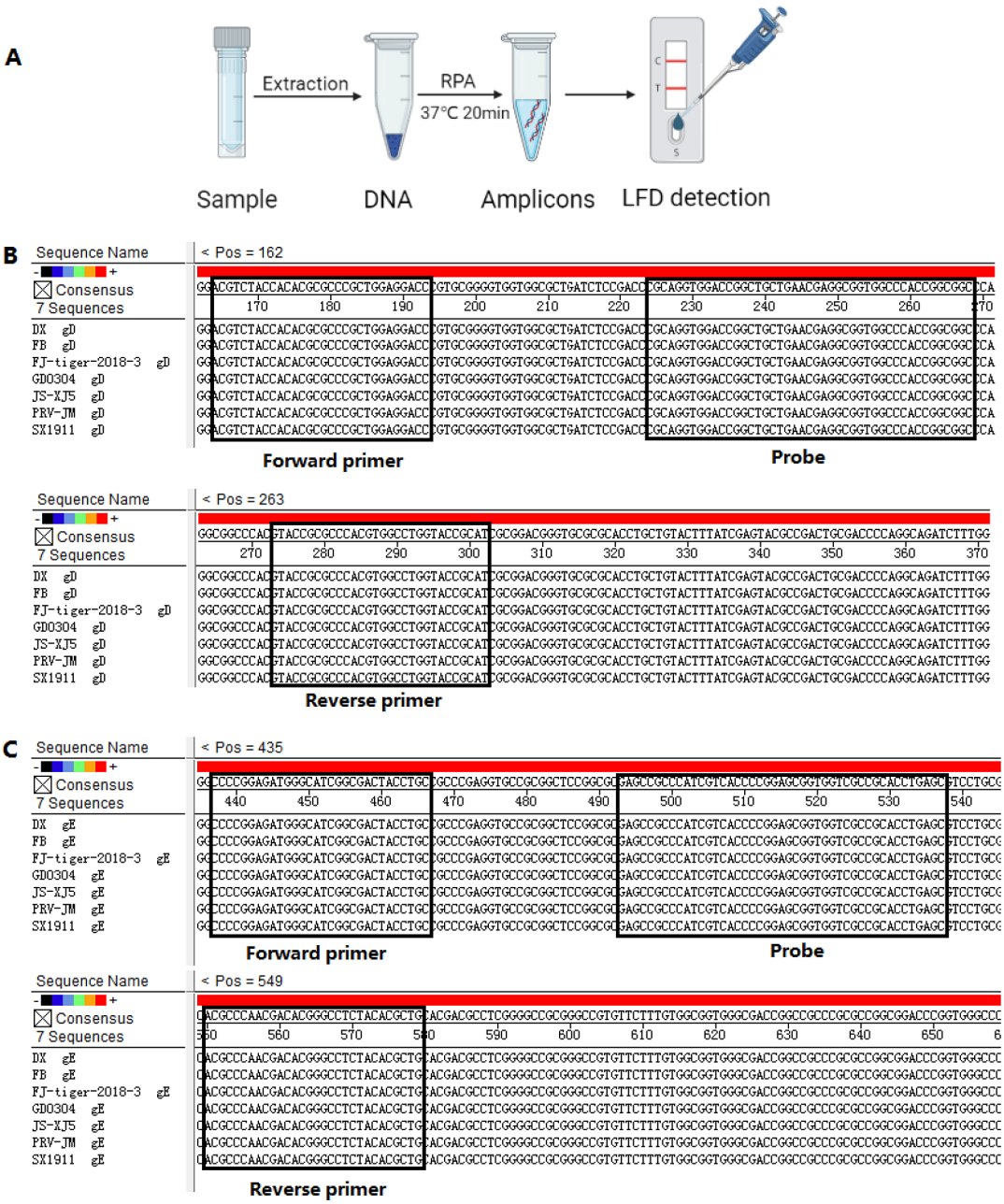

Fig. 1. Schematic representation of RPA-LFD assay for detection of PRV. A: Workflow of RPA-LFD assay. B: Alignment of target sequences of several PRV strains. Positions of the primers and probes are indicated in the red box.

biotin by Shanghai Sangon Biotech (Shanghai, China) and used for the development of the RPA-LFD assay. The probes were designed based on the sequences of the RPA primers (Table 1). Alignment of the primers and probes was performed (Fig. 1B and 1C).

Optimal reaction conditions

The reaction conditions of the RPA-LFD assay were evaluated by using the gD primer-probe set. Firstly, five RPA reactions were carried out at five different reaction temperatures (25°C, 30°C, 35°C, 37°C, 40°C) independently, and the reaction time for all the RPA assays was set at 20 min. Red detection lines appeared in the reaction temperature range from 25°C to 40°C. Furthermore, the color on the test band did not change significantly between 37°C and 40°C, and reaction temperature of 37°C was used as the optimal condition according to these results (Fig. 3A). Five RPA reactions were then carried out at five different reaction times (10 min, 15 min, 20 min, 25 min, 30 min) independently. With 37°C as the optimal temperature, the results showed that positive detection bands appeared in the reaction time of 10 to 30 min, and there was no significant change in the color of the bands after 20 min (Fig. 3B). Thus, 37°C and 20 min were finally selected as the

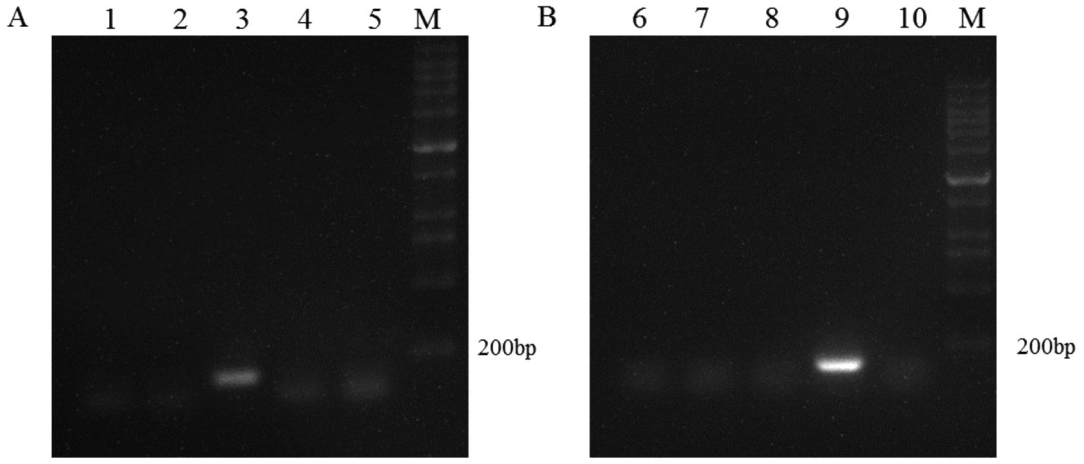

Fig. 2. Assessment of five RPA primer pairs. RPA products using five primer pairs were detected by agarose electrophoresis respectively. 1-5: PRVgD-F1/R1, PRV-gD-F2/R2, PRV-gD-F3/R3, PRV-gD-F4/R4, PRV-gD-F5/R5; 6-10: PRV-gE-F1/R1, PRV-gE-F2/R2, PRV-gE-F3/R3, PRV-gE-F4/R4, PRV-gE-F5/R5. NTC: no template control.

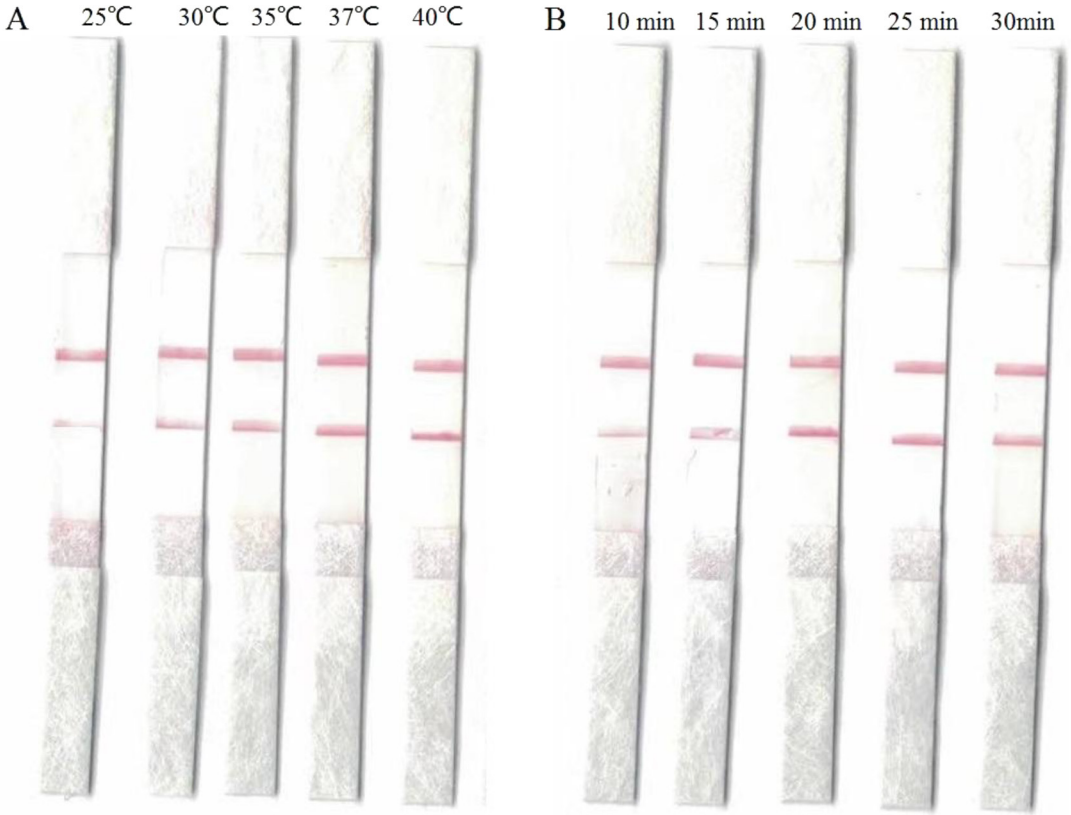

Fig. 3. Evaluation of reaction conditions. A: Different reaction temperatures (25℃, 30℃, 35℃, 37℃, 40℃) were assessed. B: Different reaction times (10 min, 15 min, 20 min, 25 min, 30min) of RPA-LFD assay were assessed.

optimal reaction temperature and time for the RPA-LFD assay.

Sensitivity Test

The 10-fold dilutions of the positive recombinant plasmids were used as a template to evaluate the sensitivity of the RPA-LFD method. The results showed that the minimum detection limit of the assay for the detection of gD gene was 10 genomic copies (Fig. 4A). While the detection limit of the assay for the gE gene was the same as that for gD (Fig. 4B).

Specificity assay

The PRV wild strain and gE-deleted vaccine both tested positive by gD RPA-LFD assay (Fig.5A). On the other hand, only the PRV wild strain tested positive by gE RPA-LFD assay (Fig. 5B). In contranst, the NTC (RNase-free water), PEDV, RV, TGEV, CSFV, PPV,

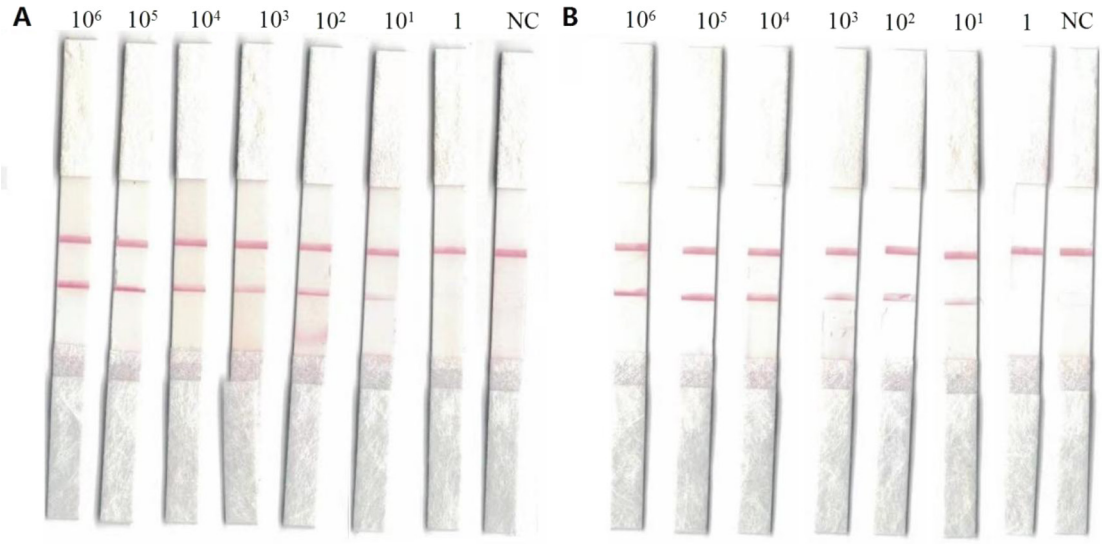

Fig. 4. Sensitivity of RPA-LFD assay. A: Seven dilutions (10^6, 10^5, 10^4, 10^3, 10^2, 10^1, and 10^0copies/µl) of PRV-gD template were used to assess the detection limit of the assay. B: Seven dilutions (10^6, 10^5, 10^4, 10^3, 10^2, 10^1, and 10^0copies/µl) of PRV-gE were used to assess the detection limit of the assay.

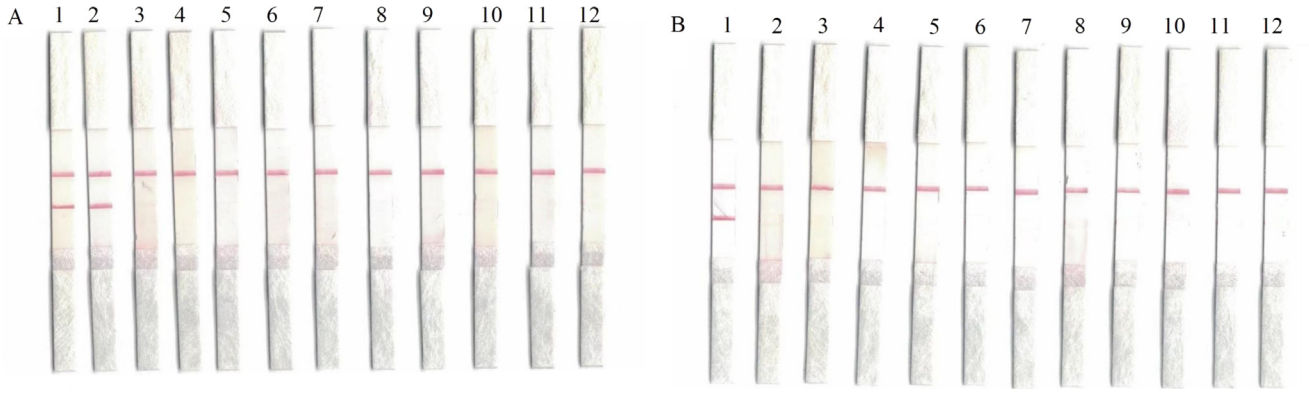

Fig. 5. Specificity of RPA-LFD assay. A: NTC (RNase-free water), PEDV, RV, TGEV, CSFV, PPV, FMDV, PCV2 and PRRSV tested negative by the PRV-gD RPA-LFD assay; both the PRV wild strain and gE-deleted vaccine tested positive by the PRV-gD RPA-LFD assay.1-12: PRV wild strain, gE-deleted vaccine strain, PEDV, RV, TGEV, CSFV, PPV, FMDV, PCV2 and PRRSV, RNase-free water, RNase-free water.
B: NTC (RNase-free water), PEDV, RV, TGEV, CSFV, PPV, FMDV, PCV2, PRRSV and PRV gE-deleted vaccine tested negative by the PRV-gE RPA-LFD assay. PRV wild strain tested positive by the PRV-gE RPA-LFD assay. 1-12: PRV wild strain, gE-deleted vaccine strain, PEDV, RV, TGEV, CSFV, PPV, FMDV, PCV2 and PRRSV, RNase-free water, RNase-free water.

FMDV, PCV2 and PRRSV showed no amplification, and only the color of the control line was displayed on the strip (Fig. 5). Therefore, the RPA-LFD method can specifically amplify the target sequence in PRV, without cross-reaction with other viral species.

Diagnostic performance of RPA-LFD assay

The previously established RPA-LFD assay and PCR was used to detect 80 samples collected from swine. The results showed that 18 were positive for PRV by the gD RPA-LFD assay, in which 2 (Tonsil-2 and Tonsil-25) were positive for gE-deleted vaccine strain and 16 were wild strain. Seventeen samples were positive for PRV by the PCR assay, in which 2 samples were positive for gE-deleted vaccine strain and 15 were positive for wild strain. One sample positive for the PRV wild strain determined by the RPA-LFD assay tested negative by PCR. The positive rate of the PRV wild strain by the RPA-LFD assay was 20%, whereas the positive rate of the PRV wild strain by the PCR assay was 18.8%. The results of the two methods were highly consistent with each other. The RPA-LFD method established in our study can be used for field detection of PRV samples. The detection results are shown in Table 2; the negative results were not included in the table. The gB gene of the PRV-positive samples confirmed by PCR was sequenced. All the sequences will be made available by the authors upon receipt of a request via email.

Table 2. Results of RPA-LFD assay and gel-based PCR assay for the detection of the clinical samples.

Sample number	RPA-LFD (gD gene)	PCR (gB gene)	RPA-LFD (gE gene)	PCR (gE gene)	Sequencing (gB gene)
Brain-2	+	+	+	+	Confirmed
Brain-5	+	+	+	+	Confirmed
Brain-7	+	+	+	+	Confirmed
Brain-8	+	+	+	+	Confirmed
Brain-14	+	+	+	+	Confirmed
Brain-17	+	+	+	+	Confirmed
Brain-18	+	+	+	+	Confirmed
Brain-22	+	+	+	+	Confirmed
Brain-25	+	+	+	+	Confirmed
Brain-35	+	+	+	+	Confirmed
Tonsil-2	+	+	–	–	Confirmed
Tonsil-6	+	+	+	+	Confirmed
Tonsil-9	+	–	+	–	NA
Tonsil-14	+	+	+	+	Confirmed
Tonsil-16	+	+	+	+	Confirmed
Tonsil-19	+	+	+	+	Confirmed
Tonsil-25	+	+	–	–	Confirmed
Tonsil-29	+	+	+	+	Confirmed

NA – not available

Discussion

In this study, a novel RPA-LFD technique was developed for distinguishing between the PRV wild strain and the gE-deleted vaccine strain. This method uses two primer-probe sets: one targets the gD gene to identify both wild and vaccine strains, while the other targets the gE gene specifically for the wild strain. In practical applications, using the RPA-LFD method of detecting the gD gene can detect all PRV strains, whether they are wild-type or vaccine strains; using the RPA-LFD method of detecting the gE gene, it is possible to distinguish between wild-type strains and vaccine strains, as vaccine strains do not possess the gE gene, while wild-type strains possess the gE gene. The RPA reaction can be completed in just 30 minutes at a consistent temperature. The test results are visible through color changes on the LF strips, allowing for simple naked-eye interpretation without the need for agarose gel electrophoresis or costly fluorescence PCR equipment. Unlike traditional PCR and real-time RPA methods, this approach eliminates the necessity for a fluorescence detection instrument (Ma et al. 2022). This new method presents a rapid, cost-efficient, and instrument-sparing approach for diagnosing PRV compared to existing techniques. The RPA-LFD method was used for the qualitative detection of PRV. Numerous studies have suggested that real-time RPA assays hold promise for quantitative detection (Yang et al. 2020). However, recent research findings have highlighted limitations in the accuracy of quantification detection using the real-time RPA method (Ma et al. 2020b, Ma et al. 2019).

Since the inception of RPA, it has been used for detecting a diverse array of infectious diseases (Boyle et al. 2013, Ma et al. 2019, Ma et al. 2020b, Ma et al. 2022), serving as a valuable complement to molecular detection methods such as PCR. In our research, we developed two single-plex RPA assays for identifying PRV wild isolates and vaccine strains. Indeed, multiplex RPA assays have been created for virus detection within a single tube to detect pathogens (Ma et al. 2020a, Wongsamart et al. 2023). However, there have been no reports of multiplex RPA assays for detecting multiple swine pathogens. There may be a future research avenue to develop a multiplex RPA assay capable of detecting various swine pathogens in a single assay tube.

In our study, online software was used to design RPA primers, and the parameters set refers to the Kit manual. The screening result demonstrated that one primer pair could work well. Sequence alignment of the primers and probes of eight PRV strains, including some variant strains, was performed. The sequence alignment analysis demonstrated that the sequence targeted by the primers was highly conserved, indicat-

ing that the RPA-LFD assay could detect the variant strains.

Nucleic acid extraction is an important step for the molecular diagnostic assay. In the present study, the Punch-it™ kit was used to isolate nucleic acid from tissue samples. There have been several studies reporting that Punch-it™ kit could be used in molecular assays (Wang et al. 2018). The nucleic acids of brain and tonsil tissues were successfully isolated using the Punch-it™ kit. The operation did not need a cumbersome centrifuge and was relatively simple. In our study, the detection result of the clinical samples confirmed the viability of the kit, indicating that the nucleic acids extracted using the kit can be directly detected by the RPA-LFD assay. In terms of downstream applications, the combination of RPA with other technologies has expanded the applications of RPA. For example, RPA can also be used to identify drug resistant genes. A recent study has devised a novel procedure to quickly predict drug resistance (DR) in *Mycobacterium tuberculosis* (TB) isolates. The study employed RPA, conducted at a temperature of 37°C for a duration of 90 minutes, to amplify three specific regions of the TB genome. Subsequently, nanopore sequencing was performed on a MinION device (Gliddon et al. 2021). Researchers have combined CRISPR effector Cas13a with RPA to develop a molecular detection platform called Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) (Kellner et al. 2019). SHERLOCK has been successfully used to detect specific strains of Zika and Dengue virus, differentiate pathogenic bacteria, genotype human DNA, and identify mutations in cell-free tumor DNA. Thus, the RPA assay combined with the CRISPR system may be used in PRV detection in the future.

Conclusions

The RPA-LFD assay established in the present study has the advantages of simple operation, short turnaround time and low cost. The method could be performed in the differential diagnosis of PRV wild strain and vaccine strain by the RPA-LFD assay and will greatly facilitate the early monitoring and prevention of PRV wild strain infection in pig herds. Interventions such as eliminating infected pigs will help control the spread of the disease.

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