



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



# Evaluation of an incubation instrument-free reverse transcription recombinase polymerase amplification assay for rapid and point-of-need detection of canine distemper virus



Jianchang Wang<sup>a,c,1</sup>, Jinfeng Wang<sup>a,c,1</sup>, Ruiwen Li<sup>b</sup>, Ruihan Shi<sup>a,c</sup>, Libing Liu<sup>a,c</sup>, Wanzhe Yuan<sup>b,\*</sup>

<sup>a</sup> Center of Inspection and Quarantine, Hebei Entry-Exit Inspection and Quarantine Bureau, Shijiazhuang 050051, China

<sup>b</sup> College of Veterinary Medicine, Agricultural University of Hebei, Baoding 071001, China

<sup>c</sup> Hebei Academy of Science and Technology for Inspection and Quarantine, Shijiazhuang 050051, China

## ARTICLE INFO

### Keywords:

CDV  
N gene  
LF probe  
LFS  
RT-RPA

## ABSTRACT

Canine distemper, caused by Canine distemper virus (CDV), is a highly contagious and fatal systemic disease in free-living and captive carnivores worldwide. Accurate, rapid and simple detection of CDV is critical to improve disease management and prevent outbreaks. In this study, a visible and incubation instrument-free reverse-transcription recombinase polymerase amplification assay combined with lateral flow strip (LFS RT-RPA) was developed to detect CDV using primers and lateral flow (LF) probe specific for the nucleocapsid (N) protein gene. The CDV LFS RT-RPA assay was performed in a closed fist using body heat for 15 min, and the products were visible to the naked eyes on the LFS within 5 min. The assay could detect CDV, and there was no cross-reaction with the other viruses tested. Using the in vitro transcribed CDV RNA as template, the analytical sensitivity was  $9.4 \times 10^1$  copies per reaction, which was the same result as that of a real-time RT-PCR. The assay performance was further evaluated by testing 32 nasal/oropharyngeal swab samples, and CDV RNA positive rate was 62.0% (20/32) by LFS RT-RPA, which was the same result as that of the real-time RT-PCR assay. The performance of the LFS RT-RPA was comparable to real-time RT-PCR, while the LFS RT-RPA assay was much faster and easier to perform. The novel CDV LFS RT-RPA assay provides an attractive and promising tool for rapid and reliable detection of CDV in the underequipped laboratory and point-of-need facility, which is of great significance in CD control in low resource settings.

## 1. Introduction

Canine distemper, caused by canine distemper virus (CDV), is a highly contagious and fatal systemic disease found worldwide not only in dogs and many other carnivores but also in some non-carnivores (Wilkes et al., 2014). CDV is a non-segmented, negative-stranded, enveloped RNA virus that belongs to the family *Paramyxoviridae* and the genus *Morbillivirus*, and is one of the most lethal infectious agents in both susceptible free-living and captive carnivores (Lednicky et al., 2004). CDV-infected dogs may develop respiratory, gastrointestinal, dermatologic, ophthalmic or neurological disorders that appear simultaneously or sequentially (Beineke et al., 2009; Decaro et al., 2004; Tan et al., 2011). The broad spectrum of clinical signs, not dissimilar from the signs observed in other respiratory and enteric diseases of dogs, hampers accurate and early clinical diagnosis of canine distemper (Seki et al., 2003). Therefore, rapid and accurate diagnosis of CDV

infection would enable veterinarians to implement appropriate strategies in time to improve disease management and prevent outbreaks, particularly within a shelter environment (Elia et al., 2015).

A substantial number of assays based on viral nucleic acid detection have been described for CD diagnosis with a varying degree of sensitivity and specificity, such as reverse transcription polymerase chain reaction (RT-PCR) (Frisk et al., 1999), nested RT-PCR (Shin et al., 2004), real-time RT-PCR (Elia et al., 2006), reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Cho and Park, 2005) and reverse transcription insulated isothermal PCR (RT-iiPCR) (Wilkes et al., 2014). However, the RT-PCR assays are cold chain dependent and require relatively sophisticated equipment with experienced technicians, making these assays unsuitable for being used in underequipped laboratory and in field (Elia et al., 2006; Frisk et al., 1999; Shin et al., 2004). Compared to the current RT-PCR assays, the use of isothermal technologies reduces the need for high precision instrumentation and

\* Corresponding author at: College of Veterinary Medicine, Agricultural University of Hebei, No.38 Lingyusi Street, Baoding, Hebei 071001, China.

E-mail address: [yuanwanzhe2015@126.com](mailto:yuanwanzhe2015@126.com) (W. Yuan).

<sup>1</sup> These authors contributed equally to this work.

**Table 1**  
Sequence of primers and probes for CDV RT-PCR, real-time RT-PCR and LFS RT-RPA assays used in the study.

Assay	Primers and probe	Sequence 5'-3'	Amplicon size (bp)	References
RT-PCR	H-F	TTAGGGCTCAGGTAGTCCA	1879	Zhao et al., 2010
	H-R	CTAAGKCCAATTGARATGTGT		
real-time RT-PCR	CDV-F	AGCTAGTTTCATCTTAACTATCAAAAT	87	Elia et al., 2006
	CDV-R	TTAACTCTCCAGAAAACATGTC		
	CDV-P	FAM-ACCCAAGAGCCGGATACATAGTTTCAATGC-BHQ1		
LFS RT-RPA	CDV-LF-F	GCTTACTTCAGACTCGGGCAAGAAATGGTTA	154	This study
	CDV-LF-R	Biotin-CAGTAGCTCGAATTGCCGGTCTCTGTGT		
	CDV-LF-P	FAM-CTTGGCATCACCAAGGAGGAAGCTCAGCTGGT(THF) TCAGAAATAGCATCCA-C3-spacer		

consistent electrical power. The results are usually produced within 60 min for the above RT-LAMP and RT-iiPCR assays, and depend on the water bath or specialized instrument, POCKIT™ Nucleic Acid Analyzer (Cho and Park, 2005; Wilkes et al., 2014). A simple, rapid, accurate and user-friendly detection platform is still needed for reliable detection of CDV in the laboratories without access to real-time PCR instrumentation and at the point-of-need (PON) diagnosis.

Recombinase polymerase amplification (RPA) is an isothermal DNA amplification technique that has been demonstrated to be rapid, specific, sensitive, and cost-effective (Daher et al., 2016; Piepenburg et al., 2006). The RPA reaction uses enzymes called recombinases that form complexes with oligonucleotide primers and pair the primers with homologous sequences in DNA. A single-stranded DNA binding protein binds to the displaced DNA strand and stabilizes the resulting loop. The primer then initiates DNA amplification by a strand-displacing DNA polymerase (Piepenburg et al., 2006). Real-time detection of the RPA amplification mainly depends on the Exonuclease III and exo probe, while the direct visual detection of the RPA products depends on the Endonuclease IV, LF probe and the opposing amplification primer labeled at 5' end with a biotin. The LF probe oligonucleotide backbone includes a 5'-antigenic label (typically a FAM group), an internal abasic nucleotide analogue (a tetrahydrofuran residue or THF) and a 3'-polymerase extension blocking group (such as a C3-spacer). The amplicons are then detected by naked eyes in the 'sandwich' assay formats, such as a lateral flow strip (LFS) that contains anti-FAM gold conjugates and biotin-ligand molecules. Our laboratory had developed a real-time RT-RPA assay based on exo probe for real-time detection of CDV while the assay still depended on the specialized instrument, Genie III (OptiGene, West Sussex, UK) (Wang et al., 2017a). Series of LFS RPA assays had been developed for the detection of porcine parvovirus (PPV), peste des petits ruminants virus (PPRV) and bovine ephemeral fever virus (BEFV) (Hou et al., 2017; Yang et al., 2017, 2016).

In this study, we developed an incubation instrument-free RPA assay for rapid, specific and sensitive detection of CDV, which was combined with LFS (USTAR, Hangzhou, China) and performed by incubating the reaction tubes in a closed fist using body heat.

## 2. Material and methods

### 2.1. Virus strains and clinical samples

Canine distemper virus (CDV-FOX-TA strain, genotype: America-2), canine parvovirus type 2 (CPV-2, CPV-b114 strain), canine coronavirus (CCoV, ATCC VR-809 strain), canine parainfluenza virus (CPIV, CPIV/A-20/8 strain), and pseudorabies virus (PRV, Barth-K61 strain) were maintained in our laboratory. Thirty-two nasal/oropharyngeal swabs were collected from 20 dogs of both sexes (various breeds and ages) from the animal hospital of the Agricultural University of Hebei and 12 raccoon dogs from the farms in Hebei Province, China from 2014 to 2016 and snap-frozen for storage at  $-80^{\circ}\text{C}$ . All the dogs and raccoon dogs clinically were suspected of being CDV infected. Fifteen samples from the dogs and 5 samples from the raccoon dogs had been tested to be CDV positive with the Ct values ranging from 16.36 to 37.03, and the

other 12 samples were CDV negative by a real-time RT-PCR (Elia et al., 2006).

### 2.2. DNA/RNA extraction

CDV, CCoV, and CPIV viral RNA was extracted using Trizol Reagent (Invitrogen, Waltham, USA), CPV-2 and PRV viral DNA was extracted using the TIANamp Virus DNA kit (Tiangen, Beijing, China), which were performed according to manufacturer's instructions, respectively. For viral RNA extraction from the nasal/oropharyngeal swabs, the swab was inoculated and vortexed in 1 mL sterile phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 10,000 rpm for 10 min at  $4^{\circ}\text{C}$ . Two hundreds microliter of the supernatant was collected for viral RNA extraction using the Trizol Reagent, and the RNA was finally eluted in 20  $\mu\text{L}$  of nuclease-free water. All RNA and DNA were quantified using ND-2000c and stored at  $-80^{\circ}\text{C}$  until use.

### 2.3. Generation of in vitro transcribed CDV RNA

The in vitro transcribed CDV RNA, which covers the nucleocapsid protein gene of CDV, was generated as described previously and diluted in ten-fold series to obtain RNA concentrations ranging from  $9.4 \times 10^4$  to  $9.4 \times 10^{-1}$  copies/ $\mu\text{L}$  (Wang et al., 2017a).

### 2.4. RPA primers and LF probe

Nucleotide sequences of different CDV genotypes (accession numbers: AB490678, AF164967, AY386316, GU138403, HQ540292, KF856711, KF914669) available in GenBank were aligned to identify regions that are highly conserved in the N gene, and the primers and LF probe were designed, which were listed in Table 1 and synthesized by a commercial company (Sangon Biotech Co., Shanghai, China).

### 2.5. LFS RT-RPA

LFS RT-RPA reactions were performed in a 50  $\mu\text{L}$  volume using a TwistAmp™ nfo kit (TwistDX, Cambridge, UK). Other components included 420 nM each RPA primer, 120 nM LF probe, 14 mM magnesium acetate, 200 U MMLV reverse transcriptase (Takara, Dalian, China), 40 U Recombinant RNase Inhibitor (Takara, Dalian, China) and 1  $\mu\text{L}$  of viral or sample RNA. All reagents except for the viral template and magnesium acetate were prepared in a master mix, which was distributed into each 0.2 mL freeze-dried reaction tube containing a dried enzyme pellet. One microliter of viral RNA was added to the tubes. Subsequently, magnesium acetate was pipetted into the tube lids, which were closed carefully, and the magnesium acetate was centrifuged into the rehydrated material using a minispin centrifuge. The sample was vortexed briefly and spun down once again, and the reaction tubes were immediately incubated in the different technician's closed fist at room temperature. The RPA was performed using the body heat for 5, 10, 15 and 20 min, and an LFS was used to detect the amplicons that were dual-labeled with FAM and biotin. The LFS contains gold particle-conjugated anti-FAM antibody (rabbit polyclonal antibody) in the

sample pad of the strip, and the gold complex forms between the dual-labeled RPA amplicons and the anti-FAM antibody. The LFS also contains the biotin-ligand at the test line and the anti-rabbit antibody at the control line. Only the amplicons captured gold particle will bound when they overflow the immobilized biotin-ligand molecules at the test line, and generate there a red band. Not-captured particles flow over the control band and will be fixed there by anti-rabbit antibodies. For each RPA reaction, 10  $\mu$ L of product was added to the sample pad of the strip, and the strip was then placed in a well of a 96-well plate containing 100  $\mu$ L of running buffer and incubated at an upright position. The final result was read visually after incubation for 5 min at room temperature. A testing sample was considered positive when both the test line and the control line were visible, and considered negative when only the control line was visible. The assay was considered invalid when the control line was not visible.

### 2.6. Analytical specificity and sensitivity analysis

Ten nanogram of RNA or DNA was used as template for the analytical specificity analysis of the CDV LFS RT-RPA assay. The assay was evaluated against a panel of pathogens dangerous to the dogs and other carnivores, CDV, CPV-2, CCoV, CPIV and PRV. Three independent reactions were performed by three different technicians.

The ten-fold serial dilutions of the in vitro transcribed RNA were used as the standard RNA for CDV LFS RT-RPA assay. One microliter of each dilution was then amplified by the LFS RT-RPA to determine the limit of detection (LOD) of the assay. Three independent reactions were performed by three different technicians.

### 2.7. Real-time RT-PCR

A real-time RT-PCR specific for CDV was performed on an ABI 7500 instrument as described previously with some modifications (Elia et al., 2006). The One Step PrimeScript<sup>®</sup> RT-PCR Kit (Takara, Dalian, China) was used, and the reaction was performed as follows: 42 °C 5 min; 95 °C for 10 s; then 40 cycles of 95 °C for 5 s and 60 °C for 35 s.

### 2.8. Validation with simulated samples

RNA extracted from 20 nasal/oropharyngeal swabs of dog and 12 nasal/oropharyngeal swabs of raccoon dog were tested by LFS RT-RPA, and the results were compared with those obtained in a real-time RT-PCR described previously (Elia et al., 2006). For the positive samples, the RT-PCR for full-length of CDV haemagglutinin (H) gene was performed using H-F and H-R as primers (Zhao et al., 2010). PCR products of the expected size (1879 bp) were purified and cloned into the pMD 19-T simple vector (TaKaRa, Dalian, China). For each sample, 3–5 positive recombinant plasmids were sequenced in both directions using primer M13 by a commercial company (Sangon Biotech Co., Shanghai, China). The sequences were compared with the H gene of different genotypes of CDV available in GenBank to identify the CDV genotypes using DNASTAR software.

## 3. Results

### 3.1. Optimization of the reaction time

To determine the optimal reaction time for the CDV LFS RT-RPA reaction, the reaction tubes were incubated in three different technicians' closed fists for 5, 10, 15 and 20 min using  $9.4 \times 10^3$  copies of in vitro transcribed RNA as template. As shown in Fig. 1, no amplified products were observed in the reactions incubated for 5 and 10 min. When the incubation time was increased to 15 min or more, the assay performance was improved, and there were no clear differences between the products observed after 15 and 20 min incubations. Similar results were observed in three independent reactions. Therefore, the

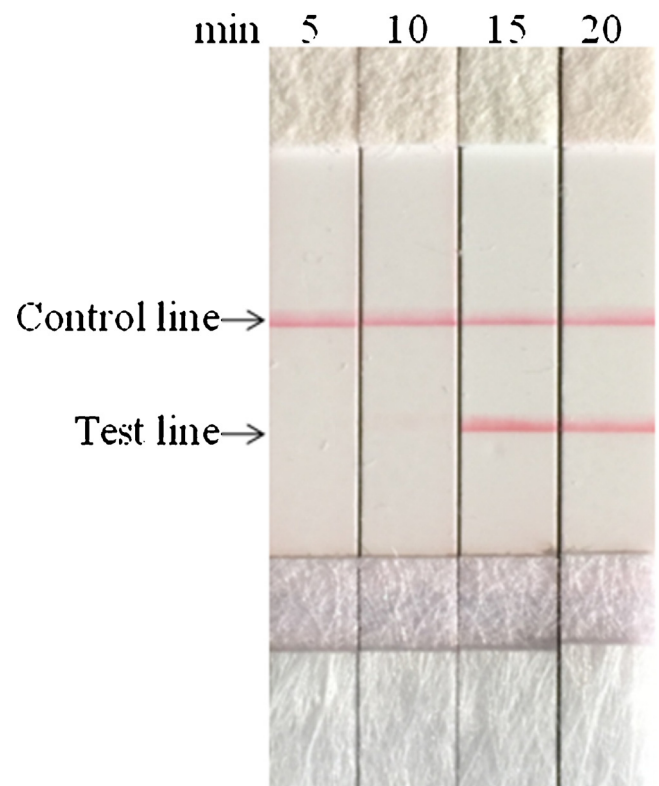


Fig. 1. Determination of the CDV LFS RT-RPA reaction time. The CDV LFS RT-RPA assay was performed by incubating the reaction tubes in a closed fist for different times as shown. The test line was clearly visible when the amplification time is longer than 15 min.

optimal incubation time for CDV LFS RT-RPA assay was set at 15 min.

### 3.2. Analytical specificity and sensitivity

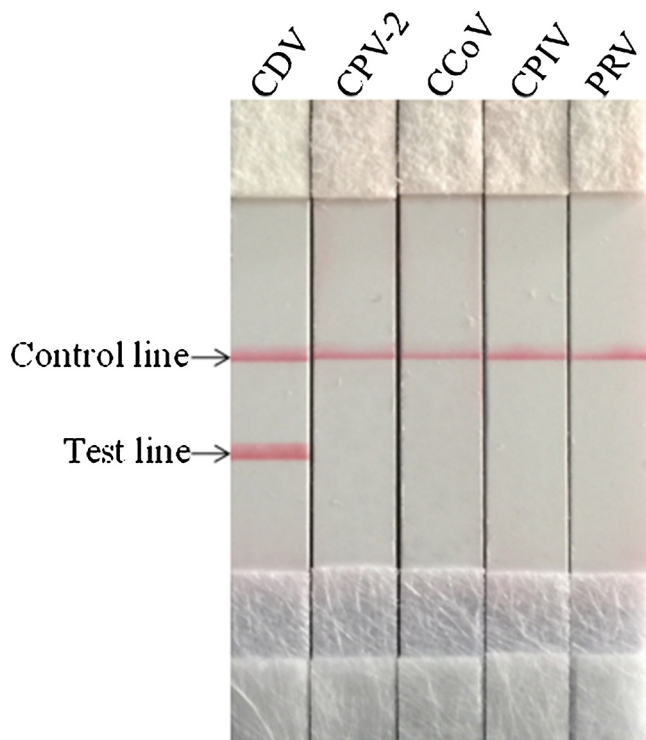
Using 10 ng of viral RNA and DNA as template, the results showed only the CDV was detected by the LFS RT-RPA while the other viruses were not detected (Fig. 2). No cross detections were observed. Three independent reactions were repeated and the above similar results were observed, demonstrating the high specificity and repeatability of the assay.

The LFS RT-RPA was performed using a dilution range from  $9.4 \times 10^4$  to  $9.4 \times 10^{-1}$  copies/ $\mu$ L of in vitro transcribed RNA as template. As shown in Fig. 3, the LOD of the LFS RT-RPA was  $9.4 \times 10^1$  copies, which was the same as the real-time RT-PCR. The LFS RT-RPA assay was performed three times by three different technicians, and results similar to those described above were obtained.

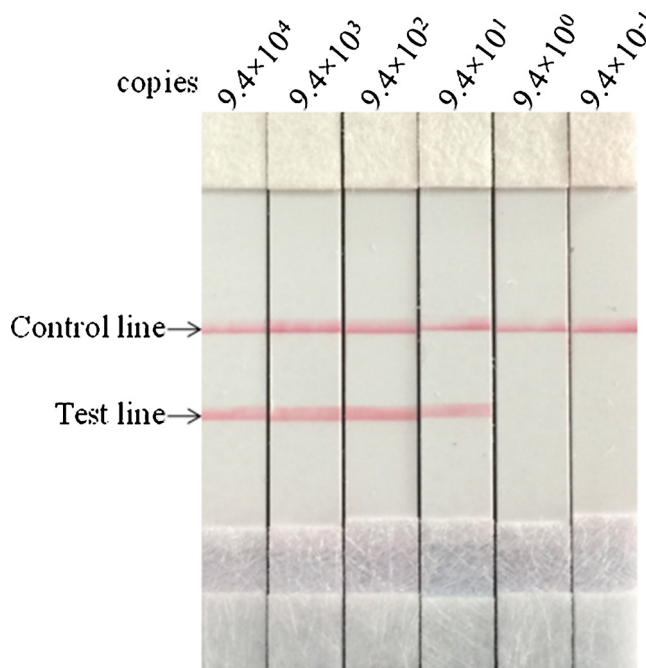
### 3.3. Evaluation of LFS RT-RPA with clinical samples

The detection results of 32 clinical samples demonstrated that the LFS RT-RPA and real-time RT-PCR showed the same performance (20 positive and 12 negative cases) (Table 2). A sample of the CDV LFS RT-RPA results is shown in Fig. 4. Further analysis demonstrated that the two assays had a diagnostic agreement of 100%. No discrepancy was found in samples (3/20) containing low levels of CDV RNA (Ct > 35, real time RT-PCR), indicating the established LFS RT-RPA reliably detected low amounts of CDV in clinical samples. Positive samples had real-time RT-PCR Ct values ranging from 16.36 to 37.03, indicating that the LFS RT-RPA was able to detect CDV RNA across the entire range of the assay. It took less than 20 min for the LFS RT-RPA assay to obtain the positive results, while real-time RT-PCR took much longer (approximately 24–55 min). These results indicated that the performance





**Fig. 2.** Analytical specificity of the CDV LFS RT-RPA assay. Reactions tubes were incubated in a closed fist for 15 min using 10 ng of viral RNA or DNA as template. The results showed LFS RT-RPA only amplified the CDV RNA, but not the other viruses tested.



**Fig. 3.** Analytical sensitivity of the CDV LFS RT-RPA assay. The sensitivity of the CDV LFS RT-RPA assay was performed using a dilution range of  $9.4 \times 10^4$ – $1.0 \times 10^{-1}$  copies of the in vitro transcribed CDV RNA. The LOD of the assay was  $9.4 \times 10^1$  copies per reaction.

of the LFS RT-RPA assay was comparable to real-time RT-PCR, but the LFS RT-RPA assay is much faster.

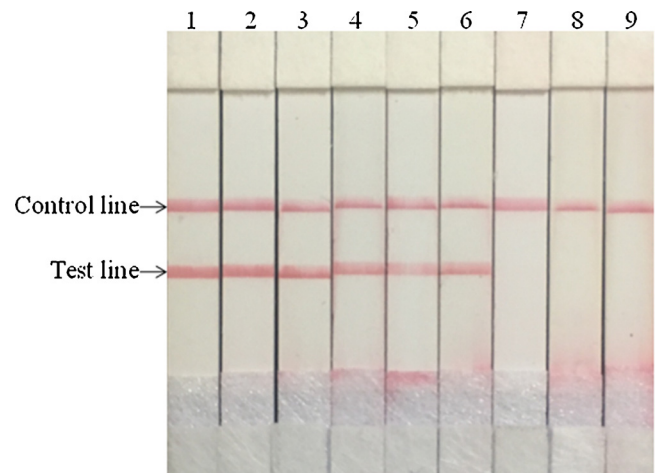
The H gene was amplified successfully for 16 samples, and unsuccessfully for the 4 samples with the Ct values ranging between

**Table 2**

Comparison of CDV LFS RT-RPA assay with real-time RT-PCR assay on the clinical nasal/oropharyngeal samples.

Sample type	Sample No.	LFS RT-RPA	real-time RT-PCR(Ct)
Dog	D1	+	34.41
	D2	+	27.85
	D3	-	> 40
	D4	+	30.96
	D5	+	16.36
	D6	-	> 40
	D7	+	37.03
	D8	-	> 40
	D9	+	28.11
	D10	+	19.28
	D11	+	35.81
	D12	+	25.67
	D13	-	> 40
	D14	-	> 40
	D15	+	24.43
	D16	+	31.06
	D17	+	31.17
	D18	+	18.47
	D19	+	28.79
	D20	+	35.04
Raccoon dog	R1	+	24.87
	R2	+	17.97
	R3	-	> 40
	R4	+	28.05
	R5	+	17.81
	R6	+	25.15
	R7	-	> 40
	R8	-	> 40
	R9	-	> 40
	R10	-	> 40
	R11	-	> 40
	R12	-	> 40

+ : positive; - : negative.



**Fig. 4.** Detection of CDV in clinical samples by LFS RT-RPA. Nine samples were selected randomly from the clinical samples. Lines 1–6 are samples that were CDV RNA-positive by the LFS RT-RPA and real-time RT-PCR assays, and lines 7–9 are samples that were CDV RNA-negative by the both assays.

34.41–37.03, which were D1, D7, D11 and D20. Comparisons of nucleotide and deduced amino acid sequences were made, and all the 16 CDV strains were characterized as Asia-1 genotype, which were highly similar to each other (97.5–100 % nt and 98.3–100% aa).

#### 4. Discussion

This study describes a visible, incubation instrument-free LFS RT-RPA assay with high sensitivity and specificity for rapid detection of

CDV. The CDV LFS RT-RPA reaction tubes were held in a closed fist for 15 min, and the results were inspected directly with the naked eyes within 5 min. Other CDV genotypes were not included in the assay except for the genotype America-2, which is deficiency of the study. RPA is tolerant to 5–9 mismatches in primer and probe showing no influence on the performance of the assay (Abd El Wahed et al., 2013; Daher et al., 2015) and there were only 2–4 mismatches in the primers and probe in this study with other CDV genotypes available in GenBank, such as strain 2784/2013 (genotype Arctic, KF914669), strain BJ-01 (genotype Asia-1, KF856711), strain 50Cb1/H (genotype Asia-2, AB490678), strain HLJ2/07 (genotype Asia-3, HQ540292), strain 5804 P (genotype European, AY386316), and strain Onderstepoort (genotype America-1, AF378705). It is assumed the assay would detect the other six genotypes of CDV basing on the facts that the LFS RT-RPA assay targeted the conserved N gene of CDV, which was further validated by the fact that the CDV strains detected in the study were identified to be Asia-1 genotype. In addition, use of Trizol for extraction in this study causes concerns about hazardous chemical waste removal for low resource settings. When using the CDV LFS RT-RPA assay in low resource settings, the use of the commercial RNA extraction kit is recommended.

RPA operates at a wide range of temperatures and does not require the reaction temperature to be precisely controlled (Piepenburg et al., 2006). TwistDx recommends an incubation temperature of 37 °C (the temperature of the human body), and others studies have shown that RPA retains reliable functionality between 31 °C and 43 °C (Lillis et al., 2014; Wu et al., 2017; Yang et al., 2016), even between 30 °C and 45 °C (Wu et al., 2017; Yang et al., 2016). Our study also showed that RPA could work well for detection of PCV2 between 34 °C and 42 °C (Wang et al., 2016). Normal human body temperature (36.1–37 °C) is within the above temperature range, and several RPA assays have been developed to perform the reaction using body heat by either holding the reaction tubes in the axilla or in closed fists (Crannell et al., 2014; Wang et al., 2017b). In this study, the CDV LFS RT-RPA assay was performed by holding the reaction tubes in a closed fist, which is one feature of the assay. The assays were performed well by three different technicians in the laboratory, office or in the field with an ambient temperature of 24.4 °C, 22.5 °C and 17.0 °C, respectively, and the temperature in the closed fists was 37.0 °C, 37.5 °C and 35.7 °C, respectively.

Most of the established LFS RPA assays either developed for DNA or performed the reactions in water baths or incubator block (Lillis et al., 2014; Wang et al., 2017b; Wu et al., 2017; Yang et al., 2017, 2016). In the LFS RT-RPA assays for PPRV and BEFV, the viral RNA was not used as the template directly, while there was an additional process to reverse transcribe the extracted RNA to cDNA prior to performing the assays, which need approximately 30–60 min (Hou et al., 2017; Yang et al., 2017). In this assay, we added MMLV (4U/μL) and RNase inhibitor (0.8U/μL) into the TwistAmp™ nfo reaction system, and the CDV LFS RT-RPA worked well with CDV RNA as the template, which is the other advantage of our assay. To our knowledge, the developed CDV LFS RT-RPA assay is the first LFS RT-RPA assay directly using RNA as the template and independent of any instrument during reaction process.

The LOD of the LFS RT-RPA was the same as that of the developed CDV real-time RT-RPA described previously (Wang et al., 2017a), while the latter assay depends on the specialized instrument. In the evaluation of the assay performances on the clinical samples, the diagnostic agreement was 100% between the CDV LFS RT-RPA and real-time RT-PCR, demonstrating excellent agreements between the two assays. Nevertheless, the LFS RT-RPA showed distinct advantages in terms of detection time and equipment requirements. The above results are encouraging, but the assay must be validated by analysis of a larger number of CDV RNA positive clinical samples.

In conclusion, a rapid, visible and incubation instrument-free method using body heat has been developed successfully for PON diagnosis of canine distemper. The good analytical specificity, analytical

sensitivity, and easy sample-to-answer protocol make the LFS RT-RPA ideal for the accurate and rapid detection of CDV RNA in an under-equipped laboratory and at the PON diagnosis, especially in low resource settings.

## Conflicts of interest

None.

## Acknowledgements

This work was supported by Natural Science Foundation Youth Project of Hebei Province (C2017325001) and Science and Technology Project Foundation of Hebei Province (16226604D) and partially funded by the Fund for One-hundred Outstanding Innovative Talents from Hebei Institution of Higher Learning (SLRC2017039).

## References

- Abd El Wahed, A., El-Deeb, A., El-Tholoth, M., El, Abd, Kader, H., Ahmed, A., Hassan, S., Hoffmann, B., Haas, B., Shalaby, M.A., Hufert, F.T., Weidmann, M., 2013. A portable reverse transcription recombinase polymerase amplification assay for rapid detection of foot-and-mouth disease virus. *PLoS One* 8, e71642.
- Beineke, A., Puff, C., Seehusen, F., Baumgartner, W., 2009. Pathogenesis and immunopathology of systemic and nervous canine distemper. *Vet. Immunol. Immunopathol.* 127, 1–18.
- Cho, H.S., Park, N.Y., 2005. Detection of canine distemper virus in blood samples by reverse transcription loop-mediated isothermal amplification. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 52, 410–413.
- Crannell, Z.A., Rohrman, B., Richards-Kortum, R., 2014. Equipment-free incubation of recombinase polymerase amplification reactions using body heat. *PLoS One* 9, e112146.
- Daher, R.K., Stewart, G., Boissinot, M., Boudreau, D.K., Bergeron, M.G., 2015. Influence of sequence mismatches on the specificity of recombinase polymerase amplification technology. *Mol. Cell. Probes* 29, 116–121.
- Daher, R.K., Stewart, G., Boissinot, M., Bergeron, M.G., 2016. Recombinase polymerase amplification for diagnostic applications. *Clin. Chem.* 62, 947–958.
- Decaro, N., Camero, M., Greco, G., Zizzo, N., Tinelli, A., Campolo, M., Pratelli, A., Buonavoglia, C., 2004. Canine distemper and related diseases: report of a severe outbreak in a kennel. *New Microbiol.* 27, 177–181.
- Elia, G., Decaro, N., Martella, V., Cirone, F., Lucente, M.S., Lorusso, E., Di Trani, L., Buonavoglia, C., 2006. Detection of canine distemper virus in dogs by real-time RT-PCR. *J. Virol. Methods* 136, 171–176.
- Elia, G., Camero, M., Losurdo, M., Lucente, M.S., Laroche, V., Martella, V., Decaro, N., Buonavoglia, C., 2015. Virological and serological findings in dogs with naturally occurring distemper. *J. Virol. Methods* 213, 127–130.
- Frisk, A.L., Konig, M., Moritz, A., Baumgartner, W., 1999. Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *J. Clin. Microbiol.* 37, 3634–3643.
- Hou, P., Zhao, G., Wang, H., He, C., Huan, Y., He, H., 2017. Development of a recombinase polymerase amplification combined with lateral-flow dipstick assay for detection of bovine ephemeral fever virus. *Mol. Cell. Probes*.
- Lednicky, J.A., Dubach, J., Kinsel, M.J., Meehan, T.P., Bocchetta, M., Hungerford, L.L., Sarich, N.A., Witecki, K.E., Braid, M.D., Pedrak, C., Houde, C.M., 2004. Genetically distant American canine distemper virus lineages have recently caused epizootics with somewhat different characteristics in raccoons living around a large suburban zoo in the USA. *Virol. J.* 1, 2.
- Lillis, L., Lehman, D., Singhal, M.C., Cantera, J., Singleton, J., Labarre, P., Toyama, A., Piepenburg, O., Parker, M., Wood, R., Overbaugh, J., Boyle, D.S., 2014. Non-instrumented incubation of a recombinase polymerase amplification assay for the rapid and sensitive detection of proviral HIV-1 DNA. *PLoS One* 9, e108189.
- Piepenburg, O., Williams, C.H., Stemple, D.L., Armes, N.A., 2006. DNA detection using recombination proteins. *PLoS Biol.* 4, e204.
- Seki, F., Ono, N., Yamaguchi, R., Yanagi, Y., 2003. Efficient isolation of wild strains of canine distemper virus in vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. *J. Virol.* 77, 9943–9950.
- Shin, Y.J., Cho, K.O., Cho, H.S., Kang, S.K., Kim, H.J., Kim, Y.H., Park, H.S., Park, N.Y., 2004. Comparison of one-step RT-PCR and a nested PCR for the detection of canine distemper virus in clinical samples. *Aus. Vet. J.* 82, 83–86.
- Tan, B., Wen, Y.J., Wang, F.X., Zhang, S.Q., Wang, X.D., Hu, J.X., Shi, X.C., Yang, B.C., Chen, L.Z., Cheng, S.P., Wu, H., 2011. Pathogenesis and phylogenetic analyses of canine distemper virus strain ZJ7 isolate from domestic dogs in China. *Virol. J.* 8, 520.
- Wang, J., Wang, J., Liu, L., Li, R., Yuan, W., 2016. Rapid detection of porcine circovirus 2 by recombinase polymerase amplification. *J. Vet. Diagn. Invest.* 28, 574–578.
- Wang, J., Wang, J., Li, R., Liu, L., Yuan, W., 2017a. Rapid and sensitive detection of canine distemper virus by real-time reverse transcription recombinase polymerase amplification. *BMC Vet. Res.* 13, 241.
- Wang, R., Zhang, F., Wang, L., Qian, W., Qian, C., Wu, J., Ying, Y., 2017b. Instant, visual, and instrument-free method for on-site screening of GTS 40-3-2 soybean based on

- body-heat triggered recombinase polymerase amplification. *Anal. Chem.* 89, 4413–4418.
- Wilkes, R.P., Tsai, Y.L., Lee, P.Y., Lee, F.C., Chang, H.F., Wang, H.T., 2014. Rapid and sensitive detection of canine distemper virus by one-tube reverse transcription-insulated isothermal polymerase chain reaction. *BMC Vet. Res.* 10, 213.
- Wu, Y.D., Xu, M.J., Wang, Q.Q., Zhou, C.X., Wang, M., Zhu, X.Q., Zhou, D.H., 2017. Recombinase polymerase amplification (RPA) combined with lateral flow (LF) strip for detection of *Toxoplasma gondii* in the environment. *Vet. Parasitol.* 243, 199–203.
- Yang, Y., Qin, X., Zhang, W., Li, Y., Zhang, Z., 2016. Rapid and specific detection of porcine parvovirus by isothermal recombinase polymerase amplification assays. *Mol. Cell. Probes* 30, 300–305.
- Yang, Y., Qin, X., Song, Y., Zhang, W., Hu, G., Dou, Y., Li, Y., Zhang, Z., 2017. Development of real-time and lateral flow strip reverse transcription recombinase polymerase amplification assays for rapid detection of peste des petits ruminants virus. *Virolog. J.* 14, 24.
- Zhao, J.J., Yan, X.J., Chai, X.L., Martella, V., Luo, G.L., Zhang, H.L., Gao, H., Liu, Y.X., Bai, X., Zhang, L., Chen, T., Xu, L., Zhao, C.F., Wang, F.X., Shao, X.Q., Wu, W., Cheng, S.P., 2010. Phylogenetic analysis of the haemagglutinin gene of canine distemper virus strains detected from breeding foxes, raccoon dogs and minks in China. *Vet. Microbiol.* 140, 34–42.