

Electronic Supplementary Material

Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR

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Table S1. Primer and probe sequences used in this study.

ID	Target Gene	Primer or Probes (5'-3')	Length (bp)	Assay use	Reference
T- RdRp F	RdRp	TAATACGACTCACTATAGGGACACCGTTTCTACAGG	300	Sample quantity control	this study
RdRp R		CGCAGGTAAGCGTAAACTCATCCAC			
RdRp qRT-F1		GGTCATGTGTGGCGGCTC			
RdRp qRT-R1		GCTGTAACAGCTTGACAAATGTTAAAG	100	Specimen confirmation	
RdRp probe ^a		CTATATGTTAAACCAGGTGGAAC			
T-N F	N	TAATACGACTCACTATAGGGCTGACAATAACCAGGATG	482	Sample quantity control	this study
N R		AGAAGAGGCTTGACTACCA			
N F1		GTGGTGGTGACGGCAAATG			
N R1		AAGTGAAGCTTCTGGGCCAG			
N Probe ^a		AAAGAGCTCAGCCCCAGATG	80	Specimen screening	(Ge et al., 2013)

Note: a: Probes were labeled at the 5' end with the reporter molecule 6-carboxyfluorescein (6-FAM) and at the 3' end with Black Hole Quencher 1 (BHQ1).

Table S2. The SL-CoV concentration in individual bat fecal sample quantified by qRT-PCR assays.

Dates	Samples	N		RdRp	
		Ct value (Mean of triplicates ± standard deviation)	Copies/g	Ct value (Mean of triplicates ± standard deviation)	Copies/g
201110	3267	36.02 ± 1.05	1.18E + 07	31.48 ± 0.08	3.42E + 07
	3262	26.92 ± 0.21	2.20E + 09	27.30 ± 0.58	5.05E + 08
	3261	25.48 ± 0.11	5.04E + 09	30.77 ± 0.45	5.39E + 07
201205	3367	30.46 ± 0.45	5.77E + 08	31.74 ± 0.17	2.89E + 07
	3371	36.09 ± 0.03	2.28E + 07	32.45 ± 0.49	1.82E + 07
	3375	37.09 ± 0.05	1.28E + 07	31.86 ± 0.38	2.68E + 07
201209	4089	36.38 ± 0.32	1.93E + 07	31.77 ± 0.24	2.83E + 07
	4079	34.32 ± 0.78	6.30E + 07	31.66 ± 0.11	3.03E + 07
	4083	32.52 ± 0.28	1.76E + 08	31.76 ± 0.44	2.85E + 07
	4090	30.87 ± 0.36	4.55E + 08	30.81 ± 0.32	5.27E + 07
	4103	30.22 ± 0.4	6.63E + 08	30.52 ± 0.51	6.34E + 07
	4080	28.58 ± 0.03	8.51E + 08	29.75 ± 0.08	1.04E + 08
	4108	29.74 ± 0.28	8.71E + 08	30.98 ± 0.3	4.71E + 07
	4097	29.53 ± 0.73	9.85E + 08	29.94 ± 0.25	9.19E + 07
	4085	29.24 ± 0.28	1.16E + 09	30.96 ± 0.47	4.78E + 07
	4087	28.86 ± 0.24	1.45E + 09	30.80 ± 0.4	5.28E + 07
	4096	26.53 ± 0.57	5.52E + 09	27.99 ± 0.09	3.24E + 08
	4084	26.48 ± 0.21	5.68E + 09	30.46 ± 0.48	6.60E + 07
	4122	24.64 ± 0.15	1.63E + 10	26.92 ± 0.21	6.45E + 08
	4091	24.55 ± 0.07	1.71E + 10	27.09 ± 0.23	5.79E + 08
	4081	23.09 ± 0.15	1.99E + 10	23.22 ± 0.35	7.06E + 09
	4110	24.25 ± 0.17	2.04E + 10	27.09 ± 0.13	5.79E + 08
	4105	22.18 ± 0.2	6.68E + 10	24.37 ± 0.54	3.35E + 09
4075	18.33 ± 0.03	3.05E + 11	19.20 ± 0.31	9.43E + 10	
4092	17.62 ± 0.09	4.58E + 11	18.28 ± 0.52	1.71E + 11	
201304	4221	33.57 ± 0.7	2.83E + 07	34.52 ± 0.34	2.81E + 06
	4249	32.91 ± 0.88	8.25E + 07	31.43 ± 0.51	2.05E + 07
	4258	32.25 ± 0.46	1.21E + 08	30.75 ± 3.58	3.19E + 07
	4224	30.56 ± 0.18	1.59E + 08	31.67 ± 0.63	1.76E + 07
	4250	30.54 ± 0.12	1.61E + 08	32.03 ± 0.66	1.40E + 07
	4255	31.24 ± 0.06	2.15E + 08	31.85 ± 0.03	1.57E + 07
	4246	31.17 ± 0.57	2.24E + 08	30.97 ± 0.13	2.77E + 07
	4254	30.99 ± 0.55	2.48E + 08	32.72 ± 0.41	8.92E + 06
	4230	30.92 ± 0.48	2.59E + 08	30.03 ± 0.32	5.07E + 07
	4213	30.75 ± 0.52	2.84E + 08	29.17 ± 0.45	8.81E + 07
	4256	30.24 ± 0.27	3.82E + 08	29.38 ± 0.25	7.70E + 07
	4228	29.96 ± 0.24	4.49E + 08	28.55 ± 0.92	1.31E + 08
	4235	28.14 ± 0.08	1.27E + 09	28.09 ± 0.79	1.78E + 08

To be continued

Genes	Dates	Samples	N		RdRp	
			Ct value (mean of triplicates \pm standard deviation)	Copies/g	Ct value (mean of triplicates \pm standard deviation)	Copies/g
		4247	27.71 \pm 0.33	1.63E + 09	27.37 \pm 0.63	2.82E + 08
		4237	27.20 \pm 0.93	2.18E + 09	26.55 \pm 0.06	4.80E + 08
		4231	23.04 \pm 0.1	1.19E + 10	23.86 \pm 0.56	2.71E + 09
201307		4872	35.43 \pm 0.1	9.72E + 06	37.05 \pm 0.73	5.48E + 05
		4937	33.95 \pm 0.02	4.54E + 07	34.03 \pm 1.66	3.84E + 06
		4834	32.51 \pm 0.07	5.19E + 07	30.69 \pm 0.91	3.32E + 07
		4829	28.24 \pm 0.42	1.20E + 09	29.12 \pm 0.59	9.15E + 07
		4952	26.35 \pm 0.37	3.55E + 09	25.92 \pm 0.05	7.19E + 08
		4832	23.79 \pm 0.78	1.55E + 10	24.78 \pm 0.23	1.50E + 09
		4841	22.56 \pm 0.03	3.13E + 10	28.31 \pm 0.65	1.54E + 08
		4900	22.56 \pm 0.41	3.13E + 10	22.24 \pm 0.28	7.70E + 09
		4943	22.38 \pm 0.43	3.49E + 10	21.94 \pm 0.31	9.36E + 09
		201405		6530	33.94 \pm 0.25	2.28E + 07
6526	30.87 \pm 0.63			2.66E + 08	29.56 \pm 0.66	6.85E + 07
6500	30.73 \pm 0.39			2.88E + 08	29.77 \pm 0.98	6.01E + 07
201410		7335	36.75 \pm 0.33	9.10E + 06	37.52 \pm 0.5	2.03E + 05
		7330	33.37 \pm 0.52	6.32E + 07	33.05 \pm 0.65	3.61E + 06
		7327	27.56 \pm 0.08	1.78E + 09	29.79 \pm 0.19	2.95E + 07
		7326	25.49 \pm 0.09	5.85E + 09	27.08 \pm 0.32	1.71E + 08
		7325	36.02 \pm 1.05	9.40E + 08	22.95 \pm 0.14	2.51E + 10

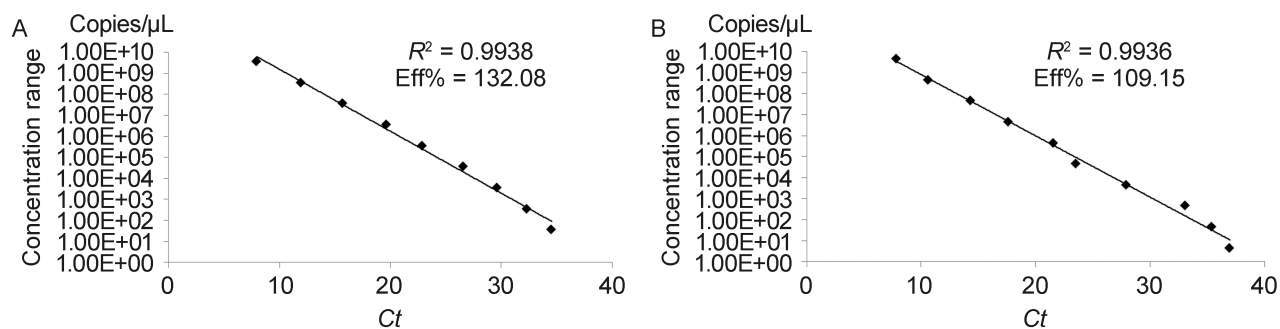


Figure S1. Plots of serial 10-fold dilutions for RNA transcripts analyzed by SL-CoV qRT-PCR assays. (A) *N* assay; (B) *RdRp* assay. Plot inserts show calculated linear correlation coefficients (R^2) and amplification efficiencies for each assay.

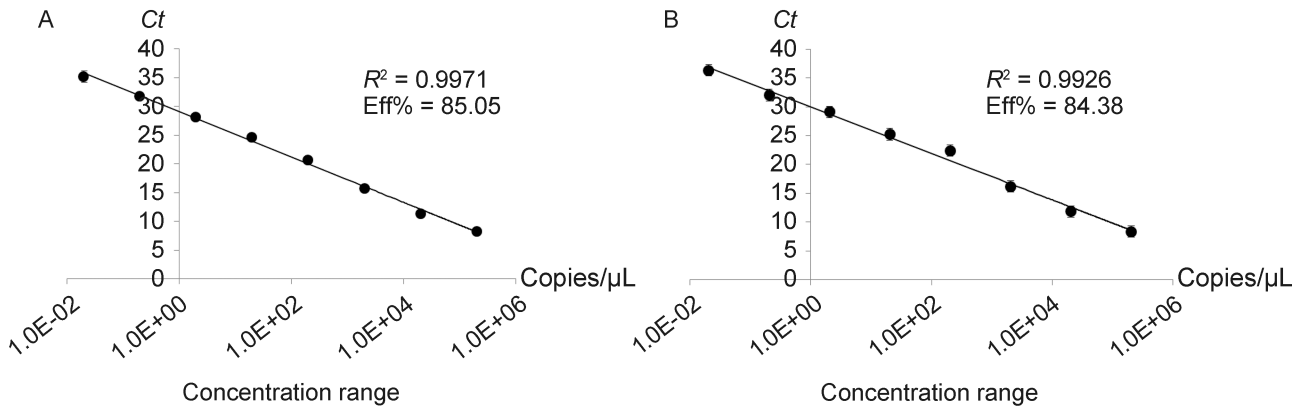


Figure S2. Plots of serial 10-fold dilutions from 2.04×10^{-2} to 2.04×10^5 copies / reaction for RNA genome of purified WIV1 analyzed by SL-CoV qRT-PCR assays. (A) *N* assay; (B) *RdRp* assay. Plot inserts show calculated linear correlation coefficients (R^2) and amplification efficiencies for each assay.

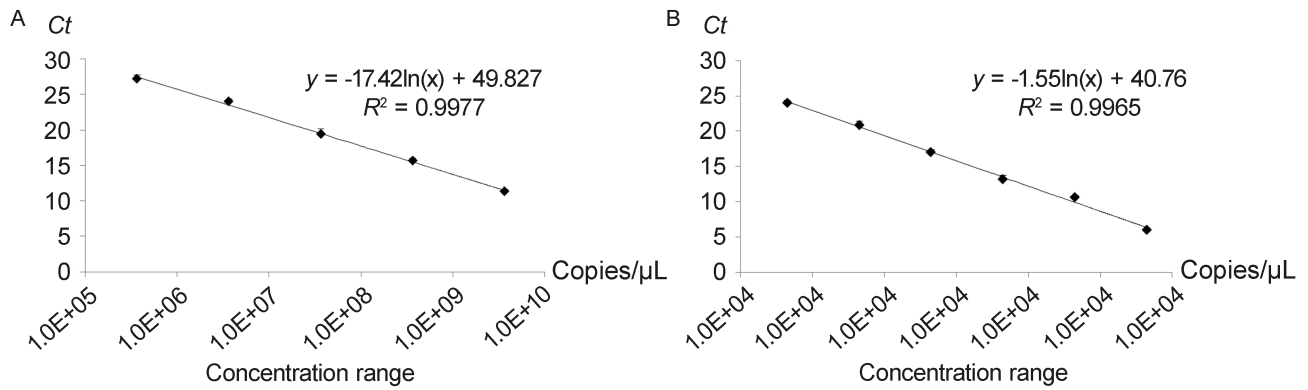


Figure S3. Standard curves of *in vitro* transcribed RNA to quantify SL-CoVs by qRT-PCR. (A) Standard curve for the *N* assay; (B) Standard curve for the *RdRp* assay.