

## Supporting information

### Two Stage, Nested Isothermal Amplification in a Single Tube

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**Table S-1:** Sequences and concentrations of ILTV and IBV LAMP primers

Virus (Target Gene)	Primer Name	Primers sequences (5' to 3')	Concentration (mM)
ILTV (Polymerase protein coding gene) <b>(Unpublished data)</b>	F3	GTGGACGACATGATATCGG	0.2
	B3	TTCAGGGTCAGCAAGTATTG	0.2
	FIP (F1c +F2)	CGCCACATTCCCTGTACTCTTTTTCTGGTTCTACGTGATTAAGG	1.6
	BIP (B1c +B2)	TGCTATCTGAATGCGCCGTTTACAAGGGTGCTAGGAACA	1.6
	Loop F	GTA <del>C</del> TTGTCCTCGCCGTC	0.8
	Loop B	AGATGTGGGCAGTGGACTA	0.8
IBV (Nucleocapsid protein coding gene) <sup>1</sup>	F3	CCACCTGGTTATAAGGTTGAYC	0.2
	B3	CTACACCATCGACRCACTG	0.2
	FIP (F1c +F2)	CATGGCTGCTAGGGACCAGATGACAAGATGAATGAGGAR <del>G</del> GG	1.6
	BIP (B1c +B2)	TGACGCCCAA <del>A</del> CTTCAACCAACGTGACACCACAGTAGTA	1.6
	Loop F	GAGCATTGCTGTAACACGC	0.8
	Loop B	TGGGCTGCACTTGA <del>A</del> ATTG	0.8

Y: C/T ; R: A/G

**Table S-2** : Summary of LAMP reaction and RPA reaction buffers compositions

<b>LAMP Reaction buffer <sup>2</sup></b>		<b>RPA reaction buffer <sup>3</sup></b>	
Ingredients	Typical Concentration	Ingredients	Typical Concentration
Tris-HCl (pH 8.8)	20 mM	Tris (pH 7.9)	50 mM
Potassium chloride (KCl)	10 mM	Potassium acetate	100 mM
Betaine	0.8 M	Phosphocreatine	50 mM
Ammonium sulphate (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10 mM	Creatine kinase	100 ng/ μL
Magnesium sulphate (MgSO <sub>4</sub> )	8 mM	Magnesium acetate	14 mM
Tween 20	0.1%	polyethylene glycol (PEG)	5%
		Dithiothreitol	2 mM
		Adenosine triphosphate (ATP)	3 mM

**Table S-3:** Data used for Probit analysis to calculate limit of detection for Closed-Tube ILTV-Penn-RAMP and IBV-RT-Penn-RAMP

<b>Genome Concentration (copies/ reaction)</b>	<b>Closed-Tube ILTV-Penn-RAMP (Number positive/tested)</b>	<b>Closed-Tube IBV-RT-Penn-RAMP (Number positive/tested)</b>
100	8/8	8/8
80	8/8	8/8
40	8/8	7/8
20	8/8	2/8
15	1/8	1/8
10	0/8	0/8
5	0/8	0/8
0	0/8	0/8

**Table S-4** : Results of clinical performance of Closed Tube-Penn RAMP, LAMP and qPCR assays for ILTV and IBV detection:

Assay	Virus	Clinical Sample No.									
		1	2	3	4	5	6	7	8	9	10
qPCR (Threshold Cycles)	ILTV	22.29	23.64	24.36	30.20	-ve	-ve	-ve	-ve	-ve	-ve
	IBV	-ve	-ve	-ve	-ve	23.63	26.34	32.15	34.3	-ve	-ve
LAMP (Threshold time)	ILTV	15.38	16.51	19.65	24.72	-ve	-ve	-ve	-ve	-ve	-ve
	IBV	-ve	-ve	-ve	-ve	22.71	25.83	28.21	28.74	-ve	-ve
Closed Tube-Penn-RAMP (Threshold time)	ILTV	4.32	9.85	10.84	15.43	-ve	-ve	-ve	-ve	-ve	-ve
	IBV	-ve	-ve	-ve	-ve	10.51	12.61	14.44	15.23	-ve	-ve

**References:**

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2. N. Kuboki, T. Sakurai, F. Di Cello, D.J. Grab, H. Suzuki, C.Sugimoto, I. Igarashi, *J. Clin. Microbiol.* 2003, **41**, 5517-5524.
3. J. Li, J. Macdonald, F. Von Stetten, *Analyst* **2019**, 144, 31-67.