Supplementary Materials

# Triplex-loop-mediated isothermal amplification combined with a lateral flow immunoassay for the simultaneous detection of three pathogens of porcine viral diarrhea syndrome in swine

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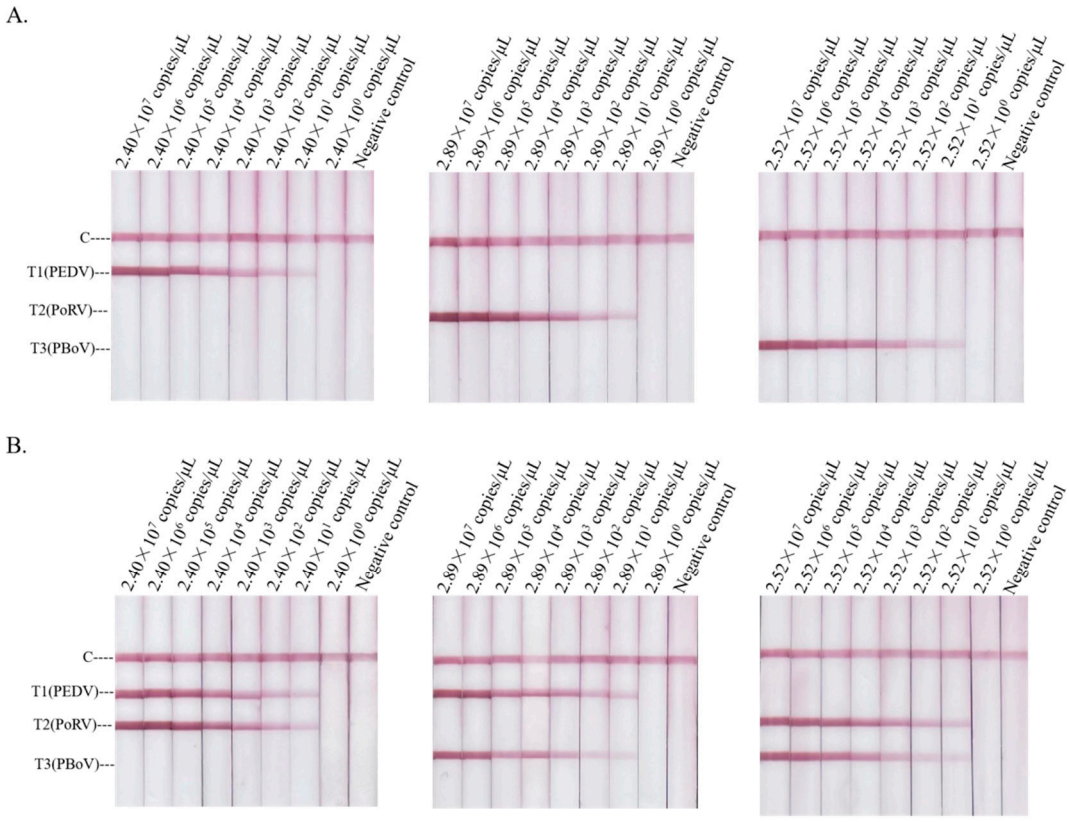


**Figure S1.** The sensitivity of single and duplex LAMP–LFD assay. (**A**) single LAMP–LFD sensitivity testing, (**B**) duplex LAMP–LFD sensitivity testing.

**Table S1.** Sequences of qPCR primers and probes for detecting PEDV, PoRV, and PBoV

| Target gene | Primer name | Sequence (5′–3′) | Fragment length |
|---|---|---|---|
| PEDV-*gp6* NC_003436.1 | Forward | AATTCTCTCAACAGCTCCCCAG | 104 bp |
| | Reverse | ATTGTTGCCATTGCCACGAC | |
| | Probe | FAM-TGCTTCACGTGCAAATTCGCGTAGCA-BHQ1 | |
| PBoV-*vp1* NC_023673.1 | Forward | AGCAACCAAGACACAACTGC | 89 bp |
| | Reverse | GTCCAAGGAAAGGCGTGTTC | |
| | Probe | Cy5-TGACGGCGGGTCTCCACGTCCTTTGCGA-BHQ2 | |
| PoRV-*vp6* KC113249.1 | Forward | GGGTTTGATTATTCGTGCGC | 128 bp |
| | Reverse | AAGCCGAATCTTTCAGCGTC | |
| | Probe | HEX-TGCCGTTAAGACGTGCTCTCACAACA-BHQ1 | |

**Table S2.** Informations of 125 animal feces samples.

| Information of 125 animal feces samples | | | | | | |
|---|---|---|---|---|---|---|
| Sample Number | Ages | Gender | Location | Time (Date/Month/Year) | Collection form | Test results |
| sample 1 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 2 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 3 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 4 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 5 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 6 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 7 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 8 | 3 months | female | farm | 5/11/2022 | feces | + |
| sample 9 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 10 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 11 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 12 | 3 months | female | farm | 5/11/2022 | feces | + |
| sample 13 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 14 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 15 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 16 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 17 | 3 months | female | farm | 5/11/2022 | feces | + |
| sample 18 | 3 months | male | farm | 5/11/2022 | feces | + |
| sample 19 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 20 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 21 | 3 months | female | farm | 5/11/2022 | feces | − |

| | | | | | | |
|---|---|---|---|---|---|---|
| sample 22 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 23 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 24 | 3 months | female | farm | 5/11/2022 | feces | − |
| sample 25 | 3 months | male | farm | 5/11/2022 | feces | − |
| sample 26 | 4 months | male | farm | 5/11/2022 | feces | + |
| sample 27 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 28 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 29 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 30 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 31 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 32 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 33 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 34 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 35 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 36 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 37 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 38 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 39 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 40 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 41 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 42 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 43 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 44 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 45 | 4 months | female | farm | 5/11/2022 | feces | + |
| sample 46 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 47 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 48 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 49 | 4 months | male | farm | 5/11/2022 | feces | − |
| sample 50 | 4 months | female | farm | 5/11/2022 | feces | − |
| sample 51 | 5 months | male | farm | 5/11/2022 | feces | − |
| sample 52 | 5 months | female | farm | 5/11/2022 | feces | − |
| sample 53 | 5 months | male | farm | 5/11/2022 | feces | − |
| sample 54 | 5 months | female | farm | 5/11/2022 | feces | − |
| sample 55 | 5 months | female | farm | 5/11/2022 | feces | − |
| sample 56 | 6 months | female | farm | 5/11/2022 | feces | − |
| sample 57 | 6 months | male | farm | 5/11/2022 | feces | − |
| sample 58 | 6 months | male | farm | 5/11/2022 | feces | − |
| sample 59 | 6 months | male | farm | 5/11/2022 | feces | − |
| sample 60 | 6 months | female | farm | 5/11/2022 | feces | − |
| sample 61 | 7 months | female | farm | 5/11/2022 | feces | − |
| sample 62 | 7 months | female | farm | 5/11/2022 | feces | − |

| | | | | | | |
|---|---|---|---|---|---|---|
| sample 63 | 7 months | female | farm | 5/11/2022 | feces | − |
| sample 64 | 7 months | male | farm | 5/11/2022 | feces | − |
| sample 65 | 7 months | female | farm | 5/11/2022 | feces | − |
| sample 66 | 8 months | male | farm | 5/11/2022 | feces | − |
| sample 67 | 8 months | male | farm | 5/11/2022 | feces | − |
| sample 68 | 8 months | female | farm | 5/11/2022 | feces | − |
| sample 69 | 8 months | female | farm | 5/11/2022 | feces | − |
| sample 70 | 8 months | female | farm | 5/11/2022 | feces | − |
| sample 71 | 9 months | female | farm | 5/11/2022 | feces | − |
| sample 72 | 9 months | female | farm | 5/11/2022 | feces | − |
| sample 73 | 9 months | female | farm | 5/11/2022 | feces | − |
| sample 74 | 9 months | male | farm | 5/11/2022 | feces | − |
| sample 75 | 9 months | female | farm | 5/11/2022 | feces | − |
| sample 76 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 77 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 78 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 79 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 80 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 81 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 82 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 83 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 84 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 85 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 86 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 87 | 3 months | female | farm | 3/12/2022 | feces | + |
| sample 88 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 89 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 90 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 91 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 92 | 3 months | male | farm | 3/12/2022 | feces | + |
| sample 93 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 94 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 95 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 96 | 3 months | male | farm | 3/12/2022 | feces | − |
| sample 97 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 98 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 99 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 100 | 3 months | female | farm | 3/12/2022 | feces | − |
| sample 101 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 102 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 103 | 4 months | female | farm | 3/12/2022 | feces | − |

| | | | | | | |
|---|---|---|---|---|---|---|
| sample 104 | 4 months | female | farm | 3/12/2022 | feces | + |
| sample 105 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 106 | 4 months | male | farm | 3/12/2022 | feces | − |
| sample 107 | 4 months | female | farm | 3/12/2022 | feces | + |
| sample 108 | 4 months | male | farm | 3/12/2022 | feces | − |
| sample 109 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 110 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 111 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 112 | 4 months | male | farm | 3/12/2022 | feces | − |
| sample 113 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 114 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 115 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 116 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 117 | 4 months | male | farm | 3/12/2022 | feces | − |
| sample 118 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 119 | 4 months | male | farm | 3/12/2022 | feces | − |
| sample 120 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 121 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 122 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 123 | 4 months | female | farm | 3/12/2022 | feces | + |
| sample 124 | 4 months | female | farm | 3/12/2022 | feces | − |
| sample 125 | 4 months | female | farm | 3/12/2022 | feces | − |

**Table S3.** Comparison of molecular methods for detection of porcine diarrhea virus

| Techniques | Detection object | Reaction time | LOD | Reference |
|---|---|---|---|---|
| duplex nanoPCR | PRV、PBoV | 80-90 min | PRV：$6.44\times10^{0}$ copies/μL<br>PBoV：$9.51\times10^{1}$ copies/μL | [1] |
| multiplex conventional PCR | PBoV G1、PBoV G2 and PBoV G3 | About 70 min | PBoV G1：$1.0\times10^{3}$ copies/μL; PBoV G2：$4.5\times10^{3}$ copies/μL<br>PBoV G3：$3.8\times10^{3}$ copies/μL | [2] |
| DPO-based multiplex real-time RT-PCR | PEDV，TGEV，PoRV，PDCoV | 75-80 min | PEDV:$8.63\times10^{2}$ copies/μL; TGEV:$1.92\times10^{2}$ copies/μL;<br>PoRV:$1.74\times10^{2}$ copies/μL; PDCoV:$1.76\times10^{2}$ copies/μL | [3] |
| Duplex real-time PCR | PEDV and PBoV3/4/5 | 45-50 min | 10 copies/μL | [4] |
| Droplet digital PCR | PEDV | 80 min | 0.26 copies/μL | [5] |
| Multiplex RT-PCR | PKV, TGEV, PEDV, PDCoV, PRV-A and PSaV | 110 min | 10 ng viral cDNAs of PKV, TGEV, PDCoV, PRV-A, PSaV;<br>1 ng cDNA of PEDV | [6] |
| Taq Man-probe-based multiplex real-time PCR | PEDV, PDCoV, PToV, and SADS-CoV | About 40 min | $1\times10^{2}$ copies/μL for each pathogen | [7] |
| multiplex RT-PCR | TGEV, PEDV, PRV-A, PDCoV, and SADS-CoV | About 50 min | $10^{3}$-$10^{4}$ copies/μL plasmid of each virus | [8] |
| Quadruple qRT-PCR | PEDV, TGEV, PDCoV, and SADS-CoV | About 35 min | 121 copies/μL for each virus | [9] |
| RT-RPA | PEDV and PDCoV | 45 min | $1\times10^{2}$ copies/μL | [10] |
| RT-LAMP-VF | PEDV | 40 min | 10 pg RNA | [11] |

| | | | | |
|---|---|---|---|---|
| RtF-RT-LAMP | PEDV strains (NK94P6 and Fukuoka-1 Tr(−)) | 40 min | $2.8 \times 10^1$ TCID50/ml and $2 \times 10^0$ TCID50/ml | [12] |
| Visual LAMP Real-Time LAMP | ASFV | 20 min | 0.1 fg | [13] |
| One-step Visual LAMP with Neutral Red | ASFV | 45 min | 10 copies/reaction | [14] |
| RT-LAMP with agarose gel electrophoresis | PEDV | 60-65 min | 0.0001ng/μL | [15] |
| Multiplex LAMP-Cas12a Assay | PEDV, TGEV, PDCoV, and SADS-CoV | 20 min | $1 \times 10^0$ copy/μL | [16] |
| duplex LAMP-LFD | PEDV、PCV 2 | 20 min | PEDV：0.1 ng/μL; PCV 2：0.246 ng/μL | [17] |
| Microfluidic-RT-LAMP chip | PEDV、PDCoV、SADS-CoV | 40 min | PEDV：$10^1$ copies/μL，；PDCoV：$10^2$ copies/μL；SADS-CoV：$10^2$ copies/μL | [18] |
| RT-LAMP | PEDV | 40 min | 50 RNA copies per reaction | [19] |
| real-time RT-LAMP | SADS-CoV | 60 min | $1.0 \times 10^1$ copies/μL | [20] |

## Reference


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