Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

A syndromic diagnostic assay on macrochannel-to-digital microfluidic platform for automatic identification of multiple respiratory pathogens

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Methods

The reproducibility of the volumes of dispensed droplets

The images of the chips were captured by the same camera after the droplets were dispersed. The camera was fixed by a stable bracket and focused on the center of each chip with the same distance to chip when taking photos. Due to mature production technology, plates are strictly parallelized. The heigh of the chamber was assumed to be consistent, therefore, allowing to measure the volume through image analysis software ImageJ (version 1.53). The total volume after elution was exactly 60 μL as the elution buffer was prestored in elution room. Thus, the volumes were calculated by the following equation:

$$Dispensed\ droplet\ volume\ (\mu L) = \frac{Dispensed\ droplet\ area}{Total\ area} \times 60\ \mu L$$

50 chips were processed for reproducibility assay in this study. The intra-assay coefficients of variation (CVs) were calculated by the 8 dispensed droplets on the same chip. The inter-assay CV represented the dispersion degree of the mean values of 50 chips.

Temperature control and monitoring

A thin-film heater and temperature sensors were embedded on the bottom printed circuit board (PCB) to generate heat and maintain a constant temperature for the qPCR reaction. The operations on the DMF chip were managed using automated control electronics (Digifluidic, Guangdong, China), which integrate a proportion integration differentiation (PID) controller and a power amplifier for temperature control. The online temperatures acquired by sensors were used as input for PID adjustment. In temperature monitoring experiment, to accurately capture the actual temperatures at each site, eight thermocouples were seamlessly sticked at the reaction sites and read the real-time temperatures every second.

Results and Discussions

Droplet volume

As shown in Fig.S3, the droplet volume count was nicely fitted (R^2=0.95) by Gauss function as follow.

$$y = Ae^{-\frac{(x-x_c)_2}{2w^2}} = 0.12 \times e^{-\frac{(x-5.55)^2}{2\times 0.34^2}}$$

Where x_c is the axis of symmetry of the Gauss distribution. It suggests that 5.55 μL is the most frequently presented droplet volume; A is the maximal relative frequency, which means 5.55 μL droplet volume was presented with the frequency of 12%; w is the full-width at the half of the maximum (FWHM), which is 0.34; e is a constant (i.e., Euler's Number).

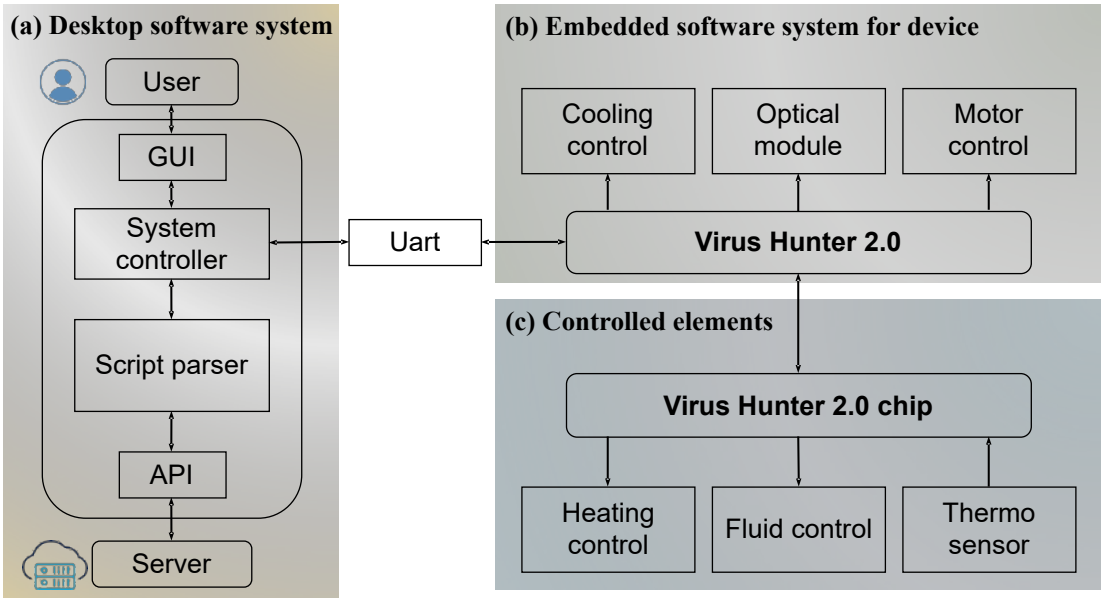

Figure S1. The software control system, composite structure diagram of VH 2.0 device and chip. (a) Desktop software system run on PC and cloud server. (b) Embedded software system for VH 2.0 device. (c) The elements to be controlled on VH 2.0 chips.

Figure S2. The on-chip “sample-answer” workflow. Sample input and preload reagents presented in dash line box. Bead moving paths are indicated by dash lines, while reagents moving path are solid lines.

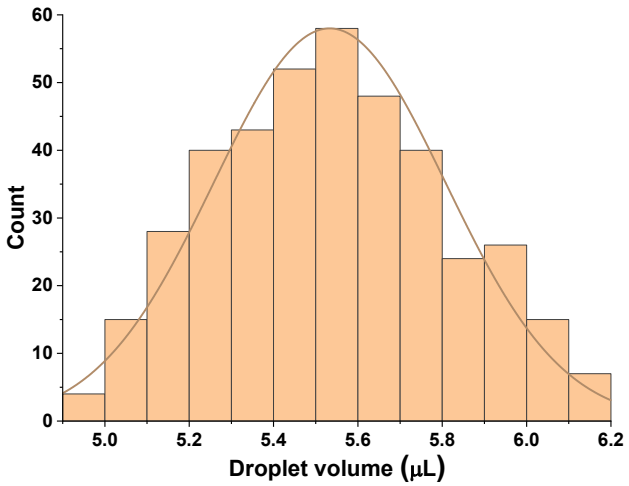

Figure S3. The droplet volume distribution over discrete 400 droplets. It well fitted (R^2=0.95) with Gauss function as the red line indicated.

Table S1. Primers and probes for RT-qPCR

Target Region	Label	Sequence
Influenza A virus M1 gene	FluA-F	GRCCGATCCTSTCACCTCTGAC
	FluA-R	GRGCATTTTGGACAAAGCGTCTACG
	FluA-P-ROX	ROX-TGTTCACGCTCACCGTGCCCAG-BHQ1
Influenza B virus hemagglutinin	FluB-F	GAAGCACTACTTTGCTCGC
	FluB-R	GATTGCAGACATTGAAGAYCTA
	FluB-P-CY5	CY5-CCTAACAACGACCATACTACGAGCA-BHQ1
SARS-CoV-2 nucleocapsid protein	CovN-F	TCACGTAGTCGCAACAGTTCAAGAA
	CovN-R	TCTCAAGCTGGTTCAATCTGTCAA
	CovN-P-FAM	FAM-TAGAATGGCTGGCAATGGCGGTGATG-HQ1
SARS-CoV-2 open reading frames 1	CovORF1-F	CACACTGGTACTGGTCAGGCAATA
	CovORF1-R	ATCTATGTGGCAACGGCAGT
	CovORF1-P-HEX	HEX-CACCGGAAGCCAATATGGATCAAGA-BHQ1

Table S2. Comparison of on-chip and off-chip complete workflow on detecting various pathogenic genes

Concentration (copies/mL)	On-chip			Off-chip		
FluB-CY5	Ct	CV	Detection rate	Ct	CV	Detection rate
1000000	24.47	1.58%	72/72	28.68	0.39%	4/4
100000	28.18	2.69%	72/72	31.51	0.99%	4/4
10000	31.39	1.50%	71/72	35.32	1.16%	4/4
1000	34.05	2.49%	70/72	38.97	2.08%	4/4
100	35.83	2.59%	18/40	/	/	/
FluA-ROX	Ct	CV	Detection rate	Ct	CV	Detection rate
1000000	24.61	3.09%	72/72	25.41	0.20%	4/4
100000	28.53	2.51%	72/72	28.59	0.57%	4/4
10000	31.22	2.54%	71/72	32.29	0.31%	4/4
1000	34.09	3.29%	70/72	35.80	0.70%	4/4
100	37.32	2.22%	29/40	39.19	3.06%	4/4
SARS-CoV-2(ORF1)-HEX	Ct	CV	Detection rate	Ct	CV	Detection rate
10000000	16.55	3.27%	16/16	21.06	0.74%	8/8
1000000	21.58	3.96%	72/72	25.02	1.00%	8/8
100000	23.70	2.39%	72/72	28.44	1.19%	8/8
10000	27.14	4.32%	71/72	32.07	1.81%	8/8
1000	30.26	3.24%	70/72	34.53	0.88%	8/8
100	33.34	2.20%	71/72	/	/	/
10	36.00	2.43%	24/72	/	/	/
SARS-CoV-2(N)-FAM	Ct	CV	Detection rate	Ct	CV	Detection rate
10000000	18.54	3.94%	16/16	19.94	1.37%	8/8
1000000	23.70	1.64%	72/72	23.79	1.30%	8/8
100000	26.49	2.22%	72/72	27.11	1.11%	8/8
10000	29.86	3.18%	71/72	31.42	1.63%	8/8
1000	32.76	3.39%	94/96	33.21	1.13%	8/8
100	35.63	4.20%	47/72	37.39	2.16%	6/8

The tests with low detection rate (<50%, printed in red) was not included in the standard curve generation.

Table S3. The summary of Pearson correlation between on- and off-chip clinic specimen

Target	Pearson's R	*p*
FluA-ROX	0.91	0.0013
SARS-CoV-2(N)-FAM	0.70	0.0002
SARS-CoV-2(ORF1)-HEX	0.93	0.0004

Table S4. Summary of the clinic specimens

Number	On-chip			Off-chip		
FluA-ROX	Mean	CV	rate	Mean	CV	rate
1	25.72	2.24%	8/8	28.34	2.08%	4/4
2	31.71	1.45%	8/8	33.58	0.20%	4/4
3	28.92	2.95%	8/8	30.71	0.91%	4/4
4	28.39	1.75%	8/8	25.45	0.23%	4/4
5	32.50	1.81%	8/8	30.68	0.56%	4/4
6	35.24	6.38%	8/8	33.65	1.34%	4/4
7	32.46	2.72%	8/8	32.28	0.73%	4/4
8	29.73	1.49%	8/8	28.85	0.35%	4/4
9	32.32	1.73%	8/8	31.68	0.70%	4/4
10	35.11	3.70%	7/8	34.90	1.31%	4/4
11	36.07	4.01%	7/8	34.23	1.25%	4/4
SARS-CoV-2(N)-FAM	Mean	CV	rate	Mean	CV	rate
1	26.51	0.99%	8/8	25.12	0.50%	4/4
2	28.96	2.36%	8/8	26.93	0.24%	4/4
3	26.59	1.94%	8/8	28.09	0.31%	4/4
4	29.90	1.85%	8/8	30.2	0.82%	4/4
5	30.82	1.11%	8/8	30.69	1.19%	4/4
6	30.67	1.35%	8/8	30.88	1.47%	4/4
7	29.93	2.40%	8/8	29.8	1.29%	4/4
8	26.39	2.14%	8/8	25.26	0.28%	4/4
9	30.84	1.73%	8/8	31.15	0.79%	4/4
10	30.58	1.86%	8/8	30.03	0.47%	4/4
11	27.88	5.23%	8/8	28.34	0.56%	4/4
SARS-CoV-2(ORF1)-HEX	Mean	CV	rate	Mean	CV	rate
1	23.91	2.88%	8/8	24.26	0.46%	4/4
2	25.42	1.42%	8/8	26.11	1.16%	4/4
3	24.04	1.57%	8/8	27.11	0.43%	4/4
4	27.29	2.64%	8/8	29.43	0.57%	4/4
5	28.09	1.08%	8/8	29.94	0.60%	4/4
6	28.28	1.28%	8/8	30.23	0.91%	4/4
7	28.32	3.17%	8/8	29.13	0.38%	4/4
8	23.16	2.39%	8/8	24.39	1.23%	4/4
9	29.07	1.41%	8/8	30.33	0.19%	4/4
10	30.46	1.80%	8/8	29.09	1.25%	4/4
11	25.65	4.46%	8/8	27.39	1.39%	4/4

Negative samples were not shown.

Table S5. Probability associated with a two-tailed distribution Student's t-test

Target	Concentration (copies/mL)	Mean Ct	Inter-assay CV	1 mon. vs 2 mon.	2 mon. vs 3 mon.	1 mon. vs 3 mon.
FluA	1000	34.09	0.76%	0.32	0.12	0.47
FluB	1000	34.03	0.71%	0.16	0.11	0.95
SARS-CoV-2 (N)	1000	32.64	0.61%	0.30	0.50	0.47
SARS-CoV-2 (ORF1)	100	33.12	0.56%	0.12	0.92	0.27