Supplementary material

Table S1. Extraction time of various protocols on the BioSprint 15 platform for original and short protocols.

Step	VET and CADOR kit			CORE kit		
	Original	**Short 4**	**Short 5**	**Original**	**Short 4**	**Short 5**
Lysis and binding	4 min 50 s	1 min 48 sec	1 min 48 s	14 min 12 s	1 min 48 sec	1 min 48 s
Collect beads	–	-	–	58 s	-	–
1st washing step	2 min 12 s	1 min 05 s	57 s	2 min 38 s	1 min 04 s	57 s
2nd washing step	2 min 12 s	1 min 05 s	57 s	1 min 41 s	1 min 04 s	57 s
3rd washing step	2 min 12 s	1 min 05 s	57 s	–	-	–
Drying of the beads	4 min 00 s	1 min 00 s	1 min 00 s	4 min 00 s	1 min 00 s	1 min 00 s
Elution	1 min 56 s	44 s	42 s	11 min 16 s	1 min 04 s	1 min 04 s
Release beads	26 s	26 s	26 s	17s	26 s	26 s
Total duration	**17 min 48 s**	**7 min 13 s**	**6 min 47 s**	**35 min 2 s**	**6 min 26 s**	**6 min 12 s**

Table S2. Reagents (in µl) and filling scheme for the rapid nucleic acid extraction protocol of three extraction kits.

	VET kit	CADOR kit	CORE kit
Sample lysis			
sample	100	100	100
lysis solution	100	100	350
Binding			
binding solution	350	400	350
beads	20	25	20
internal control	10	10	10
Wash solution 1	600	700	500
Wash solution 2	600	700	500
Wash solution 3	600	750	-[1]
Elution buffer	100	100	100

[1]washing step 3 is not included according to the manufacturer-specific recommendations.

Table S3. Test series based on a rapid extraction protocol: results of a comparative validation of different extraction kits regarding their runability in original protocol ("original") and maximal speed-optimized protocols ("short 4" and "short 5").

	VET kit*			CADOR kit*			CORE kit*		
	Original	**Short 4**	**Short 5**	**Original**	**Short 4**	**Short 5**	**Original**	**Short 4**	**Short 5**
10^{-1}	21.8	22.3	23.5	22.1	22.2	25.8	20.4	23.0	23.3
10^{-2}	26.2	26.8	28.8	26.0	26.1	30.2	25.6	26.2	26.6
10^{-3}	29.5	30.2	31.7	29.1	30.0	30.1	29.0	30.6	31.2
10^{-4}	31.4	33.8	32.5	32.0	34.0	35.0	32.0	34.1	34.1
10^{-5}	36.0	37.2	35.6	35.9	37.0	38.0	35.6	No Cq	38.7
10^{-6}	38.1	38.3	No Cq	No Cq	No Cq	38.1	No Cq	No Cq	No Cq
10^{-7}	-	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
Time	**17 min 48 s**	**7 min 13 s**	**6 min 47 s**	**17 min 48 s**	**7 min 13 s**	**6 min 47 s**	**35 min 2 s**	**6 min 26 s**	**6 min 12 s**

*Extraction kits used are: VET kit = NucleoMag® VET (Macherey-Nagel, Düren, Germany); CADOR kit = MagAttract® 96 cador Pathogen Kit (Indical, Leipzig, Germany); CORE kit = MagMAX™ CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, USA); - = not tested.

Table S4. Results (Cq-values) of the device test with five qPCR cyclers using two PPRV-specific assays (Polci-mix, PPRV-mix 6): standard protocol compared to the short protocol 5.

	Standard protocol					Short protocol 5				
	CFX96 Touch	**Aria Mx**	**MIC**	**Light Cycler 2.0**	**Light Cycler 96**	**CFX96 Touch**	**Aria Mx**	**MIC**	**Light Cycler 2.0**	**Light Cycler 96**
Polci-mix										
10^{-1}	22.1	20.2	22.3	23.8	18.3	27.0	24.8	26.5	> 35.0	24.2
10^{-2}	25.6	23.3	25.4	26.9	22.4	30.7	28.6	30.3	> 35.0	27.8
10^{-3}	29.3	26.0	28.7	31.2	26.7	34.2	32.2	33.8	No Cq	31.1
10^{-4}	32.5	29.3	32.4	33.7	30.8	38.2	35.9	37.3	No Cq	34.3
10^{-5}	36.9	33.2	37.1	34.6	35.9	No Cq	38.6	No Cq	No Cq	37.7
10^{-6}	No Cq	No Cq	No Cq	No Cq	35.2	No Cq	No Cq	No Cq	No Cq	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
PPRV-mix 6										
10^{-1}	23.0	19.8	20.9	23.6	20.8	26.8	24,3	24.3	29.1	24.3
10^{-2}	26.3	22.5	24.3	25.6	24.3	30.0	27,8	27.7	34.3	27.4
10^{-3}	29.7	26.2	27.9	28.3	27.7	33.7	30,6	31.3	> 35.0	30.8
10^{-4}	33.0	26.7	31.2	31.0	31.8	37.4	34,7	34.6	> 35.0	33.9
10^{-5}	37.1	32.0	34.2	32.6	34.6	No Cq	38,5	No Cq	No Cq	No Cq
10^{-6}	No Cq	No Cq	37.7	33.5	37.7	No Cq	No Cq	No Cq	No Cq	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
Time	**1 h 38 min**	**1 h 32 min**	**1 h 38 min**	**1 h 23 min**	**1 h 34 min**	**38 min**	**33 min**	**39 min**	**25 min**	**34 min**

Table S5. Test series for a high-speed RT-qPCR: development of the Cq-values on the BioRad CFX96 with regard to a time-optimized RT-qPCR protocol ("short") compared to a standard protocol ("standard") when using two primer-probe mixtures.

	Assay of Polci et al., 2015			**PPRV-mix 6**		
	Standard	**Short 4**	**Short 5**	**Standard**	**Short 4**	**Short 5**
10^{-1}	17.3	20.7	22.4	18.7	20.7	21.4
10^{-2}	21.0	24.1	26.2	22.3	24.4	25.5
10^{-3}	24.6	27.9	30.1	25.7	27.9	28.9
10^{-4}	28.0	31.1	34.1	29.0	31.2	32.0
10^{-5}	32.1	35.3	37.7	33.4	35.4	No Cq
10^{-6}	36.6	38.8	No Cq	36.8	No Cq	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
Time	**1 h 38 min**	**39 min**	**35 min 2 s**	**1 h 38 min**	**39 min**	**35 min 2 s**

Table S6. Test series for a high-speed RT-qPCR: development of the RFU values on the BioRad CFX96 with regard to a time-optimized RT-qPCR protocol ("short") compared to a standard protocol ("standard") when using two primer-probe mixtures.

	Assay of Polci et al., 2015			**PPRV-mix 6**		
	Standard	**Short 4**	**Short 5**	**Standard**	**Short 4**	**Short 5**
10^{-1}	9906	9729	9131	9193	8457	7114
10^{-2}	10461	9558	7330	9266	7596	5466
10^{-3}	9976	9187	7376	10023	6600	4750
10^{-4}	10207	8034	4507	9034	4324	2499
10^{-5}	9984	4391	1678	6109	1475	492
10^{-6}	8788	1515	-1.6	4777	202	91
10^{-7}	-3.8	-9,8	-2.4	5.52	16	13
Time	**1 h 38 min**	**39 min**	**35 min 2 s**	**1 h 38 min**	**39 Min**	**35 min 2 s**

Table S7. Results (Cq-values) of the test series with three various lyophilized test kits: comparison of an optimal PCR-protocol ("optimal") for the validation of the dilution series, the standard protocol ("standard protocol") of the lyophilized kit and the speed-optimized protocol ("short protocol 5") of the corresponding kit; AgPath = AgPath-ID™ One-Step RT-PCR Reagents; Qiagen = QuantiTect Multiplex-PCR Kit NO ROX; lyophilised kits: Takyon™ Dry No Rox One-Step RT Probe MasterMix kit; Qscript lyo 1-step; CAPITAL™ qRT-PCR Probe Mix.

	Optimal	Standard protocol			Short protocol 5		
PPRV	**AgPath kit**	**Takyon kit**	**Qscript kit**	**Capital kit**	**Takyon kit**	**Qscript kit**	**Capital kit**
10^{-1}	19.3	21.4	19.1	19.9	27.1	21.3	21.1
10^{-2}	22.8	25.3	22.3	23.2	31.0	24.7	24.6
10^{-3}	26.5	28.3	25.7	26.5	34.3	28.2	28.2
10^{-4}	30.0	33.3	29.0	30.3	39.0	32.0	31.7
10^{-5}	33.4	37.5	32.7	33.3	No Cq	35.0	35.2
10^{-6}	34.9	No Cq	35.2	37.6	No Cq	37.9	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
Mccp	**AgPath kit**	**Takyon kit**	**Qscript kit**	**Capital kit**	**Takyon kit**	**Qscript kit**	**Capital kit**
10^{-1}	21.2	21.4	21.8	21.8	32.6	22.1	21.9
10^{-2}	24.8	24.7	25.1	25.0	33.5	25.2	24.8
10^{-3}	28.3	28.1	28.3	28.3	37.4	28.5	28.2
10^{-4}	31.9	31.3	31.6	31.8	No Cq	31.9	31.8
10^{-5}	35.5	34.3	34.7	35.4	No Cq	35.7	34.5
10^{-6}	No Cq	No Cq	38.2	39.1	No Cq	No Cq	36.8
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
FMDV	**AgPath kit**	**Takyon kit**	**Qscript kit**	**Capital kit**	**Takyon kit**	**Qscript kit**	**Capital kit**
10^{-1}	22.0	27.6	22.1	21.8	31.6	23.8	22.7
10^{-2}	25.2	30.2	25.6	25.0	34.4	27.0	26.4
10^{-3}	28.5	34.1	28.9	28.1	38.7	30.0	29.6
10^{-4}	31.7	38.8	32.2	31.4	No Cq	33.3	33.0
10^{-5}	34.6	No Cq	34.5	33.5	No Cq	36.7	35.1
10^{-6}	37.9	No Cq	36.5	38.7	No Cq	37.0	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
GTPV	**Qiagen kit**	**Takyon kit**	**Qscript kit**	**Capital kit**	**Takyon kit**	**Qscript kit**	**Capital kit**
10^{-1}	20.6	19.5	20.3	20.0	22.1	20.3	20.0
10^{-2}	23.7	23.2	23.6	23.2	25.6	23.9	23.2
10^{-3}	27.1	26.3	26.8	26.4	28.7	27.1	26.5
10^{-4}	30.4	29.9	30.1	30.0	32.3	30.6	30.0
10^{-5}	33.5	33.7	32.7	33.8	36.3	34.0	33.2
10^{-6}	37.3	37.1	37.0	36.2	39.2	36.3	36.5
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq
Orf	**Qiagen kit**	**Takyon kit**	**Qscript kit**	**Capital kit**	**Takyon kit**	**Qscript kit**	**Capital kit**
10^{-1}	20.6	21.4	22.1	23.0	26.0	21.9	23.1
10^{-2}	23.9	24.7	25.3	26.2	29.2	25.2	26.2
10^{-3}	27.2	28.1	28.5	29.6	32.6	28.6	29.6
10^{-4}	30.8	31.3	32.0	32.7	36.2	32.4	33.1
10^{-5}	33.5	34.3	36.2	34.3	38.4	35.7	35.8
10^{-6}	36.2	No Cq	37.7	No Cq	No Cq	37.6	No Cq
10^{-7}	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq	No Cq