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Supplementary Material

Development and Clinical Evaluation of a Direct Amplification Method to Diagnose Canine Parvovirus and Canine Distemper Viral Infections in Dogs without Nucleic Acid Extraction

Xuefeng Cao[1], Guangneng Peng[1], Xiaobin Gu[1], Changliang He[1], Guizhou Yue[2], Jun Shi[3,*] and Zhijun Zhong[1,*]

[1]College of Veterinary Medicine, Key Laboratory of Animal Disease and Human Health of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
[2]College of Science, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China
[3]State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

Xuefeng Cao and Guangneng Peng have contributed equally to this work.

* Corresponding author: zhongzhijun488@126.com
shijun215@tongji.edu.cn
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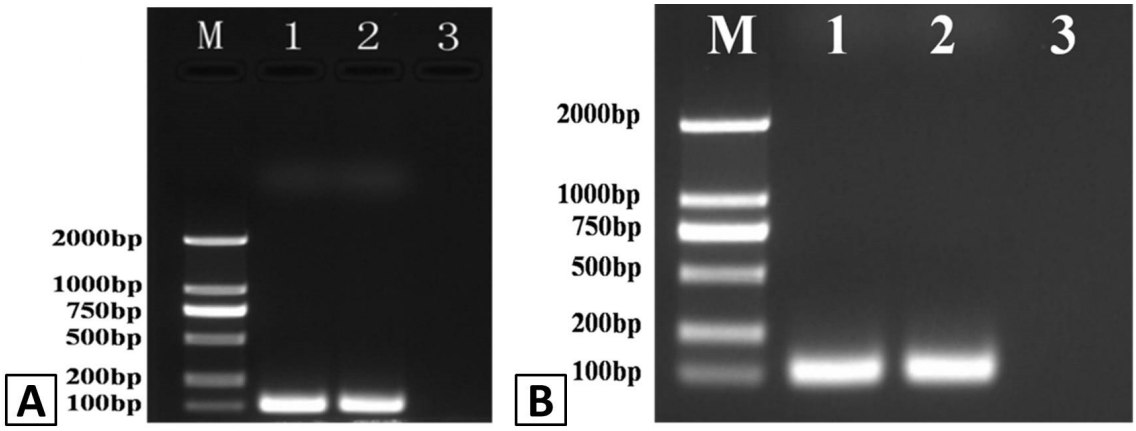
Supplementary Table I.- Intra-batch coefficient variant (CV) of the real-time Taq-Man PCR/RT-PCR for CPV and CDV.

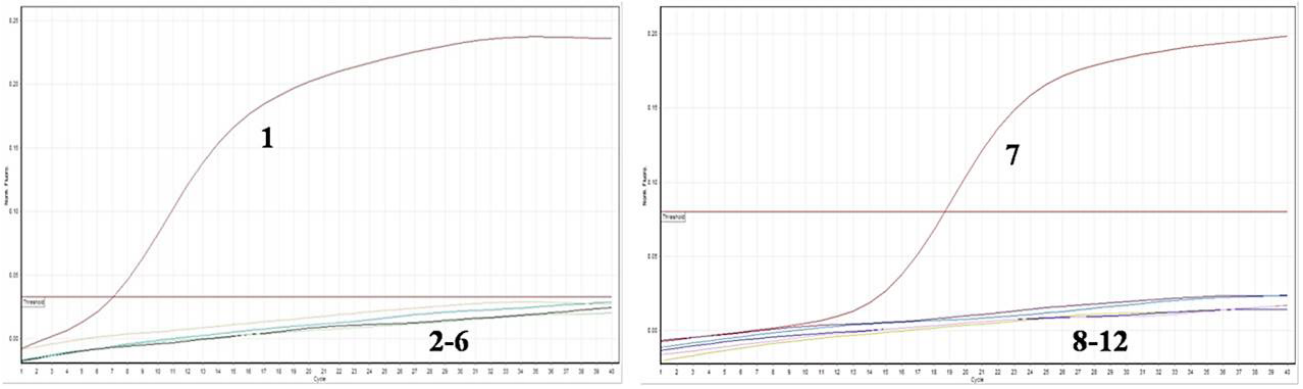
	Concentration of sample (copies·μL^{-1})	C_{t1}	C_{t2}	C_{t3}	Average of C_t	Variance	Intra-batch CV (%)
CPV	7.44×10^7	15.82	15.88	15.50	15.73	0.042	1.30
	7.44×10^5	22.75	22.85	22.79	22.79	0.003	0.22
	7.44×10^3	29.57	29.53	29.52	29.52	0.007	0.09
CDV	4.20×10^6	13.86	14.17	13.99	14.01	0.024	1.11
	4.20×10^4	20.04	19.98	20.27	20.10	0.023	0.76
	4.20×10^2	26.22	26.14	25.91	26.09	0.026	0.62

Supplementary Table II.- Inter-batch coefficient variant (CV) of the real-time Taq-Man PCR/RT-PCR for CPV and CDV.

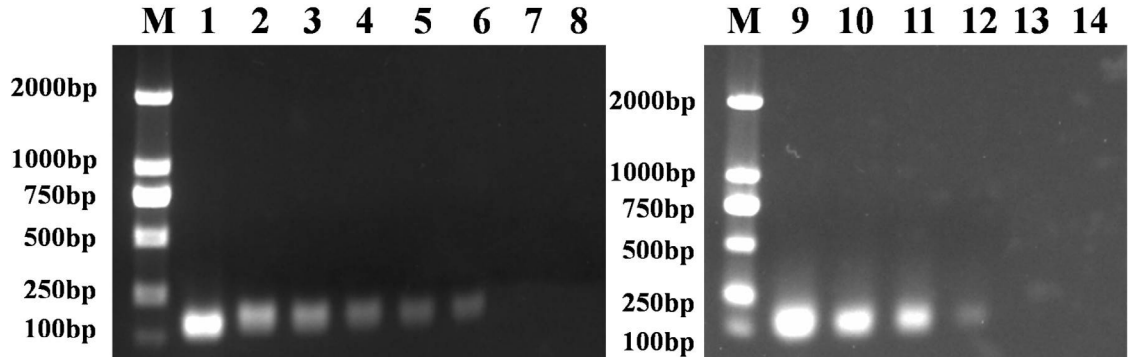
	Concentration of sample (copies·μL^{-1})	C_{t1}	C_{t2}	C_{t3}	Average of C_t	Variance	Inter-batch CV (%)
CPV	7.44×10^7	15.88	15.97	15.68	15.84	0.022	0.94
	7.44×10^5	22.86	23.20	23.03	23.03	0.029	0.73
	7.44×10^3	29.55	29.77	29.44	29.59	0.028	0.57
CDV	4.20×10^6	13.90	14.27	14.12	14.06	0.021	1.02
	4.20×10^4	20.13	20.21	20.43	20.26	0.024	0.77
	4.20×10^2	26.11	26.27	26.04	26.14	0.014	0.45



Supplementary Fig. S1. Detection of CPV and CDV plasmid standards by PCR and RT-PCR. PCR products (111 bp and 100 bp) were visualized by gel electrophoresis. A: Lane 1, Marker; Lanes 2 and 3, CPV plasmid; Lane 4, negative control; B: Lane 1, Marker; Lanes 2 and 3, CDV plasmid; Lane 4, negative control.



Supplementary Fig. S2. Real-time PCR specificity was validated using nucleic acids from CDV, CAV-1, CAV-2, CPIV, RABV and plasmid standards for CPV; all the samples tested negative except for the CPV plasmid standard. Real-time RT-PCR specificity was validated using nucleic acids from CPV, CAV-1, CAV-2, CPIV, RABV and the plasmid standard for CDV; all the samples tested negative except for the CDV plasmid standard. 1, CPV plasmid standard; 2–6, CDV, CAV-1, CAV-2, CPIV and RABV nucleic acids; 7, CDV plasmid standard, 8–12, CPV, CAV-1, CAV-2, CPIV and RABV nucleic acids.



Supplementary Fig. S3. Sensitivity of PCR and RT-PCR for detecting CPV and CDV plasmids. The serial 10-fold plasmid dilutions (CPV: 7.44×10^{8} copies·μL^{-1} to 7.44×10^{2} copies·μL^{-1}, CDV: 4.20×10^{7} copies·μL^{-1} to 4.20×10^{3} copies·μL^{-1}) were visualized by gel electrophoresis. A minimum of 7.44×10^{3} copies of CPV (A) and 4.20×10^{4} copies of CDV (B) were detected. M, 2K Marker; 1–7, equal dilutions of the CP.