Development of plasmid reference material to improve the accuracy of quantitative detection of *Bovine Parvovirus*

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Supplementary Table

Table S1. Nanodrop detection results of BPV plasmid RM

Gene	A260/A280	A260/A230
BPV-VP1	1.92	2.01

Table S2. Primer and probe sequences of BPV-VP1 dPCR

Assay	Type	Sequence（5'-3'）	length（bp）
Assay A	Forward	CAAGCACATCCAATCAAC	198
	Reverse	CCACAATGTTCTCGCTAA	
	Probe	FAM-CGTCCATCCGCCAGTTGAGTA-BHQ1	
Assay B	Forward	CCAGTACCAGGAAACGGAGAC	118
	Reverse	GCATGTATTCCGGTCTCCAA	
	Probe	HEX-CCTCAACATCTACGTCACCGGACAA-BHQ1	

Table S3. The partition volumes of different dPCR platforms

Platform	Partition volumes	Partition number	Individual reaction volume(μL)
A[1]	0.8 nL	Approx.23000	22
B[2]	0.85 nL	Approx.20000	20
C[3]	0.82 nL	Approx.26000	40

Table S4. Detection results of dPCR positivity in low concentration samples

Concentration (copies/reaction)	Mean (copies/reaction)	Positive/Total
4	3.76	18/20
6	6.05	20/20
8	8.26	20/20

Table S5. DPCR detection results for low concentration samples (6 copies/reaction)

Repetitions	Concentration (copies/reaction)	Repetitions	concentration (copies/reaction)
1	1.76	11	7.48
2	3.74	12	11
3	9.24	13	3.74
4	7.26	14	1.76

5	5.72	15	5.5
6	11.44	16	3.74
7	5.5	17	3.74
8	3.74	18	3.52
9	9.46	19	3.96
10	9.24	20	9.46
Mean		6.05	
standard deviation (s)		3.04	
LOD		9	
LOQ		30	

Table S6. Homogeneity assessment of BPV plasmid RM

Unit	Concentration (copies/μl)	
	Rep1	Rep2
1	2.24×10^6	2.09×10^6
2	2.15×10^6	2.09×10^6
3	2.06×10^6	2.22×10^6
4	2.11×10^6	2.18×10^6
5	2.20×10^6	2.32×10^6
6	2.25×10^6	2.44×10^6
7	2.16×10^6	2.26×10^6
8	2.16×10^6	2.16×10^6
9	2.20×10^6	2.07×10^6
10	2.27×10^6	2.40×10^6
11	2.22×10^6	2.18×10^6
Mean	2.20×10^6	
$M_{between}$	1.27×10^{10}	
M_{within}	6.87×10^9	
F	1.85	
$F_{0.05(10,11)}$	2.85	
s_{bb}	5.39×10^4	
s_r	8.29×10^4	
u'_{bb}	3.83×10^4	
$u_{hom\ rel}$	1.74%	

Table S7. The long-term stability assessment of BPV plasmid RM

Events		Concentration (copies/μL)
Time (month)	0	2.21×10^6
	1	2.27×10^6
	2	2.25×10^6
	3	2.12×10^6

6	2.39×10^6
9	2.19×10^6
12	2.39×10^6
Mean	2.26×10^6
$\lvert\beta_1\rvert$	1.09×10^4
$s(\beta_1)$	9.04×10^3
$t_{0.95,3}\times s(\beta_1)$	2.32×10^4
conclusion	$\lvert\beta_1\rvert < t_{0.95,3}\times s(\beta_1)$
u_{lts}	1.09×10^5
$u_{lts\ \mathrm{rel}}$	4.80%

Table S8. The reference value of the BPV plasmid RM.

Unit	Concentration (copies/μL)		
	Rep1	Rep2	Rep3
1	2.22×10^6	2.24×10^6	2.09×10^6
2	2.04×10^6	2.15×10^6	2.09×10^6
3	2.38×10^6	2.44×10^6	2.47×10^6
4	2.04×10^6	2.06×10^6	2.22×10^6
5	2.11×10^6	2.16×10^6	2.18×10^6
6	2.05×10^6	2.08×10^6	2.04×10^6
7	2.20×10^6	2.32×10^6	2.32×10^6
8	2.34×10^6	2.25×10^6	2.44×10^6
9	2.07×10^6	2.16×10^6	2.26×10^6
10	2.16×10^6	2.16×10^6	2.10×10^6
11	2.20×10^6	2.20×10^6	2.07×10^6
12	2.27×10^6	2.40×10^6	2.39×10^6
mean		2.21×10^6	
u_{A}		3.67×10^4	
$u_{\mathrm{A}\ rel}$		1.67%	

Table S9. Components of the uncertainty of BPV plasmid RM

Components	value
$u_{\mathrm{A\ rel}}$	1.67%
$u_{\mathrm{B\ rel}}$	1.71%
$u_{\mathrm{char\ rel}}$	2.39%
$u_{lts\ rel}$	4.80%
$u_{hom\ rel}$	1.74%
$u_{\mathrm{CRM\ rel}}$	5.64%
$U_{rel}= u_{\mathrm{CRM\ rel}} \cdot k$	11.27%
Reference values (copies/μL)	2.21×10^6
Expanded uncertainty (copies/μL)	2.49×10^5

References

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