

Supplementary material

Table S1. BVDV determination in unprocessed bovine lung lavages. Storage samples of lots 1104, 1108 and 1111 were subjected to RNA isolation and qRT-PCR was performed using the cador BVDV RT-PCR Kit.

Sample	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
1104	40.23	n/d	n/d	31.41	31.46	0.08 (0.24%)
	invalid			31.41		
	invalid			31.57		
1108	invalid	n/d	n/d	32.19	32.26	0.05 (0.16%)
	invalid			32.31		
	invalid			32.28		
1111	invalid	n/d	n/d	32.76	32.43	0.23 (0.71%)
	invalid			32.27		
	invalid			32.27		

Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default; *invalid*, no signal detected; *n/d*, not determined.

Table S2. Calibration curve with BVDV spiked in BVDV-negative lung lavage. Storage samples of lot 1102 were spiked with BVDV to yield indicated titers [TCID₅₀/ml], subjected to RNA isolation and qRT-PCR was performed using the cador BVDV RT-PCR Kit.

BVDV titer in sample [log ₁₀ TCID ₅₀ /ml]	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
0.00	invalid	n/d	n/d	33.34	33.54	0.17 (0.51%)
	invalid			33.76		
	invalid			33.52		
1.00	invalid	n/d	n/d	32.80	32.97	0.13 (0.38%)
	invalid			33.01		
	invalid			33.10		
2.00	invalid	39.70	0.59 (1.50%)	30.37	30.38	0.28 (0.91%)
	39.10			30.05		
	40.29			30.73		
2.50	37.03	37.74	0.95 (2.51%)	32.59	32.54	0.05 (0.14%)
	37.12			32.48		
	39.08			32.55		
4.00	31.96	31.77	0.25 (0.80%)	31.97	32.07	0.14 (0.43%)
	31.41			32.26		
	31.94			31.97		
5.50	26.89	26.82	0.05 (0.20%)	30.91	30.75	0.12 (0.38%)
	26.8			30.63		
	26.76			30.71		
6.00	24.53	24.48	0.06 (0.25%)	33.56	33.45	0.16 (0.49%)
	24.51			33.22		
	24.39			33.57		
7.00	20.44	20.37	0.09 (0.44%)	28.69	28.41	0.46 (1.61%)
	20.42			27.77		
	20.24			28.78		

Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default; *invalid*, no signal detected; *n/d*, not determined.

Table S3. Calibration curve with BVDV spiked in ddH₂O. Sterile ddH₂O was spiked with BVDV to yield indicated titers [TCID₅₀/ml], subjected to RNA isolation and qRT-PCR was performed using the cadov BVDV RT-PCR Kit.

BVDV titer in sample [log ₁₀ TCID ₅₀ /ml]	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
---	<i>invalid</i>	<i>n/d</i>	<i>n/d</i>	31.72	31.78	0.05 (0.15%)
	<i>invalid</i>			31.79		
	<i>invalid</i>			31.84		
1.00	<i>invalid</i>	<i>n/d</i>	<i>n/d</i>	31.90	31.82	0.06 (0.17%)
	<i>invalid</i>			31.77		
	<i>invalid</i>			31.80		
2.50	39.23	39.5	0.27 (0.68%)	32.27	32.19	0.06 (0.18%)
	<i>invalid</i>			32.15		
	39.77			32.14		
4.00	31.66	31.78	0.10 (0.32%)	32.18	32.19	0.04 (0.13%)
	31.91			32.24		
	31.77			32.14		
5.50	26.86	26.82	0.04 (0.14%)	30.97	31.06	0.07 (0.23%)
	26.83			31.14		
	26.77			31.08		
7.00	20.93	20.94	0.07 (0.33%)	29.47	29.49	0.10 (0.35%)
	20.86			29.38		
	21.03			29.63		

Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default; *invalid*, no signal detected; *n/d*, not determined.

Table S4. Determination of the minimal amount of BVDV necessary for infection of cell cultures. MDBK cells were inoculated with the indicated titers. 7 dpi culture supernatant and cells were subjected to RNA isolation and qRT-PCR was performed using the cadov BVDV RT-PCR Kit. Final titers were extrapolated by comparing mean CT values with the generated calibration curve.

Sample	Inoculation titer [log ₁₀ TCID ₅₀]	Calculated BVDV titer [log ₁₀ TCID ₅₀]	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
medium	3.00	5.30	27.1	27.09	0.02 (0.08%)	30.42	30.44	0.06 (0.18%)
			27.11			30.52		
			27.06			30.39		
cells	3.00	6.61	22.14	22.12	0.02 (0.10%)	29.62	29.44	0.15 (0.51%)
			22.13			29.25		
			22.09			29.46		
medium	1.00	0.00	<i>invalid</i>	<i>n/d</i>	<i>n/d</i>	32.28	32.39	0.08 (0.25%)
			37.22			32.45		
			<i>invalid</i>			32.45		
cells	1.00	2.40	<i>invalid</i>	38.16	0.60 (1.57%)	31.72	31.91	0.17 (0.54%)
			37.56			31.88		
			38.76			32.14		

Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default; *invalid*, no signal detected; *n/d*, not determined.

Table S5. BVDV quantification in lung lavage inoculated cell cultures. MDBK cells were exposed to lung lavage lots 1104, 1108 and 1111 and 7 dpi and 14 dpi the cellular fractions were subjected to RNA isolation and qRT-PCR was performed using the cadov BVDV RT-PCR Kit. Final titers were extrapolated by comparing mean CT values with the generated calibration curve.

Sample	Dpi	Calculated BVDV titer [log ₁₀ TCID ₅₀ /ml]	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
1104	7	6.56	22.27	22.31	0.03 (0.13%)	26.92	28.27	0.95 (3.37%)
			22.32			28.93		
			22.34			28.95		
1104	14	6.29	23.32	23.33	0.01 (0.06%)	28.06	28.83	0.55 (1.91%)
			23.32			29.09		
			23.35			29.33		
1108	7	4.94	28.55	28.46	0.07 (0.23%)	30.42	30.59	0.13 (0.42%)
			28.41			30.62		
			28.41			30.73		
1108	14	6.36	23.02	23.04	0.11 (0.48%)	30.00	29.91	0.09 (0.30%)
			22.92			29.79		
			23.19			29.94		
1111	7	5.68	25.58	25.65	0.05 (0.19%)	28.75	28.73	0.10 (0.36%)
			25.67			28.84		
			25.69			28.59		
1111	14	6.67	21.85	21.86	0.05 (0.23%)	30.10	30.06	0.28 (0.94%)
			21.81			29.69		
			21.93			30.38		

Dpi, days post infection; Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default.

Table S6. Calibrator samples. Low and high value calibrator samples with indicated titers were included in measurements of unknown samples to proof validity of the assay. Calibrators and unknown samples were prepared and treated at the same time and in the same way.

Calibrator	Replicate	BVDV titer in sample [log ₁₀ TCID ₅₀ /ml]	BVDV Ct values	Mean of BVDV Ct values	SD (CV) of BVDV Ct values	Control Ct values	Mean of control Ct values	SD (CV) of control Ct values
low	1	2.50	38.93	36.46	2.47 (6.77%)	31.93	32.08	0.31 (0.97%)
			33.99			31.79		
			invalid			32.51		
low	2	2.50	36.58	37.23	0.65 (1.73%)	32.89	33.15	0.18 (0.56%)
			37.87			33.26		
			invalid			33.30		
low	3	2.50	38.03	38.30	0.27 (0.69%)	31.84	31.90	0.05 (0.17%)
			38.56			31.90		
			invalid			31.97		
high	1	5.50	27.84	27.90	0.08 (0.29%)	29.96	30.16	0.15 (0.50%)
			27.84			30.21		
			28.01			30.32		
high	2	5.50	27.7	27.67	0.09 (0.34%)	31.73	31.47	0.21 (0.66%)
			27.76			31.22		
			27.54			31.45		
high	3	5.50	27.88	27.83	0.04 (0.13%)	31.20	30.88	0.29 (0.93%)
			27.79			30.50		
			27.82			30.93		

Dpi, days post infection; Ct, cycle threshold; SD, Standard deviation of the mean; CV, coefficient of variation [%]; Control, internal control included in the RT-PCR Kit by default; *invalid*, no signal detected.

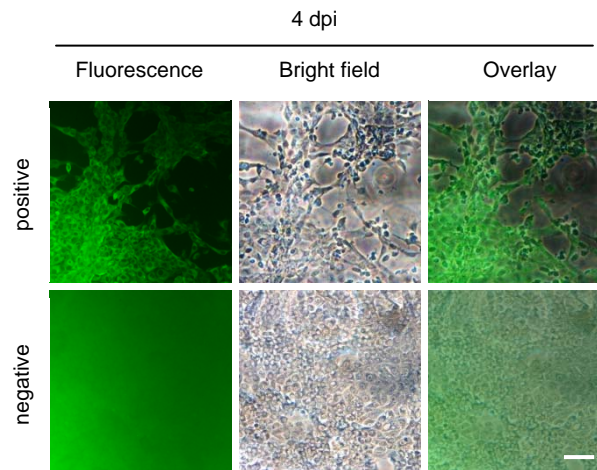


Fig. S1. BVDV detection in positive and negative control samples by IFT with anti-BVDV FITC-coupled antibodies. MDBK cells were inoculated with BVDV or buffer, respectively, and growth on chamber slides was initiated 4 days before microscopic examination with an Eclipse E400 fluorescence microscope (Nikon, Germany) at 100-200x magnification under fluorescent light. Photographs were taken using the AxioCam MRC and analyzed with AxioVision Rel 4.7 (Zeiss, Jena, Germany). Scale bar 100 μ m.