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A loop-mediated isothermal amplification coupling with a lateral flow dipstick for rapid and specific detection of fowl adenovirus serotype-4

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ABSTRACT

Fowl adenovirus serotype-4 (FAdV-4) has been recognized as a predominant threat to the broilers aged from three to five weeks. Hydropericardium syndrome (HPS) is one of its major clinical diseases by FAdV-4 resulting in heavy economic losses. In this study, a loop-mediated isothermal amplification coupling with a lateral flow dipstick (LAMP-LFD) was developed for rapid and specific detection of fowl adenovirus serotype-4. The optimized LAMP-LFD can be completed in 60 min at 65 °C. The minimum detection limits of PCR, real-time PCR, nested PCR and LAMP-LFD are 1×10^4 copies/μl, 1×10^2 copies/μl, 10 copies/μl and 10 copies/μl respectively. Moreover, the specificity of the LAMP-LFD assay is satisfactory and does not produce cross reactions with other species. In field samples, 150 samples were assayed by PCR and LAMP-LFD. They agreed on the diagnosis "positive" in 13% of clinical samples, and they agreed on the diagnosis "negative" in 85% of clinical samples. Their probability of agreement is $p_0 = 147/150 = 13\% + 85\% = 98\%$. LAMP-LFD can potentially be modified and applied as a diagnostic tool for FAdV-4 infection especially in resource-limited areas, such as small breeding farms and basic veterinary labs to offer an affordable diagnostic.

1. Introduction

Fowl adenoviruses (FAdVs), genus Aviadenovirus in the family Adenoviridaea, have been classified into five species from FAdV-A to FAdV-E according to restriction enzyme digestion patterns. These five species could also be grouped into 12 serotypes (FAdV-1 to FAdV-8a and FAdV-8b to FAdV-11) based on the cross-neutralization test (https://talk.ictvonline.org/taxonomy/). The pathogenic FAdVs are associated with some notable poultry-farm diseases, such as hydropericardium syndrome (HPS), inclusion body hepatitis (IBH), adenoviral gizzard erosion (AGE), hepatitis-hydropericardium syndrome (HHS), as well as many other diseases. Among these diseases, HPS caused by FAdV-4 has a mortality rate ranging from 10% to 100% (Pan et al., 2017; Vera-Hernandez et al., 2016). Major symptoms caused by HPS include clear, straw-colored fluid in the pericardial sac, congested kidney and enlarged liver (Guan et al., 2018; Liu et al., 2016). Since mid-2015, massive outbreaks of HPS have occurred on poultry farms in most provinces of China, causing significant economic losses and seriously threatening the poultry industry (Guan et al., 2018; Zhao et al., 2015).

FAdVs are non-enveloped icosahedral viruses with a linear, double-stranded DNA genome approximately 45 kb in length (Chiocca et al., 1996; Grgic et al., 2011; Griffin and Nagy, 2011; Gunes et al., 2012; Marek et al., 2012; Ojkic and Nagy, 2000). The genome is protected by a nucleocapsid formed by the viral major structural proteins, Penton, Hexon, and Fiber (Russell, 2009). The major capsid protein, Hexon and the DNA polymerase gene are commonly used to detect FAdVs by PCR-based assays (Kajan et al., 2011). In previous studies, the 52 K gene was used as a target fragment to detect FAdVs by real-time PCR (Gunes et al., 2012). The 52 K gene, encoding a protein that plays a role in viral DNA encapsidation, is highly conserved and is an ideal target for FAdV-4 detection (Gunes et al., 2013).

In recent years, different FAdV-4 detection methods have been developed by using molecular techniques, serological techniques, and other conventional microbiological techniques (Schachner et al., 2018). Molecular techniques, including restriction endonuclease analysis (REA), DNA probe *in situ* hybridization, polymerase chain reaction (PCR), real-time quantitative PCR (qPCR), and a high-resolution melting (HRM)-curve analysis, play pivotal roles in detection and differentiation of FAdVs. However, these methods have not been widely

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used due to the complexity of their operation. The serological techniques, such as the agar gel precipitation test (AGPT) and enzyme-linked immunosorbent assay (ELISA) are also cumbersome and inefficient because of the need to prepare viral antigens when using these techniques. Other conventional microbiological techniques, such as virus isolation, require skilled personnel, special facilities and professional laboratory services (Li et al., 2017). The use of the above-mentioned detection methods is somewhat limited in practice, particularly for small-scale farms and resource limited regions. Thus, a simple, rapid, inexpensive, and sensitive diagnostic methods is urgently needed for FAdV-4 detection.

In order to address the drawbacks of existing detection methods, a promising alternative strategy for nucleic acid detection, loop-mediated isothermal amplification (LAMP), was developed in 2000 (Mori et al., 2013; Mori and Notomi, 2009; Notomi et al., 2000; Yang et al., 2010). It has been applied to a variety of microbiological diagnostic fields due to its high sensitivity, ease of use and low dependence on equipment (Notomi et al., 2000; Zhang et al., 2014). Therefore, the LAMP assay has also been employed for detecting many poultry pathogens in previous studies, including Newcastle disease virus (NDV) (Pham et al., 2005), infectious bronchitis virus (IBV) (Chen et al., 2010), avian influenza virus (AIV) (Postel et al., 2010), Marek's disease virus (MDV) (Wozniakowski et al., 2011) and others. It is a universal nucleic acid isothermal amplification method that uses either two or three primer pairs and *Bst* DNA polymerases to amplify large quantities of target DNA sequences at a constant temperature ranging from 60 ℃ to 65 ℃ (Notomi et al., 2000; Zhang et al., 2014). The whole procedure requires no special apparatus and is carried out using a water bath or a heating block (Zhang et al., 2014). It is worth noting that high tolerance to assay inhibitors is a major advantage of LAMP (Francois et al., 2011). Moreover, the positive amplification results can be easily observed with naked eye, by observing a white precipitate of magnesium pyrophosphate in the mixture or by adding colorimetric dyes. In addition, the chromatographic lateral flow dipstick (LFD) format has been also applied to reveal LAMP products in a simpler and faster way for colorimetric readout with the naked eye. Therefore, the LAMP-LFD has considerable promise for viral detection in a variety of samples (Zhang et al., 2014).

The objective of this study was to develop a LAMP-LFD diagnostic system for detecting of FAdV-4 by targeting the 52 K gene. Also, different types of PCR-based methods, including conventional polymerase chain reaction (PCR) (Gunes et al., 2012), nested PCR (nPCR), real-time quantitative PCR (qPCR) (Gunes et al., 2012) and LAMP-LFD have been compared and evaluated for specificity and sensitivity. The LAMP-LFD assay provided to be a useful diagnostic technique for FAdV-4 infection, and it is highly suitable for most chicken breeding environments in China and other underdevelopment regions.

2. Materials and methods

2.1. Viruses and samples

FAdV-4 SC-Neijiang strain (GenBank Accession number KY927938) was isolated from a broiler chicken farm where all diseased chickens showed clinical signs of HPS in Sichuan province, China, in 2016. The serotype of the virus was identified as FAdV-4 based on Hexon gene sequence according to the ICTV system (Guan et al., 2018). FAdV-7 and FAdV-8b strain obtained from Professor Peng Zhao (Shandong Agricultural University). These FAdVs were propagated in a Leghorn male hepatoma (LMH) cell line. The cells were grown in Dulbecco's modified Eagle medium supplemented with 8% fetal bovine serum (HyClone, South Logan, UT, USA) and 0.1% TransSafe Mycoplasma Prevention Reagent at 37 ℃ in a humidified 5% CO_2 atmosphere. The cells were observed daily for cytopathic effects (CPE) in preparation for the next step. The Marek's disease virus (MDV) FC-126 strain, Infectious bursal disease virus (IBDV) B87 strain and fowl pox virus (FPV) quail-adapted strain were obtained from commercial vaccines (HILE, China). 150 clinical samples, stored in our own laboratory, were used in clinical evaluation of the LAMP-LFD assay.

2.2. DNA extraction

When an 80% cytopathic effect (CPE) was observed in infected cells, the supernatants were collected by centrifugation at 4000 × g for 20 min at 4 ˚C after three freeze-thaw cycles. Viral DNA was extracted from the cell culture supernatants with the TIANGEN Genomic DNAKit (Beijing, China) according to the manufacturer's instructions. The quality of the extracted genomic DNA samples was measured by Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, United States). The viral DNA was stored at −20 ˚C for the next step.

2.3. Primer design

The 52 K gene of FAdV-4 SC-Neijiang strain was compared with seven other FAdV-4 reference strains (GenBank Accession number KU558760, KU558761, KU558762, KU569295 KU569296 KX421401 and KX421404) to screen out a conserved sequence of 467 bp for use as the detection target. The selected fragment was used as the template for designing primers *via* Primer Premier 6 software (PRIMER Biosoft International, United States). All oligonucleotides used in this study are listed in Table 1 and Fig. 1.

2.4. Preparation of DNA standards

To confirm the copy number of viral DNA, a pair of primers (52 K-F: ACGTACATGGAGCACCCGG and 52 K-R: CCTTGGGGAGGTCGGTTCTC) was designed to amplify the partial 52 K gene fragment from the genomic DNA of the SC-neijiang strain using PCR. The PCR products were purified with Universal DNA Purification Kit (TIANGEN, Beijing, China). Then the purified products were cloned into pMD-18 T vector (TaKaRa, Japan) and the copy number of pMD-18T-52 K was calculated. Subsequently, plasmids were extracted using TIANprep Mini Plasmid Kit146 (TIANGEN, Beijing, China) and the DNA of plasmids were serially diluted 10-fold in concentrations ranging from 1.02×10^9 to 1.02×10^2 copies/μl. They were used as the templates to construct a standard curve by Bio-Rad real-time PCR system and software (Bio-Rad CFX Maestro 1.1, 3.0, USA). The copy number of viral DNA was determined based on the standard curve. Then, a series of 10-fold dilutions of viral DNA (at concentrations ranging from 1×10^9 copies/μl to 1×10^0 copies/μl) were used as the positive control template for PCR-based methods and LAMP-LFD.

Table 1
Oligonucleotides used in this study targeting the 52 K gene of FAdV-4.

Primer	Primer type	Sequences (5' to 3')	
Conventional PCR	PCR-F	TGTACGAYTTTGTSCARAC	Gunes et al. (2012)
	PCR- R	TARATGGCGCCYTGCTC	
Nested PCR	nPCR-F	GCATAGAGCAGCAGGTAT	This study
	nPCR-R	CGAACTCATCCTCCTCTC	
Real-time PCR	qPCR-F	ATGGCKCAGATGGCYAAGG	Gunes et al. (2012)
	qPCR-R	AGCGCCTGGGTCAAACCGA	
LAMP	F3	CGTGGCTGAGAGACCTGAT	This study
	B3	TGCACCCCCAAGTCCAG	
	FIP	Biotin-TCGTGCACACCGCCGATAC CATGATCGTGACCGACCCG	
	BIP	FITC[a]-CAAGTTGGCCGCGAAGAAC GCCTGCATCACCCGGTAGA	

[a] Fluorescein isothiocyanate.

Fig. 1. Primer-binding sites and orientations of primers targeting for 52 K gene of FAdV-4. The boxes and arrows showed the position and directions of two outer primers (F3, B3), a forward inner primer FIP (F1c + F2), a backward inner primer BIP (B1c + B2).

2.5. Conventional PCR and real time PCR

The PCR reaction system contained 12.5 μl 2 × TSINGKE Master Mix (blue) (TSINGKE, Beijing, China), 1 μl DNA, 1 μl of each pair of primers and 9.5 μl sterilized double-distilled water in a final volume of 25 μl. All reactions were carried out in Biomatra TRIO Cycler (Biometra GmbH, Germany) and pre-set for an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at a temperature of 94 °C for 30 s, annealing for 20 s at 55 °C, extension for 15 s at 72 °C, with a final extension temperature of 72 °C for 10 min to evaluate the PCR primers (PCR-F/PCR-R). Nested PCR consisted of two amplification rounds, using PCR primers (PCR-F/PCR-R) as outer primers in the first amplification round and primers nPCR-F/nPCR-R in the second round. Briefly, for nested PCR, 1 μl of the first PCR products were used as a template in the nested PCR. The protocol began with a preliminary denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 10 s at 55 °C, extension for 15 s at 72 °C, and a final extension temperature of 72 °C for 10 min. The products were analyzed by electrophoresis using a 1% agarose gel, stained with ethidium bromide, and photographed under a UV transilluminator. For real-time PCR, the total reaction volume of 20 μl contained 10 μl of SsoFast EvaGreen Supermix (Vazyme, Beijing), 0.8 μl (10 μM) of each primer (qPCR-F and qPCR-R), 1 μl of DNA templates and 7 μl sterile double distilled water. All reactions were performed using Bio-Rad real-time PCR system and software (Bio-Rad CFX Maestro 1.1, 3.0, USA) according to the following protocol: 95 °C for 5 min, with 40 cycles of 95 °C for 10 s, followed by 60 °C for 30 s, 72 °C for 30 s, and a fluorescence read at 72 °C at the end of each cycle and a melt curve analysis with a temperature gradient of 0.1 °C s^{-1} increasing to 95 °C. The results were presented in Bio-Rad real-time PCR software. All

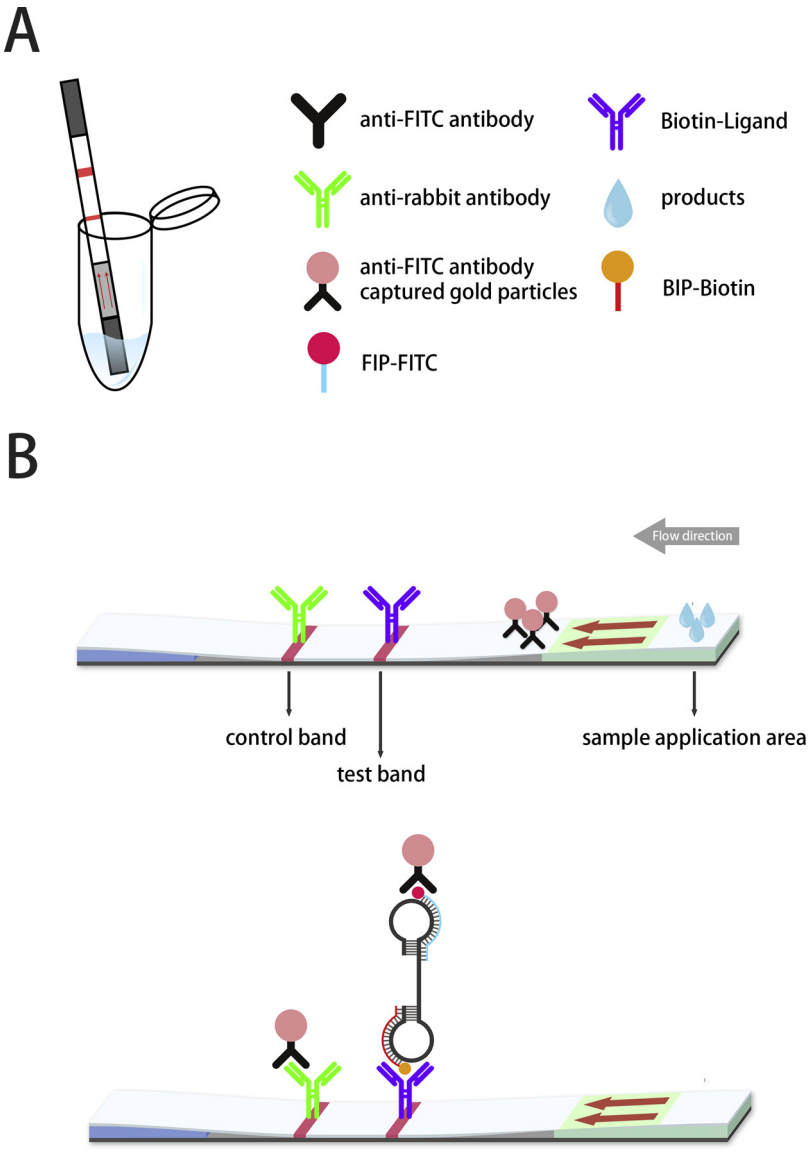

Fig. 2. The schematic diagram of the LFD.

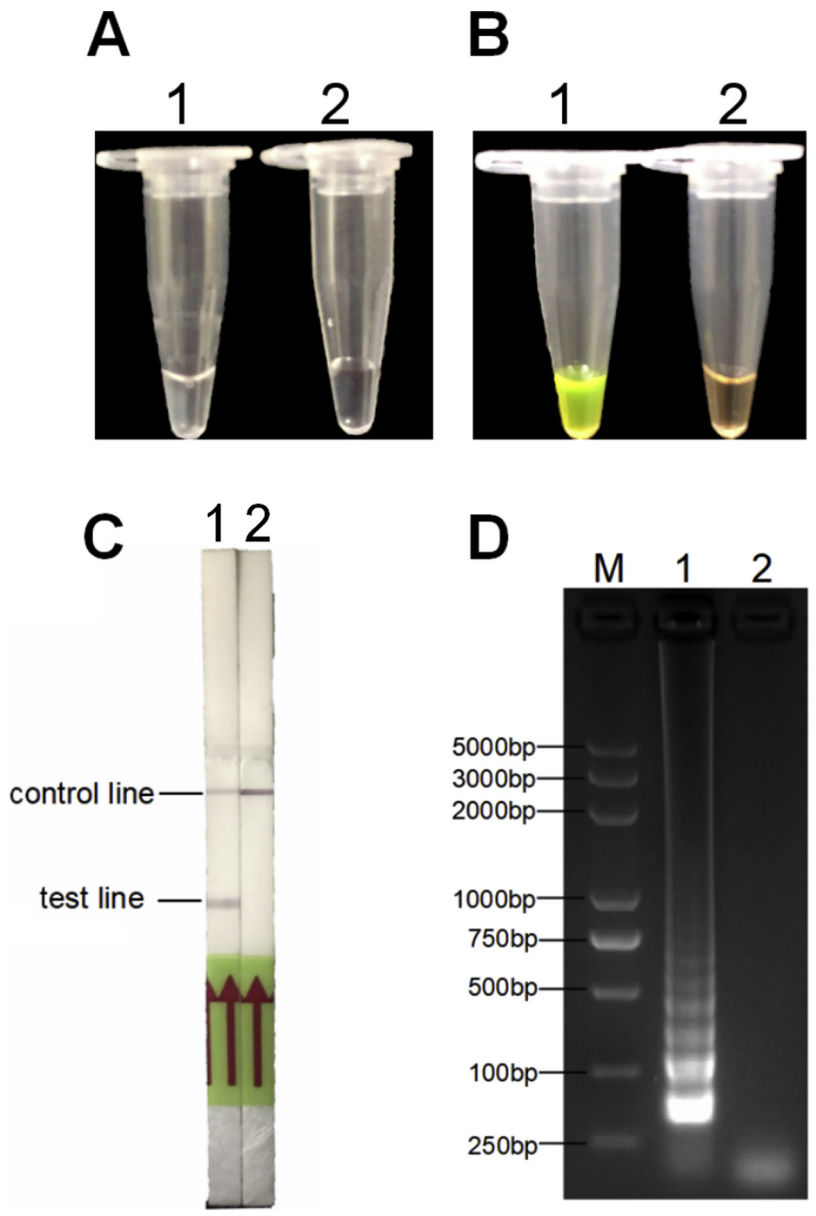

Fig. 3. Monitoring methods of LAMP-LFD assay. (A) Visual monitoring by observing precipitates. (B) Visual monitoring by observing the color change after adding SYBR Green I. (C) Lateral flow dipstick detection. (D) Agarose Gel electrophoresis. M, 5,000-bp DNA marker; Tube, lane and line 1, genomic DNA of FAdV-4 (positive control); Tube, lane and line 2, ddH_2O (negative control). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

samples were tested three times to enhance the reliability of the results.

2.6. Optimization of the RT-LAMP-LFD assay

A range of concentrations of deoxyribonucleotide triphosphates (dNTPs) (0.8–1.8 mM) (TaKaRa), $MgSO_4$ (2–10 mM), F3/B3 (0.2–1.0 μM), FIP/BIP (0.8–2.4 μM), Bst 2.0 WarmStart® DNA polymerase (0.16–0.64 U/μl) (New England Biolab® Inc., M0538S, Beijing, China), different incubation times (20–90 min) and temperatures (55–65 °C) were assessed. To establish the optimal LAMP system for detecting FAdV-4, the reactions were performed in the Bio-Rad real-time PCR after the addition of 1 μl of Eva Green nucleic acid stain (Coolaber, Beijing, China). The normal LAMP reactions were performed in 0.2 mL sterilized microtubes in a temperature-controlled water bath and the reaction was terminated by heat inactivation at 80 °C for 5 min. After amplification, the turbidity caused by the white precipitate of magnesium pyrophosphate in the mixture could be visualized with the naked eye and also observed upon addition of 1 μl of 1000 × SYBR Green I nucleic acid stain (Solarbio®, Beijing, China) to the mixture. In normal lighting, the color resulting from amplification (positive) was green, while the negative reaction was orange. For further confirmation, LAMP products were also analyzed by electrophoresis using a 2% agarose gel, stained with ethidium bromide, and photographed under a UV transilluminator. In order to detect the LAMP products using LFD strips, the 5′- BIP biotinylated primer and the fluorescein isothiocyanate (FITC) - labeled FIP replaced the BIP and FIP, respectively, for the subsequent LFD assay steps under the same reaction conditions as LAMP. In addition, there was no final heat inactivation step after the incubation step. After the amplification step, 5 μl of hybridized products were transferred to a new tube containing 80 mL of assay buffer (Milenia® GenLine HybriDetect). The LFD strip was immersed into the mixture for approximately 5 min, according to the manufacturer's instructions, and then the results were observed and recorded (Fig. 2A). In the positive samples, the biotinylated LAMP products were

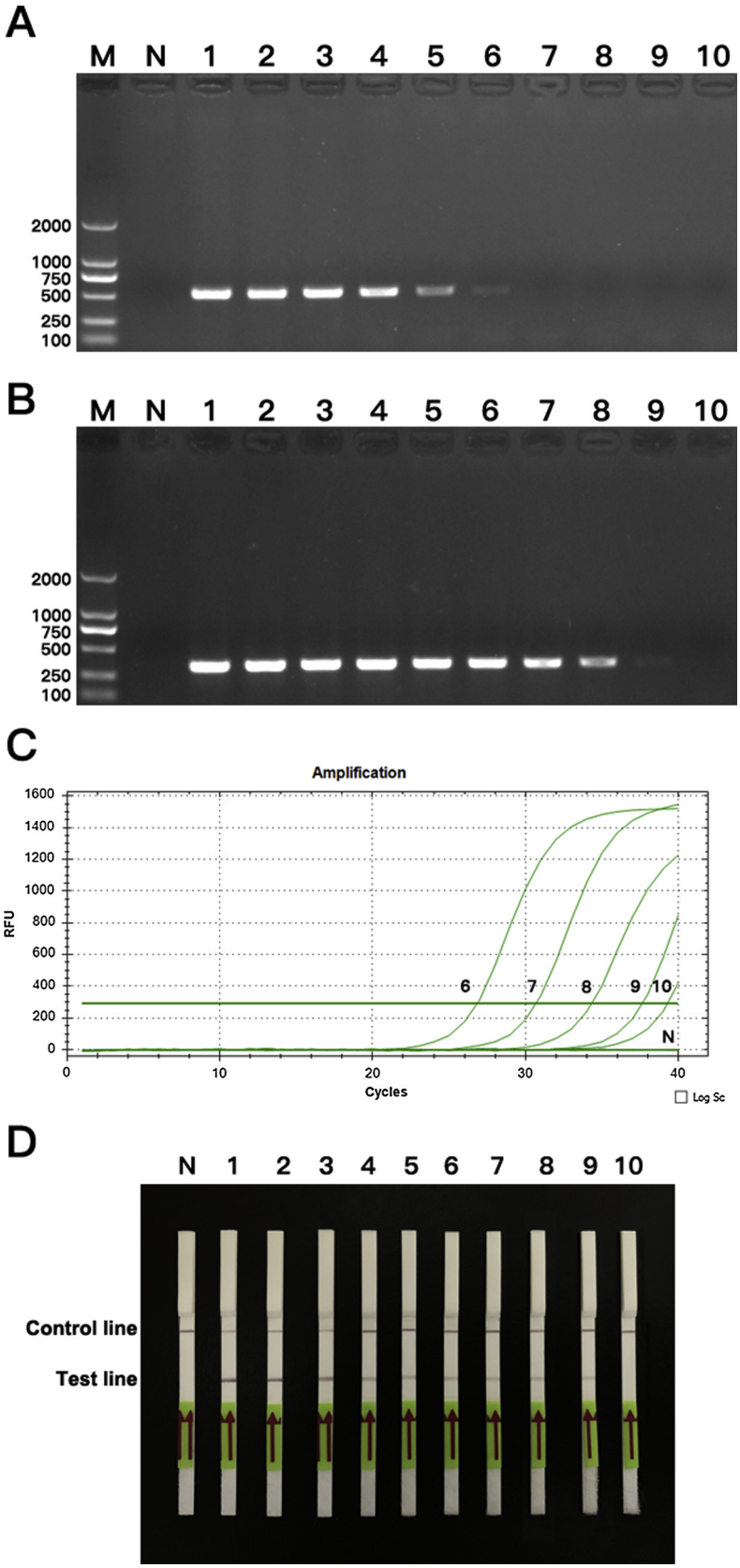

Fig. 4. Compare sensitivity of PCR (A), nested PCR (B), the real-time PCR (C) and LAMP-LFD (D) for the detection of FAdV-4. Concentration gradient of template DNA (copies/μl) were as follows: Lane N: double-distilled H_2O; Lane 1: 1×10^9; Lane 2: 1×10^8; Lane 3: 1×10^7; Lane 4: 1×10^6; Lane 5: 1×10^5; Lane 6: 1×10^4; Lane 7: 1×10^3; Lane 8: 1×10^2; Lane 9: 1×10^1; Lane 10: 1; Lane M: 2000-bp DNA marker.

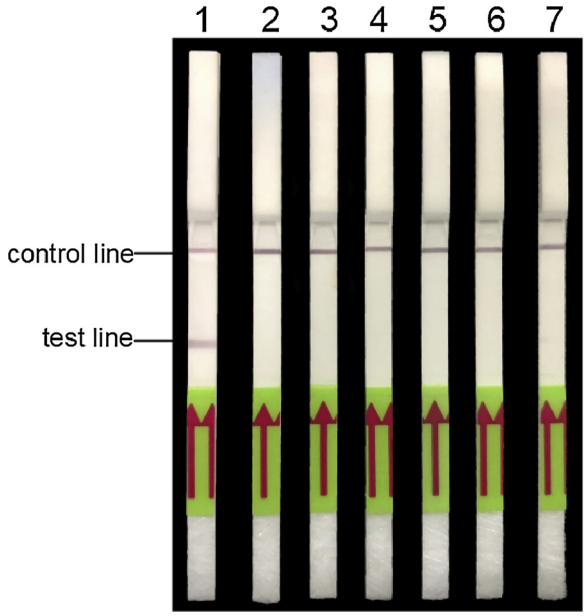

Fig. 5. Specificity of LAMP-LFD to detect FAdV-4. Lane 1: FAdV-4; Lane 2: FAdV-7; Lane 3: FAdV-8b; Lane 4: MDV; Lane 5: IBDV; Lane 6: FPV; Lane 7: double-distilled H_2O.

hybridized with FITC – labeled FIP, which bound first to the gold-labeled FITC-specific antibodies in the sample application area of the dipstick. When the complexes flowed over the immobilized biotin-ligand molecules at the test band, the complexes generated a red band over time. In the negative sample, the hybridization products were fixed by species-specific antibodies without capturing gold particles as they flowed over the control band. As time went by, the control band turned redder. The schematic diagram of the LFD is showed in Fig. 2B. There were three replicates in each assay.

2.7. Sensitivity and specificity of LAMP-LFD assay

In order to evaluate the sensitivity of these PCR-based assays (LAMP-LFD, nested PCR, qPCR and PCR), the viral DNA used to confirm the copy number was serially diluted 10-fold resulting in DNA concentrations ranging from 1×10^9 copies/μl to 1×10^0 copies/μl. These templates were then subjected to all PCR-based assays in the conditions described above. When the reactions were completed, the products were observed as described above. To determine the specificity of LAMP-LFD for the target 52 K gene, FAdV-4 and other viral species (FAdV-7, FAdV-8b, MDV, IBDV and FPV) were used to test the LAMP-LFD assay using the optimal reaction conditions described above. The LAMP reaction results were visualized using the LFD. The tests in the present study were repeated at least three times.

2.8. Clinical evaluation of LAMP-LFD assay

150 suspected samples from 10 breeder-broiler farm in China were LAMP-LFD assayed and the results were compared with those from PCR assay. Tissue samples (liver, heart and kidney) of dead chickens were homogenized to extract DNA. The LAMP reaction products were analyzed by LFD strips, and the results of PCR were analyzed by 1% AGE.

3. Results

3.1. Optimization of LAMP-LFD assay

In the attempts to optimize the LAMP-LFD reaction system, various concentrations of the reagents mentioned above were prepared for use in detecting the 52 K gene. The results showed that the optimal system contained 2.5 μl 10 × Isothermal Amplification Buffer, 4 mM $MgSO_4$, 1.2 mM dNTPs, 0.2 μM F3/B3, 1.6 μM FIP/ BIP, 8U *Bst* 2.0 WarmStart DNA polymerase and 1 μl target DNA, and the optimal reaction condition was incubation for 60 min at 65 °C. In natural lighting, the positive products were visualized with the naked eye because the turbidity caused by the white precipitate of magnesium pyrophosphate in the mixture in positive samples was easily visible; while the reaction mixture in negative control samples remained clear (Fig. 3A). After the addition of SYBR Green in the tubes, the positive products turned green, while the negative control turned orange (Fig. 3B). The results of LAMP-LFD are presented in Fig. 3C. The positive products produced two red bands and the negative control only produced one line. The results of LAMP assay were analyzed by 2% AGE (Fig. 3D), in which the typical ladder-like patterns of the positive amplification can be observed.

Table 2
Comparison of LAMP-LFD and PCR for the detection of FAdV-4 in clinical samples.

		PCR		
		Positive	Negative	Row totals
LAMP-LFD	Positive	20 (0.13)	3 (0.02)	23 (0.15)
	Negative	0 (0)	127 (0.85)	127 (0.85)
	Column totals	20 (0.13)	130 (0.87)	150

3.2. Comparison of sensitivity between LAMP-LFD and PCR assays

The sensitivity of the LAMP-LFD assay was compared with that of PCR, nested PCR, and qPCR. Their templates were the 10-fold serial dilutions of the viral DNA. Using conventional PCR, 1×10^4 copies/μl DNA was the detection limit, and the results could be seen using 1% agarose gel electrophoresis (Fig. 4A). The detection limit of qPCR is 1×10^2 copies/μl (Fig. 4C). Sensitivity was further assessed by nested PCR (Fig. 4B) and LAMP-LFD assays (Fig. 4D). They successfully detected as low as 10 copies/μl DNA. Comparatively, the LAMP-LFD assays were 1000-fold more sensitive than conventional PCR, 10-fold more sensitive than qPCR and had same detection limit as nested PCR.

3.3. Specificity of LAMP-LFD assay

To determine the specificity of LAMP-LFD for the target 52 K gene, FAdV-4 strains and other viral species (FAdV-7, FAdV-8b, MDV, IBDV and FPV) were tested. The results showed that FAdV-4 strain tested with LAMP-LFD gave positive results. In contrast, the same primers did not yield positive reactions when tested on with other strains, including FAdV-7, FAdV-8b, MDV, IBDV and FPV (Fig. 5). Thus, the results demonstrated that amplification of the 52 K gene using our LAMP-LFD assay was highly specific for FAdV-4.

3.4. Comparative analysis of field samples

To confirm the applicability of the LAMP-LFD assay in the field, 150 field samples were assayed for the 52k gene using the LAMP-LFD and PCR assay. Using LAMP-LFD assay, 23 field samples were positive and 127 samples were negative. For the positive samples, 20 samples can be simultaneous detected by PCR and LAMP-LFD, whereas 3 samples can only be detected by LAMP-LFD. They were further detected by nested PCR. DNA Sequence analysis demonstrated that the 3 samples were positive showing that LAMP-LFD has higher sensitivity in field samples. The two methods agreed on the diagnosis “positive” in 13% of clinical samples, and they agreed on the diagnosis “negative” in 85% of clinical samples. Their probability of agreement is thus $p_0 = 147/150 = 13\% + 85\% = 98\%$. (Table 2).

4. Discussion

Previous studies have reported that hexon genes targeting LAMP method can detect FAdVs, but no LFD has been combined for colorimetric readout (Xie et al., 2011). The present study selected the 52 K gene for FAdV-4 detection in LAMP-LFD combined system. The 52 K gene was chosen based on its highly conserved and great specificity for FAdV-4 (Gunes et al., 2012). This study involved the design of highly selective primers (F3/B3/FIP/BIP), the 5′-BIP biotinylated primer, and the fluorescein isothiocyanate (FITC)-labeled FIP. A series of concentrations of reagents and the reaction conditions were optimized. Briefly, the LAMP-LFD reaction was completed within 60 min at 65 °C, which is faster than other methods that require 1.5–2 h (excluding the gel electrophoresis step), such as the cross-priming amplification (CPA) (Niczyporuk et al., 2015) and the real-time PCR assays for FAdVs (Gunes et al., 2012). Also, the LAMP-LFD detection limit was 10 copies/μl of FAdV-4 genomic DNA which is 1000 times more sensitive than conventional PCR and 10 times more sensitive than the qPCR assay. Even though it has the same detection limit as nested PCR, it can potentially require less than one half of the time and reagents. In field sample, PCR and LAMP-LFD were chosen for comparison because PCR is a conventional detection method in pathogen detection. The two methods agreed on the diagnosis “positive” in 13% of clinical samples, and they agreed on the diagnosis “negative” in 85% of clinical samples. Their probability of agreement is 98%. The results showed the LAMP-LFD assay was more sensitive than PCR and provided a useful tool for FAdV-4 infection detection in field samples. In previous studies (Khunthong et al., 2013), there was only a 5′- BIP biotinylated primer used in the LAMP amplification step. After amplification, another FITC-labeled probe was added to the tubes and incubation was continued for 5–10 min. During this process, aerosol pollution increased. In this study, two labeled primers were simultaneously added to the amplification system to reduce the reaction time and aerosol pollution. Also, the same primers did not produce positive reactions when other strains (FAdV-7, FAdV-8b, MDV, IBDV and FPV) were tested, demonstrating that the specificity of the LAMP-LFD method developed in the present study was acceptable. As a paper-based readout method for LAMP, LFD has several advantages in sensitivity and specificity because the results of LAMP-LFD amplification depend on the specific hybridization between sequences and avoid the false positive results caused by non-specific amplification (Zhang et al., 2014). In addition, the LAMP-LFD assay allows for objective assessment of the results rather than relying on subjective observation with the naked eye.

In recent years, various PCR based molecular techniques were developed to detect FAdVs, such as the cross-priming amplification (Niczyporuk et al., 2015), real-time PCR (Gunes et al., 2012), and duplex-PCR assays (Niczyporuk et al., 2010). However, these methods have limitations such as being messy, time-consuming, not applicable in resource-limited regions and unsuitable for on-field testings. In this study, the entire amplification was carried out in a water bath and a lateral flow test strip was designed for reporting results. In addition, there are other advantages, such as simple steps, short operating time and eliminating the use of harmful reagents and delicate instruments. These advantages make it possible for LAMP-LFD to be applied in resource-limited areas, such as small breeding farms and basic veterinary labs. In summary, the LAMP-LFD system developed in this study provided a better solution for detecting FAdV-4 due to its rapid, reliable and ease-to-use practices, along with uncompromised sensitivity and specificity when compared with other molecular detection methods.

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Authors' contributions

Xiwen Zhai, Xueran Mei and Hongning Wang conceived and designed the experiments, Xiwen Zhai, Xuan Wu, Yiming Tian, Long Zhou and Lei Zuo performed the experiments, Xiwen Zhai and Xin Yang analyzed and interpreted the data, Xiwen Zhai and Xiaoxiao Han wrote the paper.

Compliance with ethical standards

The authors declare that they have no conflict of interest.

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