

Supplementary Materials

**Combining a Lateral Flow Immunoassay with Triplex Loop-Mediated
Isothermal Amplification for the Concurrent Identification of Three Bovine
Diarrhea Syndrome Viruses**

Table S1. Sequences of LAMP primers for detecting BVDV, BRV, and BPV.

Virus	Primer	Sequence (5' to 3')
BVDV-5'UTR M31182.1	F3	GCCATGCCCTTAGTAGGACT
	B3	AGCACCTATCAGGCTGTA
	FIP	CGAACCACTGACGACTACCCTGTTTTGGTAGCAACA GTGGTGAGTT
	BIP	AAGGTCTCGAGATGCCACGTGTTTTCTGCTTTTACCT GGGCGAC
	LF	5'-Biotin-GGGCTTAAGCCATCCAACG
	LB	5'-ROX-GGCATGCCCAAAGCACA
	BRV-VP6 JN790188.1	F3
B3		CGCTGCAAGTTATCCTCC
FIP		ACAGATGTCACATTTGCTAGCATTTTTTCTGCAGTTT GTGAATCAGTG
BIP		AGACAAGAATACGCGATACCAGTAGTTTTTTGATGG TGAATAGTTAGTGATC
LF		5'-Biotin-GTTTCTCTAGCGTCGGCAAG
LB		5'-FAM-CCAGTTTTTCCCCCAGGTATG
BPV-VP2 JN191349.1		F3
	B3	GTCTTGCTGCGTTTTCCAAT
	FIP	ATCCTACTCGCACGGCTCCCTTTTGCCTGGTGACC ATGGTAAC
	BIP	CTACGGGGGACAGCAACCAGTTTTGTCTTCTGCTG CCGATG
	LF	5'-Biotin-GGAGACGTCTGCTCCGTTA
	LB	5'-Digoxin-TGCTGAGACTGAGATACTCCG

Table S2. Sequences of qPCR primers for detecting BVDV, BRV, and BPV.

Virus	Primer	Sequence (5' to 3')
BVDV-5'UTR M31182.1	Forward Primer	AGGACTAGCATAATGAGG
	Reverse Primer	TCGAGACCTTTATTCCAA
	Probe	5'-FAM-CGAACCACTGACGACTACCCT-BQH1-3'
BRV-VP6 JN790188.1	Forward Primer	GACTCACGCTTAGAATTG
	Reverse Primer	CGCGTATTCTTGTCTAAC
	Probe	5'-FAM-ATCAGTGCTTGCCGACGCTA-BQH1-3'
BPV-VP2 JN191349.1	Forward Primer	CTGAGACTGAGATACTCC
	Reverse Primer	CATCCACCATTCTTTGTAG
	Probe	5'-FAM-CATCGGCAGCAGAAGGACAAC-BQH1-3'

Table S3. The informations of 156 animal sample.

Sample Number	Test results
Sample 1	-
Sample 2	-
Sample 3	-
Sample 4	-
Sample 5	-
Sample 6	-
Sample 7	-
Sample 8	-
Sample 9	-
Sample 10	-
Sample 11	+
Sample 12	-
Sample 13	-
Sample 14	-
Sample 15	-
Sample 16	-
Sample 17	-
Sample 18	-
Sample 19	+
Sample 20	-
Sample 21	-
Sample 22	-
Sample 23	-
Sample 24	-
Sample 25	+
Sample 26	-
Sample 27	-
Sample 28	+
Sample 29	+

Sample 30	-
Sample 31	-
Sample 32	-
Sample 33	-
Sample 34	-
Sample 35	-
Sample 36	-
Sample 37	-
Sample 38	+
Sample 39	-
Sample 40	-
Sample 41	-
Sample 42	-
Sample 43	-
Sample 44	-
Sample 45	-
Sample 46	+
Sample 47	-
Sample 48	+
Sample 49	-
Sample 50	-
Sample 51	-
Sample 52	-
Sample 53	-
Sample 54	-
Sample 55	-
Sample 56	-
Sample 57	-
Sample 58	-
Sample 59	-
Sample 60	-
Sample 61	+
Sample 62	-
Sample 63	-
Sample 64	-
Sample 65	-
Sample 66	-
Sample 67	-
Sample 68	-
Sample 69	-
Sample 70	-
Sample 71	-
Sample 72	-
Sample 73	+

Sample 74	+
Sample 75	-
Sample 76	-
Sample 77	-
Sample 78	-
Sample 79	-
Sample 80	-
Sample 81	-
Sample 82	-
Sample 83	-
Sample 84	+
Sample 85	-
Sample 86	-
Sample 87	-
Sample 88	-
Sample 89	-
Sample 90	-
Sample 91	-
Sample 92	-
Sample 93	-
Sample 94	-
Sample 95	-
Sample 96	-
Sample 97	-
Sample 98	-
Sample 99	-
Sample 100	+
Sample 101	-
Sample 102	-
Sample 103	-
Sample 104	-
Sample 105	-
Sample 106	+
Sample 107	-
Sample 108	-
Sample 109	-
Sample 110	-
Sample 111	-
Sample 112	-
Sample 113	-
Sample 114	-
Sample 115	-
Sample 116	-
Sample 117	+

Sample 118	+
Sample 119	-
Sample 120	-
Sample 121	-
Sample 122	-
Sample 123	-
Sample 124	-
Sample 125	-
Sample 126	-
Sample 127	-
Sample 128	-
Sample 129	+
Sample 130	+
Sample 131	-
Sample 132	-
Sample 133	-
Sample 134	-
Sample 135	-
Sample 136	-
Sample 137	-
Sample 138	-
Sample 139	-
Sample 140	-
Sample 141	-
Sample 142	-
Sample 143	-
Sample 144	-
Sample 145	-
Sample 146	-
Sample 147	-
Sample 148	-
Sample 149	+
Sample 150	-
Sample 151	-
Sample 152	-
Sample 153	-
Sample 154	-
Sample 155	-
Sample 156	-

Table S4. Comparison of molecular methods for detection of porcine diarrhea virus.

Techniques	Detection object	Reaction time	LOD	Reference
CRISPR-LwCas13a	BVDV	30 min	10 ³ pM	[1]
HCR-AuNP colorimetric assay	BVDV	120 min	0.008 TCID ₅₀	[2]
DPO-nanoPCR	BRV, BPV, BVDV	75 min	BRV: 9.40×10 ² copies/μL; BPV: 5.14×10 ³ copies/μL; BVDV: 4.09×10 ¹ copies/μL	[3]
multiplex real-time fluorescent quantitative PCR	BVDV, BRV, BCoV	50 min	BVDV: 1.19×10 ² copies/μL; BRV: 3.89×10 ¹ copies/μL; BCoV: 3.74×10 ¹ copies/μL	[4]
Real-time RT-PCR assay	BVDV	75 min	5.2 RNA molecules per reaction	[5]
multiplex real-time fluorescence-based quantitative PCR assay	BToV, BEV, BNoV, BCoV, BRV, BVDV	35 min	BNoV: 1.91 copies/μL; BEV: 96.0 copies/μL; BToV: 12.8 copies/μL; BRV: 16.4 copies/μL; BCoV: 18.2 copies/μL; BVDV: 65.3 copies/μL	[6]
multiplex droplet digital PCR	BEV, BCoV, BRV	83 min	BEV: 2.7 copies/μL; BCoV: 1 copies/μL; BRV: 2.4 copies/μL	[7]
TaqMan real-time PCR	BVDV, BoHV-1, BPIV3, BRSV, IDV	40 min	100 copies/μL for each virus	[8]
cross-linking and non-crosslinking probe-gold nanoparticle hybridization assays	BVDV	40 min	CL: 6.83 ng/reaction; NCL: 44.36 ng/reaction	[9]
RT-LAMP	BVDV	40 min	About 70 PFU/mL	[10]
RT-RPA combined with LFD	BVDV, BPIV3	25 min	BVDV: 50 RNA molecules per reaction; BPIV3: 34 RNA molecules per reaction	[11]
RPA	BVDV, BoHV-1	30 min	BVDV, BoHV-1: 1×10 ¹ copies/μL	[12]
Triple LAMP-LFD	BVDV, BRV, BPV	30 min	BVDV: 2.62×10 ¹ copies/μL; BRV: 2.43×10 ¹ copies/μL; BPV: 2.50×10 ¹ copies/μL	This Study

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