基于 CRISPR/Cas12a 技术的猪流行性腹泻病毒检测方法的建立

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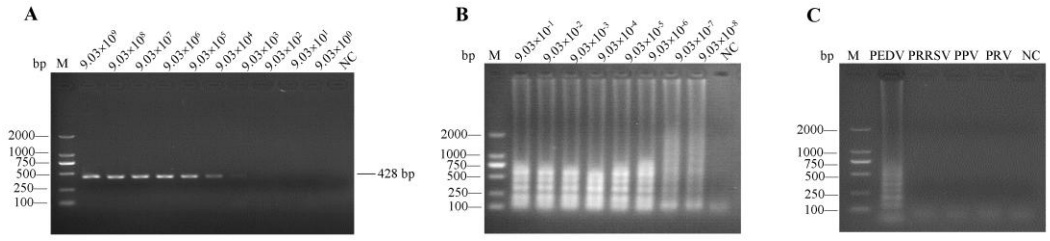
附表 1 引物和探针信息

Supplementary Table 1. Primer and probe information

类型	名称	序列（5’-3’）
Type	Name	Sequence (5’-3’)
	crRNA1	GGAAGCTAGCCCATGCGTCAATCTACAACAGTAGAAAT
	crRNA2	CTGGAAGCTAGCCCATGCGTATCTACAACAGTAGAAAT
	crRNA3	CATAAGGATGCTGAAAGCGAATCTACAACAGTAGAAAT
crRNA	crRNA4	CCACAACCGAATGCTATTGAATCTACAACAGTAGAAAT
	crRNA5	AGCGCGTCAGTTTCAGGATTATCTACAACAGTAGAAAT
	crRNA6	GTGGCCTTGGCGACTGTGACATCTACAACAGTAGAAAT
PCR	PEDV-M-F	GGATACTTTGGCCTCTTGTGT
	PEDV-M-R	CCAACCAGTGCCAGATGAAG
LAMP	PEDV-M-F3	TGTAGTGCTTCAGTATGGC
	PEDV-M-R3	CAACCGAATGCTATTGACAA
	PEDV-M-FIP	ACACAAGAGGCCAAAGTATCCAGTACTCTGTGTTCTTGTATGGT
	PEDV-M-BIP	CATGGGCTAGCTTCCAGGTCCACAGCATAAGAGTGATGC
	PEDV-M-LF	TAGAATAGCCATCTTGAC
	PEDV-M-LB	CTGGGTCTTTTTCGCTTTCAGCA
Probe	JOE Probe	5’JOE-GTATCCAGTGCG-3’BHQ1
	ROX Probe	5’ROX-GTATCCAGTGCG-3’BHQ2



A：PCR 产物琼脂糖凝胶电泳图；B：LAMP 产物琼脂糖凝胶电泳图；C： RT-LAMP/LAMP 产物琼脂糖凝胶电泳图。扩增模板浓度单位为 copies/μL；PEDV：猪流行性腹泻病毒，PRRSV：猪繁殖与呼吸综合征病毒，PRV：猪伪狂犬病毒，PPV：猪细小病毒

A: Agarose gel electrophoresis image of PCR products. B: Agarose gel electrophoresis image of LAMP products. C: Agarose gel electrophoresis of RT-LAMP/LAMP products. The concentration unit for the amplified template is copies/μL. PEDV: Porcine epidemic

diarrhea virus, PRRSV: Porcine reproductive and respiratory syndrome virus, PRV: Pseudorabies virus, PPV: Porcine parvovirus

附图 1 琼脂糖凝胶电泳图

Supplementary Fig. 1 Agarose gel electrophoresis images.

附表 2 PEDV 的多种检测方法

Supplementary Table 2 Various detection methods for PEDV.

方法 Methods	检测靶标 Detection targets	检测下限 Detection limits	特异性依赖于 Specificity (relies on)	参考文献 References
多重 RT-PCR	*M* 基因（质粒）	6.48 ×10^5 copies/μL	引物设计和技术本身	Niu et al., 2022[1]
TaqMan 探针多重 RT-PCR	*M* 基因（质粒）	100 copies/μL	引物和探针设计	Ren et al., 2024a[2]
TaqMan 探针多重 RT-PCR	*M* 基因（质粒）	1 × 10^2 copies/μL	引物和探针设计	Ren et al., 2024b[3]
四重 RT-qPCR	*M* 基因（质粒）	100 copies/μL	设计的引物和探针	Ye et al., 2025[4]
多重 RT-qPCR	*ORF1a* 基因（病毒及质粒）	5 copies/μL	设计的引物和探针	Baek et al., 2024[5]
多重 TaqMan 探针 RT-qPCR	*M* 基因（质粒）	2.18 × 10^2 copies/μL	设计的探针	Xin et al., 2024[6]
TaqMan 多重 RT-qPCR	*M* 基因（质粒）	5 copies/μL	TaqMan 多重 qPCR 技术的设计和优化	Song et al., 2024[7]
RT-RAA	*N* 基因	10 拷贝/反应	设计的引物和探针	Wu et al., 2022[8]
RT-LAMP	*N* 基因	0.0001 ng/μL	引物设计和 LAMP 技术本身	Li et al., 2022[9]
RT-LAMP	*M* 和 *S* 基因（质粒和病毒）	100 拷贝/反应	引物设计和 LAMP 技术本身	Liu et al., 2025[10]
纸基微流控芯片结合 LAMP	*ORF1a/b* 基因（病毒及质粒）	4.82 × 10^2 copies/μL	LAMP 技术本身	Li et al., 2025[11]
双通道芯片数字 PCR（cdPCR）	*M* 基因（质粒）	0.99 ± 0.07 copies/μL	引物探针设计和 cdPCR 技术本身	Zhang et al., 2025[12]
多重数字 PCR	*N* 基因（质粒）	3.00 拷贝/反应	引物设计和数字 PCR 技术本身	Han et al., 2025[13]
双抗体夹心定量 ELISA DAS-qELISA	N 蛋白	0.05ng/mL 重组 N 蛋白；103.02 TCID50/mL	抗体的选择和 ELISA 技术本身	Han et al., 2024[14]
双抗体夹心 ELISA DAS-ELISA	N 蛋白	0.05ng/mL 重组 N 蛋白；102.59 TCID50	抗体的高亲和力和表位的保守性	Zhao et al., 2025[15]
间接 ELISA	S 蛋白	1:4800	抗体的高亲和力和表位的保守性	Ma et al., 2025[16]
纳米抗体基础阻断 ELISA	S 蛋白	1:32 阳性猪血清	纳米抗体的选择和 ELISA 技术本身	Yang et al., 2025[17]
RT-LAMP-CRISPR/Cas12a	*ORF3* 基因（质粒）	1 copy/μL	Cas12a 系统的高特异性识别	Liu et al., 2022[18]
RT-RAA-CRISPR/Cas12a	*S* 基因	100 拷贝/反应	crRNA 设计和 Cas12a 系统的高特异性识别	Qian et al., 2022[19]
RT-ERA-	*ORF3* 基因（质粒和病	2 个病毒基因拷贝	crRNA 序列特异性	Yang et al.,

CRISPR/Cas12a	毒）			2021[20]
RAA-CRISPR/Cas13a	*ORF3* 基因（质粒）	10 copies/μL	crRNA 序列特异性和 Cas13a 系统的高特异性识别	Yin et al., 2022[21]
RPA-CRISPR/Cas13a	*M* 基因（质粒和病毒）	10 copies/μL	crRNA 序列特异性和 Cas13a 系统的高特异性识别	Wang et al., 2024[22]

参考文献

[1] Niu JW, Li JH, Guan JL, et al. Development of a multiplex RT-PCR method for the detection of four porcine enteric coronaviruses [J]. Front Vet Sci, 2022, 9: 1033864.

[2] Ren J, Li FJ, Yu X, et al. Development of a TaqMan-based multiplex real-time PCR for simultaneous detection of porcine epidemic diarrhea virus, *Brachyspira hyodysenteriae*, and *Lawsonia intracellularis* [J]. Front Vet Sci, 2024, 11: 1450066.

[3] Ren J, Zu CC, Li Y, et al. Establishment and application of a TaqMan-based multiplex real-time PCR for simultaneous detection of three porcine diarrhea viruses [J]. Front Microbiol, 2024, 15: 1380849.

[4] Ye CW, Xu JR, Fan SS, et al. Establishment and application of a quadruple RT-qPCR method for simultaneous detection of porcine enteric coronaviruses [J]. Front Vet Sci, 2025, 12: 1714780.

[5] Baek JH, Lee YM, Vu ND, et al. A multiplex real-time RT-qPCR assay for simultaneous detection of porcine epidemic diarrhea virus, porcine deltacoronavirus, and swine acute diarrhea syndrome coronavirus [J]. Arch Virol, 2024, 169(4): 82.

[6] Xin ZH, Li SH, Lu X, et al. Development and clinical application of a molecular assay for four common porcine enteroviruses [J]. Vet Sci, 2024, 11(7): 305.

[7] Song WB, Feng YX, Zhang JL, et al. Development of a multiplex reverse transcription-quantitative PCR (qPCR) method for detecting common causative agents of swine viral diarrhea in China [J]. Porc Health Manag, 2024, 10: 12.

[8] Wu XH, Liu YJ, Gao LG, et al. Development and application of a reverse-transcription recombinase-aided amplification assay for porcine epidemic diarrhea virus [J]. Viruses, 2022, 14(3): 591.

[9] Li CH, Liang JL, Yang D, et al. Visual and rapid detection of porcine epidemic diarrhea virus (PEDV) using reverse transcription loop-mediated isothermal amplification method [J]. Animals, 2022, 12(19): 2712.

[10] Liu Z, Li LL, Fang MT, et al. Distinguished loop-mediated isothermal amplification assay to detect porcine epidemic diarrhea virus genotypes I and II [J]. Vet Sci, 2025, 12(5): 399.

[11] Li RF, Tian XQ, Cao WY, et al. Development of a paper-based microfluidic chip for point-of-care detection of PEDV [J]. Vet Sci, 2025, 12(5): 427.

[12] Zhang Y, Dong FT, Zhang YH, et al. Establishment and application of a dual chip digital PCR assay for detection of PDCoV and PEDV [J]. Front Vet Sci, 2025, 12: 1655079.

[13] Han X, Chen KX, Qiu H, et al. Establishment of multiplex digital PCR assay for detection of four porcine enteric coronaviruses [J]. Int J Mol Sci, 2025, 26(17): 8731.

[14] Han WG, Ma ZQ, Li ZW, et al. A novel double antibody sandwich quantitative ELISA for detecting porcine epidemic diarrhea virus infection [J]. Appl Microbiol Biotechnol, 2024, 108: 482.

[15] Zhao Y, Wei F, Liu ZY, et al. Development of double-antibody sandwich ELISA for detecting PEDV N protein [J]. J Microbiol Meth, 2025, 236: 107186.

[16] Ma GM, Hu GL, Ren GC, et al. Development of an indirect ELISA based on the PEDV S protein and evaluation of its correlation with neutralizing antibody and immune protection [J]. Transbound Emerg Dis, 2025, 2025: 6185969.

[17] Yang XQ, Yang JY, Su XN, et al. Nanobody-based epitope mapping and establishment of a blocking ELISA for S protein of porcine epidemic diarrhea virus [J]. Int J Biol Macromol, 2025, 333: 148110.

[18] Liu JJ, Tao DG, Chen XQ, et al. Detection of four porcine enteric coronaviruses using CRISPR-Cas12a combined with multiplex reverse transcriptase loop-mediated isothermal amplification assay [J]. Viruses, 2022, 14(4): 833.

[19] Qian BX, Liao K, Zeng DX, et al. Clustered regularly interspaced short palindromic repeat/Cas12a mediated multiplexable and portable detection platform for GII genotype porcine epidemic diarrhoea virus rapid diagnosis [J]. Front Microbiol, 2022, 13: 920801.

[20] Yang KK, Liang YQ, Li YN, et al. Reverse transcription–enzymatic recombinase amplification coupled with CRISPR-Cas12a for rapid detection and differentiation of PEDV wild-type strains and attenuated vaccine strains [J]. Anal Bioanal Chem, 2021, 413(30): 7521-7529.

[21] Yin DD, Yin L, Guo H, et al. Visual detection and differentiation of porcine epidemic diarrhea virus wild–type strains and attenuated vaccine strains using CRISPR/Cas13a-based lateral flow strip [J]. Front Cell Infect Microbiol, 2022, 12: 976137.

[22] Wang YY, He DL, et al. Field-deployable porcine epidemic diarrhea virus diagnostics utilizing CRISPR-Cas13a [J]. Virulence, 2024, 15: 2429022.