Supporting Information

Quick plant sample preparation methods using a micro-homogenizer for the detection of multiple citrus pathogens

Chia-Wei Liu [1], Sohrab Bodaghi [2], Georgios Vidalakis [2,*] and Hideaki Tsutsui [1,3,*]

[1] Department of Mechanical Engineering, University of California, Riverside, CA, 92521, USA

[2] Department of Microbiology and Plant Pathology; University of California, Riverside, CA, 92521, USA

[3] Department of Bioengineering; University of California, Riverside, CA, 92521, USA

* Corresponding authors. Email addresses: htsutsui@engr.ucr.edu (H. Tsutsui) and vidalg@ucr.edu (G. Vidalakis)

Table S1. Comparison of quick and portable extraction techniques for qPCR-based assays.

Method/Kit	Specimen input	Extraction strategy and processing time	Pros/Cons	Reference
Qiagen RNeasy/DNeasy plant mini kits	Plant lysates (External tools, such as TissueRuptor or TissueLyser, are required to break down the tissues)	Spin columns with silica-based membrane for DNA/RNA purification. Time per run or per prep: 30 min - 1 hr	Well-established method for pure and high yields of DNA/RNA.$6.98 - $10.44 per sample. Multiple times of centrifugation is required.	[34-37]
FTA PlantSaver card	Plant tissue is physically crushed on the card, and the DNA binds to the card matrix.	A special coating on the FTA card protects the DNA from degradation and allows the cards to be stored at room temperature. Purification time: < 30 min	Fast sampling and purification. $6.66 per 4-sample-area card. The chance of getting contamination is high as there are no physical borders between each sampling area.	[38,39]
Microneedle patch	Plant leaf is physically punctured by the patch, and the DNA is collected within needle tips.	A microneedle patch made of polyvinyl alcohol (PVA) can isolate plant or pathogenic DNA from	Quick extraction within a minute. It takes overnight to complete the fabrication process of a microneedle	[40,41]

		different plant species within a minute.	patch. The soft feature of a PVA microneedle patch might limit its use in dealing with thicker/tougher leaves, such as avocados and lemons.	
This work	Plant lysate is prepared with an OmniLyse micro-homogenizer.	Dipping untreated chromatography paper disks in the guanidine-based lysate for quick nucleic acid capture (5 min), followed by wash (1 min) and elution (1 min).	A ¼-inch disk cut from Whatman chromatography paper costs just $0.0007 per sample [a]. No pretreatment is required for the paper. Quick extraction taking only 7 min.	-

[a] Calculated based on the price provided on the official website of Sigma-Aldrich (https://www.sigmaaldrich.com/US/en/product/aldrich/wha3001861).

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Table S2. Oligonucleotide primers and qPCR probes used for singleplex and multiplex qPCR/RT-qPCR assays.

Isolates	Primers/probes	Sequence 5' – 3'	Nucleotide position	Amplicon size (bp)	Ref.
COX	Forward	AATCTGACCTTCTTTCCCATGC	32-53	162	[6, 7]
	Reverse	AAGTGATTGTTACGACCACGAAGA	194-171		
	Probe	ATCCAGATGCTTACGCTGG	96-114		
CTV	Forward	TGTGTGCGGATTTCTTGACTG	701-721	135	[7]
	Reverse	TTCCCAAGCTGCCTGACATT	833-814		
	Probe	AAGCGAGGGGCTGAT	784-798		
CPsV	Forward 1	TCACAAATCAGTGAGGAATTGAGC	60-83	154	[7]
	Forward 2	CACAAATCAGTGATGAATTGAGCC	61-84		
	Reverse 1	GCAAACCCAGCATATCTCACAG	214-193		
	Reverse 2	CGCAAACCCAGCATATCTTACAG	215-193		
	Probe	TCTCAAGATTGATATAGACAAC	119-140		
CLBV	Forward	TTCAAGAACTGGATTTGAATTTGC	7,711-7,735	161	[7]
	Reverse	TGCACAGAATTGCCTCACAGT	7,874-7,853		
	Probe	AAGTTGTGGATCAAGAAG	7,738-7,756		
CEVd	Forward	GTCCAGCGGAGAAACAGGAG	181-200	105	[6]
	Reverse	AGAGAAGCTCCGGGCGA	286-270		
	Probe	TCCTTCCTTTCGCTGCT	212-228		
HSVd	Forward	GAGACGCGACCGGTGG	216-231	88	[6]
	Reverse	GCTCAAGAGAGGATCCGCG	304-286		
	Probe	TCACCTCTCGGTTCGTC	234-250		
CBCVd	Forward	GGGAACATACCTGAAGAGGGATC	113-135	89	[6]
	Reverse	TTCCGGTGCTGGACGC	202-187		
	Probe	TCTCTTCAGACTCGTCGAGG	146-165		
S. citri	Forward	ATTGCAGCACCTGCAACTGTAG	112-133	114	[48]
	Reverse	TGTTTTTACAACTCCTTGCACTGC	225-202		

	Probe	ACAGCGTTAGAA GCTAAT	175-192		

Nucleotide position is based on GenBank accessions: COX-CX297817, CTV-M76485, CPsV-AF036926, CLBV-NC_003877, CEVd-HQ284019, CBCVd-KC121568, HSVd-KJ810553, and S. citri-FJ755921.

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Table S3. Kits and protocols used for singleplex and multiplex qPCR/RT-qPCR assays.

Pathogen	Assay	Composition	Thermal-cycling protocol
S. citri	Singleplex qPCR iTaq™ Universal Probes One-Step Kit (Bio-Rad, USA)	6 µL 2X PCR buffer 0.576 µL primer/probe mix 0.424 µL ddH2O 5 µL DNA template.	Incubation stage (2 min at 50 °C and 10 min at 95 °C) and 40 repeating cycles (15 sec at 95 °C and 1 min at 60 °C).
CLBV, CPsV, CTV, CEVd, HSVd, CBCVd	Singleplex RT-qPCR AgPath-ID One-Step RT-PCR kit (Thermo Fisher Scientific, USA)	6.25 µL 2X RT-PCR buffer 0.6 µL primer/probe mix 0.5 µL 25X RT-PCR enzyme mix 2.65 µL ddH2O 2 µL RNA template.	Incubation stage (10 min at 45 °C and 10 min at 95 °C) and 40 repeating cycles (15 sec at 95 °C and 45 sec at 60 °C).
CLBV+CPsV+CTV, CEVd+HSVd+CBCVd, Multiple infections w/ 6 viroids [a]	Multiplex RT-qPCR QuantiFast Multiplex RT-PCR kit (Qiagen, USA)	6.25 µL 2X RT-PCR buffer 0.58 µL primer/probe mix 0.125 µL 25X RT-PCR enzyme mix 0.045 µL ddH2O 5 µL of RNA template	Incubation stage (20 min at 50 °C and 5 min at 95 °C) and 40 repeating cycles (15 sec at 95 °C and 1 min at 60 °C)

[a] 6 viroids: CEVd+CBCVd+HSVd+CBLVd+CDVd+CVd-V

Table S4. A list with the true positive rates that achieved by different serial dilutions and controls (N = 9).

	True positive rate	
	CTV	COX
Positive Control	100%	100%
Negative Control	0%	100%
Water Control	0%	0%
G (undiluted)	0%	0%
G-10x	33.3%	33.3%
G-100x	100%	100%
G-1000x	100%	100%
T (undiluted)	0%	0%
T-10x	0%	11.1%
T-100x	100%	100%
T-1000x	100%	100%
H (undiluted)	0%	0%
H-10x	0%	0%
H-100x	100%	100%
H-1000x	100%	100%

Table S5. Comparison of Cq values for COX between MagMAX-treated samples and paper disk-treated samples in testing healthy citrus plants.

Category	Host	COX-MagMAX (N = 9)	COX-Paper disk (N = 9)	Cq difference of COX
Healthy source	*Citrus excelsa*	16.05 ± 0.35	20.47 ± 0.41	4.42
	Mexican lime	16.39 ± 0.60	20.58 ± 0.37	4.19
	Pineapple sweet orange	15.56 ± 0.36	20.20 ± 0.20	4.64
	Rusk citrange	16.45 ± 0.36	20.44 ± 0.16	3.99

	Rough lemon	15.73 ± 0.50	20.41 ± 0.16	4.69
	Sour orange	16.33 ± 0.24	20.81 ± 0.52	4.48
	Citron	16.01 ± 0.34	20.35 ± 0.27	4.34

Table S6. Comparison of target / COX Cq values between MagMAX-treated samples and paper disk-treated samples in detecting single-infected citrus plants.

Pathogen	Target-MagMAX (N = 9)	Target-Paper disk (N = 9)	ΔCq of target	COX-MagMAX (N = 6)	COX-Paper disk (N = 6)	ΔCq of COX
CLBV	22.52 ± 0.77	33.81 ± 0.95	11.29	16.78 ± 0.40	20.76 ± 0.69	3.99
CPsV	25.18 ± 1.00	33.99 ± 2.92	8.82	16.57 ± 0.55	20.67 ± 0.22	4.09
CEVd	30.05 ± 0.71	34.85 ± 0.57	4.80	16.72 ± 0.16	20.41 ± 0.40	3.69
HSVd	26.21 ± 0.50	31.17 ± 0.73	4.97	16.96 ± 0.05	20.75 ± 0.25	3.79
CBCVd	23.49 ± 0.95	29.44 ± 0.99	5.96	17.17 ± 0.70	20.99 ± 0.10	3.82
S. citri	30.98 ± 0.96	35.68 ± 1.35	4.70	17.94 ± 0.47	23.27 ± 0.66	5.33

Table S7. Comparison of target Cq values between MagMAX-treated samples and paper disk-treated samples in detecting multi-infected citrus plants.

Source	Pathogen	Target-MagMAX (N = 9)	Target-Paper disk (N = 9)	ΔCq of target	COX-MagMAX (N = 6)	COX-Paper disk (N = 6)	ΔCq of COX
3207-8	CEVd	30.83 ± 0.40	35.13 ± 0.56	4.30	18.62 ± 0.50	22.91 ± 0.16	4.29
	HSVd	29.43 ± 0.60	33.72 ± 0.69	4.28			
	CBCVd	28.23 ± 0.41	31.74 ± 0.21	3.51			
3210-44	CEVd	31.09 ± 1.03	35.24 ± 0.91	4.15	17.53 ± 0.48	22.40 ± 0.30	4.87
	HSVd	29.41 ± 0.48	34.10 ± 0.15	4.70			
	CBCVd	29.35 ± 1.02	34.08 ± 1.15	4.72			
3210-46	CEVd	31.01 ± 0.29	35.70 ± 0.50	4.69	17.90 ± 0.33	22.58 ± 0.42	4.68
	HSVd	29.45 ± 0.35	33.85 ± 0.53	4.40			
	CBCVd	28.70 ± 0.89	32.77 ± 1.22	4.07			
3300-7	CLBV	23.68 ± 0.12	30.06 ± 0.55	6.38	17.68 ± 0.23	22.22 ± 0.51	4.54
	CPsV	25.34 ± 0.36	30.81 ± 0.76	5.47			
	CTV	22.36 ± 0.43	26.54 ± 0.21	4.18			

Table S8a. Effects of storage conditions on nucleic acids stored on paper disks (Healthy sources, N = 9). True positive rates were 100% except for rough lemon (1mRT: 66.7%) and citron (1mRT: 66.7%).

Healthy sources	Fresh	1wRT	1w4C	1mRT	1m4C
Citrus excelsa	20.47 ± 0.41	21.36 ± 0.48 *** ΔCq = 0.89	20.60 ± 0.31 ns ΔCq = 0.14	21.18 ± 0.64 * ΔCq = 0.71	20.58 ± 0.39 ns ΔCq = 0.11
Mexican lime	20.58 ± 0.37	20.89 ± 0.27 ns ΔCq = 0.31	21.04 ± 0.36 * ΔCq = 0.46	21.45 ± 0.24 *** ΔCq = 0.87	21.02 ± 0.78 ns ΔCq = 0.44
Pineapple sweet orange	20.20 ± 0.20	20.88 ± 0.61 ** ΔCq = 0.68	20.46 ± 0.52 ns ΔCq = 0.26	20.74 ± 0.65 * ΔCq = 0.54	20.56 ± 0.61 ns ΔCq = 0.36
Rusk citrange	20.44 ± 0.16	20.94 ± 0.47 ** ΔCq = 0.50	20.36 ± 0.31 ns ΔCq = -0.08	21.09 ± 0.81 * ΔCq=0.65	20.43 ± 0.63 ns ΔCq = -0.01
Rough lemon	20.41 ± 0.16	20.74 ± 0.50 ns ΔCq = 0.33	20.23 ± 0.32 ns ΔCq = -0.18	20.16 ± 0.42 ns ΔCq = -0.25	20.42 ± 0.47 ns ΔCq=0.01
Sour orange	20.81 ± 0.52	21.33 ± 0.66 ns ΔCq = 0.52	20.88 ± 0.38 ns ΔCq = 0.07	21.20 ± 0.54 ns ΔCq = 0.38	21.03 ± 0.96 ns ΔCq=0.22
Citron	20.35 ± 0.27	20.54 ± 0.31 ns ΔCq = 0.19	20.62 ± 0.48 ns ΔCq = 0.27	20.19 ± 0.45 ns ΔCq = -0.16	20.58 ± 0.45 ns ΔCq = 0.23

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4^{o}C storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S8b. Effects of storage temperature on Cq values.

Healthy sources	One week (1w)		One month (1m)	
Citrus excelsa	RT > 4C	**	RT > 4C	*
Mexican lime	RT < 4C	ns	RT > 4C	ns
Pineapple sweet orange	RT > 4C	ns	RT > 4C	ns
Rusk citrange	RT > 4C	**	RT > 4C	ns
Rough lemon	RT > 4C	*	RT < 4C	ns
Sour orange	RT > 4C	ns	RT > 4C	ns
Citron	RT < 4C	ns	RT < 4C	ns

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ $4^{o}C$ storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S8c. Effects of storage period on Cq values.

Healthy sources	RT		4C	
Citrus excelsa	1m < 1w	ns	1m < 1w	ns
Mexican lime	1m > 1w	***	1m < 1w	ns
Pineapple sweet orange	1m < 1w	ns	1m > 1w	ns
Rusk citrange	1m > 1w	ns	1m > 1w	ns
Rough lemon	1m < 1w	*	1m > 1w	ns
Sour orange	1m < 1w	ns	1m > 1w	ns
Citron	1m < 1w	ns	1m < 1w	ns

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ $4^{o}C$ storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S9a. Effects of storage conditions on nucleic acids stored on paper disks (single-infected sources, N = 9). True positive rates were 100% except for CEVd (1w4C: 88.9%), CLBV (1mRT: 83.3%), HSVd (1mRT: 83.3%), and S. citri (1mRT: 88.9%).

Single-infected sources	Fresh	1wRT	1w4C	1mRT	1m4C
CLBV	33.81 ± 0.95	35.45 ± 2.11 * ΔCq = 1.64	35.35 ± 1.29 * ΔCq = 1.54	35.20 ± 1.42 * ΔCq = 1.39	35.65 ± 0.90 *** ΔCq = 1.84
CPsV	33.99 ± 2.92	32.94 ± 0.98 ns ΔCq = -1.05	32.23 ± 1.74 ns ΔCq = -1.76	35.00 ± 1.40 ns ΔCq = 1.01	32.22 ± 1.28 ns ΔCq = -1.77
CEVd	34.85 ± 0.57	34.18 ± 1.43 ns ΔCq = -0.67	34.87 ± 0.82 ns ΔCq = 0.02	34.43 ± 1.22 ns ΔCq = -0.42	34.87 ± 1.02 ns ΔCq = 0.02
HSVd	31.17 ± 0.73	29.62 ± 0.72 *** ΔCq = -1.55	29.16 ± 0.67 *** ΔCq = -2.01	31.49 ± 2.49 ns ΔCq = -0.32	29.57 ± 0.77 *** ΔCq = -1.6
CBCVd	29.44 ± 0.99	27.78 ± 0.70 *** ΔCq = -1.66	28.55 ± 0.50 * ΔCq = -0.89	27.91 ± 0.63 ** ΔCq = -1.53	27.90 ± 0.36 *** ΔCq = -1.54
S. citri	35.68 ± 1.35	35.22 ± 0.79 ns ΔCq = -0.46	35.15 ± 1.14 ns ΔCq = -0.53	36.48 ± 1.10 ns ΔCq = 0.80	35.29 ± 1.18 ns ΔCq = -0.39

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4^{o}C storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S9b. Effects of storage temperature on Cq values.

Single-infected sources	One week (1w)		One month (1m)	
CLBV	RT > 4C	ns	RT < 4C	ns
CPsV	RT > 4C	ns	RT > 4C	***
CEVd	RT < 4C	ns	RT < 4C	ns
HSVd	RT > 4C	ns	RT > 4C	*
CBCVd	RT < 4C	*	RT < 4C	ns
S.citri	RT > 4C	ns	RT > 4C	*

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4^{o}C storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S9c. Effects of storage period on Cq values.

Single-infected sources	RT		4C	
CLBV	1m < 1w	ns	1m > 1w	ns
CPsV	1m > 1w	***	1m < 1w	ns
CEVd	1m > 1w	ns	1m > 1w	ns
HSVd	1m > 1w	*	1m > 1w	ns
CBCVd	1m > 1w	ns	1m < 1w	**
S.citri	1m > 1w	*	1m > 1w	ns

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4^{o}C storage.

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S10a. Effects of storage conditions on nucleic acids stored on paper disks (Multi-infected sources, N = 9). True positive rates were 100% except for CLBV (1wRT: 66.7%), CPsV (1wRT: 66.7%), CTV (1wRT: 77.8%), CLBV (1w4C: 88.9%), CLBV (1mRT: 0%), CPsV (1mRT: 0%); CLBV (1m4C: 66.7%), and CPsV (1m4C: 0%).

Multi-infected sources		Fresh	1wRT	1w4C	1mRT	1m4C
3207-8	CEVd	35.13 ± 0.56	36.20 ± 0.68 ** ΔCq = 1.07	37.35 ± 0.89 *** ΔCq = 2.22	34.96 ± 0.53 ns ΔCq = -0.17	35.89 ± 0.47 ** ΔCq = 0.76
	CVd IIa (HSVd)	33.72 ± 0.69	34.46 ± 0.49 * ΔCq = 0.74	35.14 ± 0.81 ** ΔCq = 1.42	33.17 ± 0.76 ns ΔCq = -0.55	33.75 ± 0.69 ns ΔCq = 0.03
	CVd IV (CBCVd)	31.74 ± 0.21	32.50 ± 0.75 * ΔCq = 0.76	32.52 ± 0.65 ** ΔCq = 0.78	31.84 ± 0.87 ns ΔCq = 0.10	32.04 ± 0.39 ns ΔCq = 0.30
3210-44	CEVd	35.24 ± 0.91	36.51 ± 1.43 * ΔCq = 1.27	36.78 ± 0.78 ** ΔCq = 1.54	34.92 ± 1.47 ns ΔCq = -0.32	36.15 ± 1.21 ns ΔCq = 0.91
	CVd IIa (HSVd)	34.10 ± 0.15	34.58 ± 1.00 ns ΔCq = 0.48	34.64 ± 0.53 * ΔCq = 0.54	33.03 ± 1.33 * ΔCq = -1.07	34.29 ± 1.15 ns ΔCq = 0.19
	CVd IV (CBCVd)	34.08 ± 1.15	35.08 ± 1.87 ns ΔCq = 1.00	34.71 ± 1.51 ns ΔCq = 0.63	34.07 ± 1.92 ns ΔCq = -0.01	34.62 ± 2.11 ns ΔCq = 0.54
3210-46	CEVd	35.70 ± 0.50	36.09 ± 0.71 ns ΔCq = 0.39	37.59 ± 1.76 * ΔCq = 1.89	35.15 ± 1.09 ns ΔCq = -0.55	36.05 ± 1.37 ns ΔCq = 0.35
	CVd IIa (HSVd)	33.85 ± 0.53	34.32 ± 0.63 ns ΔCq = 0.47	35.17 ± 0.38 *** ΔCq = 1.32	32.87 ± 1.15 * ΔCq = -0.98	33.57 ± 1.32 ns ΔCq = -0.28
	CVd IV (CBCVd)	32.77 ± 1.22	33.61 ± 1.75 ns ΔCq = 0.84	33.91 ± 1.97 ns ΔCq = 1.14	33.28 ± 2.04 ns ΔCq = 0.51	32.76 ± 1.83 ns ΔCq = -0.01

3300-7	CLBV	30.06 ± 0.55	29.76 ± 0.18 ns ΔCq = -0.30	31.37 ± 1.47 * ΔCq = 1.31	ΔCq = n/a	31.26 ± 0.87 ** ΔCq = 1.20
	CPsV	30.81 ± 0.76	30.93 ± 0.42 ns ΔCq = 0.12	32.34 ± 0.1.13 ns ΔCq = 1.53	ΔCq = n/a	ΔCq= n/a
	CTV	26.54 ± 0.21	26.10 ± 0.21 ** ΔCq = -0.44	26.91 ± 0.45 * ΔCq = 0.37	31.32 ± 0.78 *** ΔCq = 4.78	27.43 ± 0.40 *** ΔCq = 0.89

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4^{o}C storage; n/a: Not applicable

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S10b. Effects of storage temperature on Cq values.

Multi-infected sources		One week (1w)		One month (1m)	
3207-8	CEVd	RT < 4C	**	RT < 4C	**
	CVd IIa (HSVd)	RT < 4C	*	RT < 4C	ns
	CVd IV (CBCVd)	RT < 4C	ns	RT < 4C	ns
3210-44	CEVd	RT < 4C	ns	RT < 4C	ns
	CVd IIa (HSVd)	RT < 4C	ns	RT < 4C	*
	CVd IV (CBCVd)	RT > 4C	ns	RT < 4C	ns
3210-46	CEVd	RT < 4C	*	RT < 4C	ns
	CVd IIa (HSVd)	RT < 4C	**	RT < 4C	ns
	CVd IV (CBCVd)	RT < 4C	ns	RT > 4C	ns

3300-7	CLBV	RT < 4C	*	n/a	n/a
	CPsV	RT < 4C	*	n/a	n/a
	CTV	RT < 4C	**	RT > 4C	***

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4°C storage; n/a: Not applicable

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

Table S10c. Effects of storage period on Cq values.

Multi-infected sources		RT		4C	
3207-8	CEVd	1m < 1w	***	1m < 1w	***
	CVd IIa (HSVd)	1m < 1w	***	1m < 1w	**
	CVd IV (CBCVd)	1m < 1w	ns	1m < 1w	ns
3210-44	CEVd	1m < 1w	*	1m < 1w	ns
	CVd IIa (HSVd)	1m < 1w	*	1m < 1w	ns
	CVd IV (CBCVd)	1m < 1w	ns	1m < 1w	ns
3210-46	CEVd	1m < 1w	*	1m < 1w	ns
	CVd IIa (HSVd)	1m < 1w	**	1m < 1w	**
	CVd IV (CBCVd)	1m < 1w	ns	1m < 1w	ns
3300-7	CLBV	n/a	n/a	1m < 1w	ns
	CPsV	n/a	n/a	n/a	n/a
	CTV	1m > 1w	***	1m > 1w	*

1w: Extracts w/ one-week storage; 1m: Extracts w/ one-month storage;

RT: Extracts w/ room-temperature storage; 4C: Extracts w/ 4°C storage; n/a: Not applicable

Statistical analysis (Student's t-test) was performed using GraphPad Prism (Dotmatics Inc., San Diego, CA). *, **, and *** denote p-values < 0.05, 0.01, and 0.001, respectively), and 'ns' refers to 'no significance'.

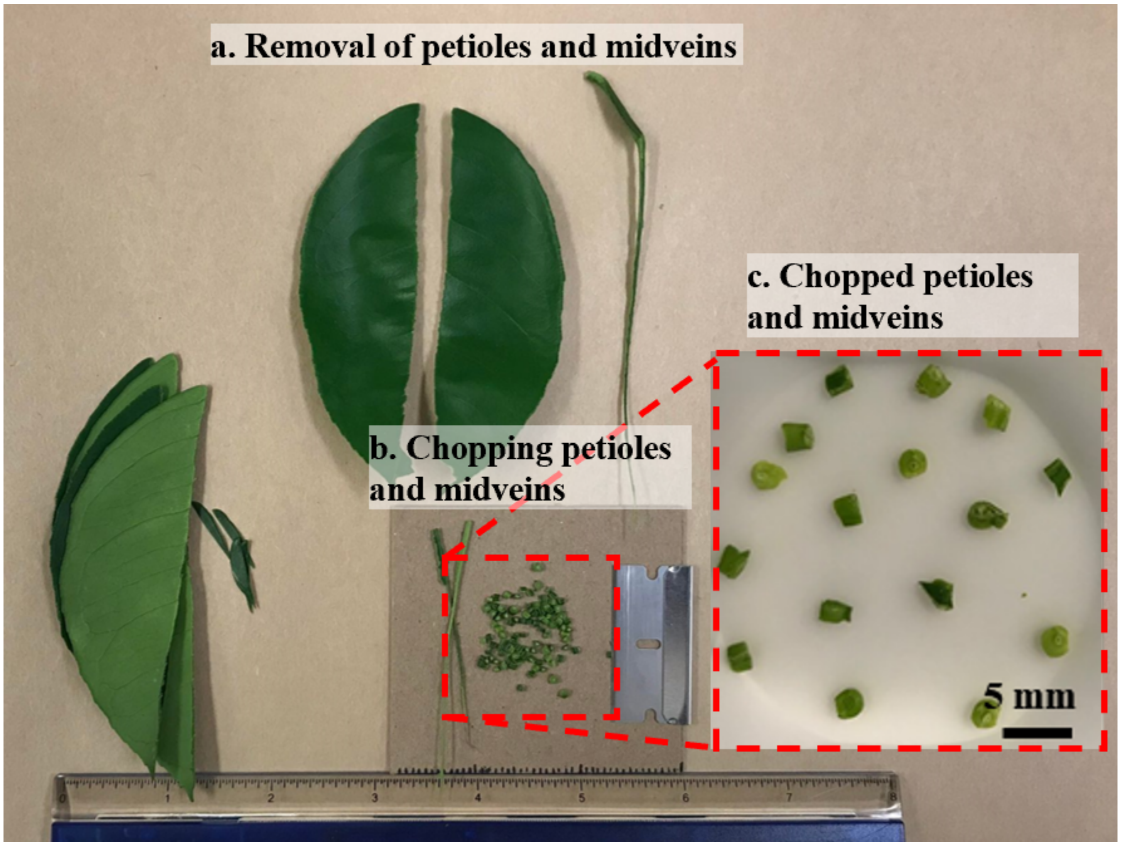
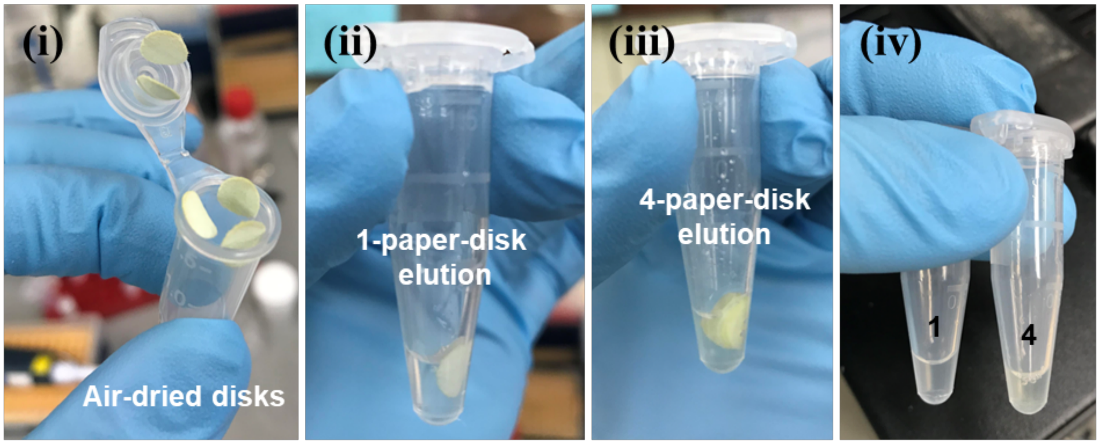
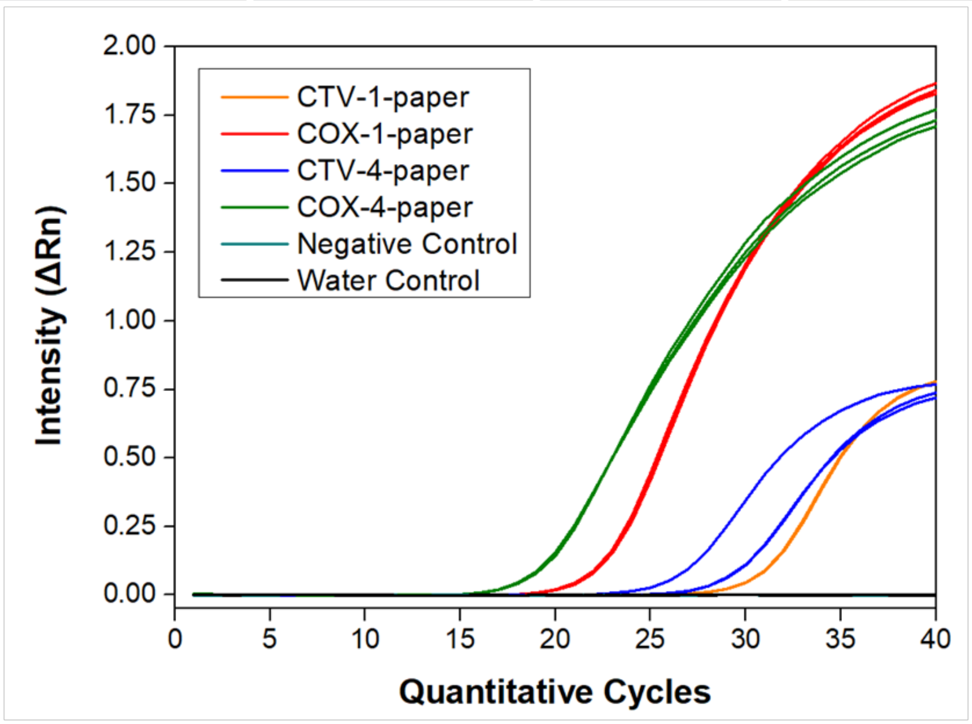


Figure S1. Illustration of the stepwise sample pretreatment of citrus leaves: (a) Removal of petioles and midveins, (b) chopping petioles and midveins, and (c) chopped petioles and midveins.

a.



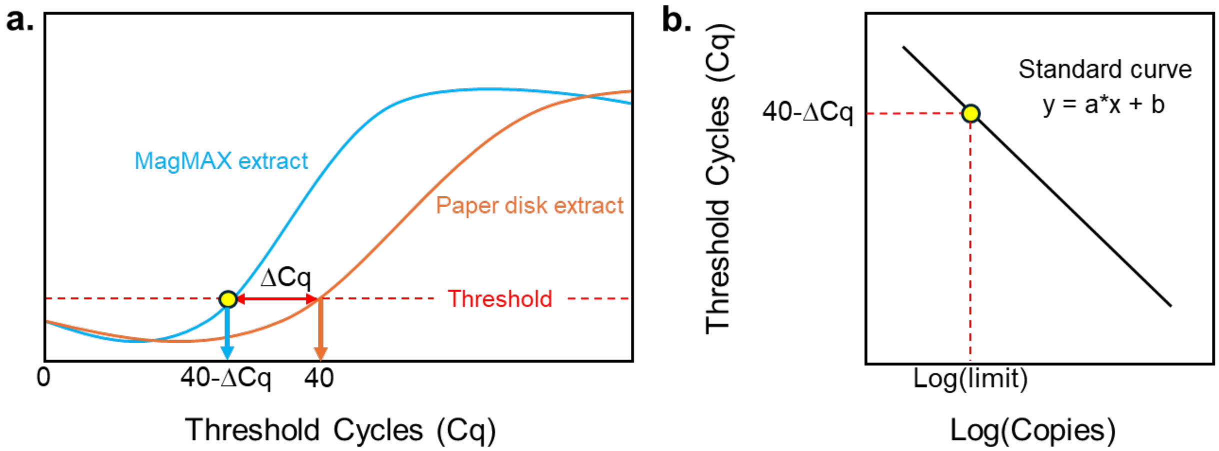
b.



c.

	dsDNA Conc.	RNA Conc.	CTV Cq	COX Cq
1-paper-disk	0.316 ng/uL	3.9 ng/uL	30.53±0.02	22.01±0.07
4-paper-disk	1.29 ng/uL	14.5 ng/uL	28.14±1.58	18.99±0.07

Figure S2. Comparison between 1-paper-disk extract and 4-paper-disk extract. (a) Elution of paper disks: (i) air-dried paper disks; (ii) elution with 1 paper disk; (iii) elution with 4 paper disks, and (iv) extracts from 1 paper disk (left) and 4 paper disks (right). (b) Real-time curves of the RT-qPCR assay. (c) Concentrations of dsDNA and RNA measurement using Qubit (N=1) and the average Cq values with standard deviation from RT-qPCR assay (N=3).



c.

	Equation of the standard curve	Singleplex assay (ΔCq)	Multiplex assay (ΔCq)	Singleplex LoD Copies/reaction	Multiplex LoD Copies/reaction	Ref.
CTV	Single: y = -3.37x + 44.09 Multi: y = -3.58x + 42.35	10.08	4.18	$1.6*10^4$	$6.7*10^1$	[7]
CLBV	Single: y = -3.43x + 36.74 Multi: y = -3.61x + 40.61	11.29	6.38	$2.2*10^2$	$8.6*10^1$	[7]
CPsV	Single: y = -3.60x + 43.03 Multi: y = -3.23x + 41.55	8.82	5.47	$2.0*10^3$	$1.5*10^2$	[7]
CEVd	Single: y = -3.56x + 43.23 Multi: y = -0.98x + 36.80	4.80	4.38	$1.8*10^2$	$1.6*10^1$	[6]
HSVd	Single: y = -3.60x + 42.73 Multi: y = -3.55x + 40.44	4.97	4.46	$1.4*10^2$	$2.4*10^1$	[6]
CBCVd	Single: y = -3.65x + 42.32 Multi: y = -3.32x + 42.50	5.96	4.10	$1.9*10^2$	$9.7*10^1$	[6]

[6] Osman, Fatima, et al. *Journal of Virological Methods* 220 (2017): 40-52.

[7] Osman, Fatima, et al. *Journal of Virological Methods* 245 (2015): 64-75.

Figure S3. Estimation of limits of detection of the paper disk extraction method. (a) Illustration of RT-qPCR real-time curves for MagMAX and paper disk extracts. Assuming a constant difference in Cq values (ΔCq) between the paper disk and MagMAX extracts, a Cq value of 40 is taken as the limit at which qPCR would return a negative result for the paper disks, and ΔCq is subtracted from 40 to find the corresponding Cq value for MagMAX. (b) Illustration of how the copy numbers were obtained from the standard curves. The standard curves are adopted from plasmid serial dilution assay [6, 7]. The MagMAX Cq value is then found on the standard curve for its primers, and then the corresponding copy count is determined. (c) Estimation of the limits of detection of the paper disk method for singleplex and multiplex assays of all the viruses and viroids. These are the estimated copy counts, below which the paper disk method is unable to provide a positive result.