Integrated microfluidic detection system for automated and rapid diagnosis of high-risk human papillomavirus

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Supplemental Information

Supplemental Table 1. The primer set sequences for *β-actin* qPCR assay

Item	Sequences (5'-3')
FP	AGCGAGCATCCCCCAAAGTT
BP	GGGCACGAAGGCTCATCATT

Supplemental Table 2. The primer set sequences for *HPV18* qPCR assay[1].

Item	Sequences (5'-3')	Genome position	
FP	GCATGGACCTAAGGCAACAT	E7 oncogene region	3-22
BP	GAAGGTCAACCGGAATTTCAT		56-76

Supplemental Table 3. The primer sets sequences for *HPV LAMP* assay[2,3].

Type	Item	Sequences (5'-3')	Genome position
HPV16	FIP	GTGGCCCTGTGCTCGTTG-TCTATGGTTACCTCTGATGCC	6593–6576/6528–6548
	BIP	CACGCAGTACAAATATGTCA-CCCCATGTCGTAGGTACTCC	6644–6665/6738–6719
	F3	CAAATTATTTTCCTACACCTAGTGG	6502–6526
	B3	GTCATAACGTCTGCAGTTAAGG	6802–6781
	LF	GCTGCCATATCTACTTCAGAAACTACA	6672–6698
HPV18	FIP	GGCACCATATCCAGTATCTACCATA ATTGCCCCCCTTTAGAACT	6209-6233/6163-6181
	BIP	TGCAAGATACTAAATGTGAGGTACC GCAGACATTTGTAAATAATCAGGAT	6250-6274/6304-6328
	F3	CGCGTCCTTTATCACAGG	6142-6159
	B3	TGGAATCCCCATAAGGATC	6336-6348
	LF	TCACCATCTTCCAAAACTG	6190-6208
	LB	ATTGGATATTTGTCAGTCT	6275-6293
HPV39	FIP	AACATATACCATTGTTGTGGCCC-TTCCATGGTAACCTCTGATTCCC	6612–6590/6530–6552
	BIP	CTACCCGTAGTACCAACTTTAC-CCACGTGCCTGGTATATTCC	6646–6667/6744–6725
	F3	GTTCTGTATACTGCCCCTCTC	6502–6522
	B3	GACATAACATCAGTTGTTAATGTGAC	6808–6783
	LF	CCTTATGTAGCCAATAAGGC	6585–6566
HPV45	FIP	ATGACATAACCTCTGCAGTTAAAGT TGTGGAGGAATATGATTTACAGTT	6763-6787/6717-6740
	BIP	AATTGGAATTTTGGTGTCCCTCCAC TGATTGCACAAAACGATA	6820-6844/6871-6888
	F3	ACTAAGTTTAAGCACTATAGTAGAC	6691-6715
	B3	CCTTTTGACAGGTAACAGC	6892-6910
	LF	AGTGCACAACTGAAAA	6744-6759
	LB	ACCACCTACTACAAGTTTAGTGGA	6843-6866
HPV52	FIP	ATTATTGTGGCCCTGCGCACG-TTCTATGGTAACCTCAGAATCCC	6620–6600/6548–6570
	BIP	ACCACTCGTAGCACTAACATGAC-TCGCCATGACGAAGGTATTCCT	6663–6685/6757–6735
	F3	GCCACTGTACAAAGCAGTGC	6507–6526
	B3	TGAATGTATGTCATAACATCAGCTG	6829–6805
	LB[f]	GCTGAGGTTAAAAAGGAAAGCACA	6693–6716

Note: Dashes in the FIP and BIP primer sequences indicate the two regions linked by a TTTT linker.

Supplemental Table 4. The exact Tt values of the *specificity* experiments results.

Item	Time Threshold (min)
HPV16	19.91
	20.91
	20.43
HPV18	17.27
	17.92
	17.65
HPV39	9.02
	8.95
	8.61
HPV45	8.06
	7.65
	7.68
HPV52	11.01
	10.53
	10.81

Supplemental Table 5. The exact Tt values of the *stability* experiments results.

Item	No.	Time Threshold (min)	
		Strong	**Weak**
HPV16	1	9.12	14.97
	2	9.16	15.76
	3	9.33	15.82
	4	9.57	16.17
	5	9.85	16.20
	6	9.98	16.34
	7	10.02	16.72
	8	10.15	16.89
	9	10.15	16.92
	10	10.33	17.23
	11	10.57	17.27
	12	10.57	17.85
	SD	0.5056	0.7933
	C.V.	5.11%	4.80%
HPV18	1	11.74	14.00
	2	11.49	14.38
	3	10.91	14.14
	4	10.84	12.59
	5	10.70	14.52
	6	10.57	13.52
	7	10.46	13.59
	8	11.63	13.87
	9	11.29	12.97
	10	10.50	13.87
	11	10.67	14.62
	12	10.88	12.97
	SD	0.4504	0.6477
	C.V.	4.10%	4.71%
HPV39	1	7.13	12.49
	2	7.37	12.63
	3	7.37	12.87
	4	7.89	13.01
	5	7.96	13.14
	6	7.99	13.18
	7	7.99	13.21
	8	8.02	13.35
	9	8.02	13.69

	10	8.09	13.93
	11	8.09	14.00
	12	8.57	14.18
	SD	0.3957	0.5430
	C.V.	5.03%	4.08%
HPV45	1	7.58	11.49
	2	7.61	11.87
	3	7.65	11.91
	4	7.68	11.91
	5	7.68	12.04
	6	7.71	12.22
	7	7.89	12.70
	8	7.92	12.73
	9	7.92	12.73
	10	8.02	12.80
	11	8.20	12.94
	12	8.37	13.59
	SD	0.2482	0.5969
	C.V.	3.16%	4.81%
HPV52	1	5.62	8.20
	2	5.72	8.95
	3	5.76	9.06
	4	5.86	9.16
	5	6.00	9.16
	6	6.03	9.36
	7	6.17	9.40
	8	6.24	9.64
	9	6.27	9.67
	10	6.37	9.74
	11	6.51	9.81
	12	6.55	9.85
	SD	0.3109	0.4720
	C.V.	5.10%	5.06%

Supplemental Table 6. The exact Cycle threshold values and converted Time Threshold values of the HPV18 qPCR experiments results.

No.	Cycle Threshold	Time Threshold (min)
1	21.7	16.47
2	20.66	15.77
3	20.98	15.99
4	20.78	15.85
5	20.6	15.73
6	20.64	15.76
7	21.92	16.61
8	20.55	15.70
9	19.59	15.06
10	19.71	15.14
11	20.48	15.65
12	19.48	14.99
13	17.64	13.76
14	20.42	15.61
15	23.12	17.41
16	20.48	15.65
17	20.54	15.69
18	21.09	16.06
19	19.98	15.32
20	20.77	15.85
21	20.32	15.55
22	20.13	15.42
23	20.08	15.39
24	21.3	16.20
25	22.15	16.77
26	22.08	16.72
27	24.14	18.09
28	22.23	16.82
29	21.8	16.53
30	22.86	17.24
31	21.84	16.56
32	22	16.67
33	21.89	16.59
34	21.57	16.38
35	21.96	16.64
36	22.02	16.68
37	28.5	21.00
38	21.98	16.65

39	21.6	16.40
40	22	16.67
41	21.97	16.65
42	23.8	17.87
43	28.77	21.18
44	21.83	16.55
45	21.88	16.59
46	21.5	16.33
47	21.28	16.19
48	21.68	16.45

Supplemental Table 7. The exact Tt values of the HPV18 LAMP experiments results.

No.	Time Threshold (min)
1	9.88
2	10.91
3	10.50
4	9.09
5	8.95
6	12.25
7	12.59
8	10.81
9	10.09
10	11.29
11	11.43
12	8.92
13	10.02
14	9.40
15	9.30
16	9.57
17	9.71
18	11.08
19	9.50
20	9.33
21	9.02
22	12.18
23	10.50
24	11.12
25	9.50
26	9.50
27	9.50
28	9.30
29	13.59
30	12.29
31	11.53
32	12.22
33	12.87
34	11.74
35	11.77
36	11.49
37	10.91
38	10.84
39	10.70

|	
40	10.57
41	10.46
42	11.63
43	11.29
44	10.50
45	10.67
46	10.88
47	12.66
48	12.90
49	12.59
50	10.05
51	9.09
52	9.23
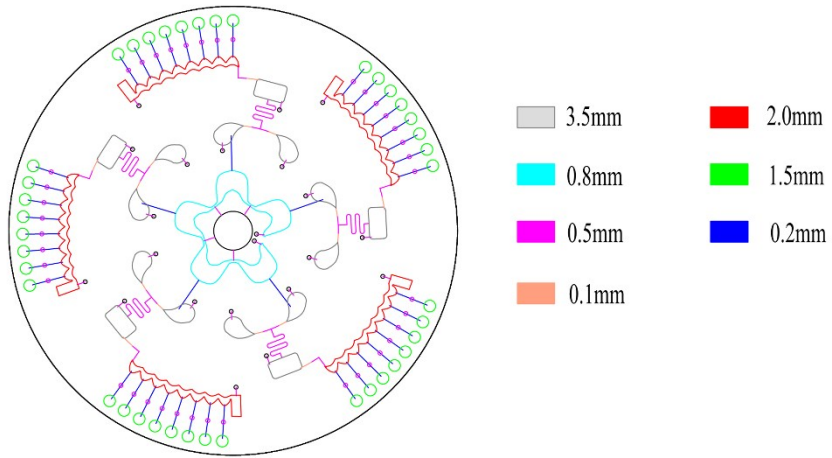
Supplemental Figure 1. Detailed dimensions of the microfluidic chip. The line represents the microchannel and the closed region represents the chamber. The depth is distinguished by different color.
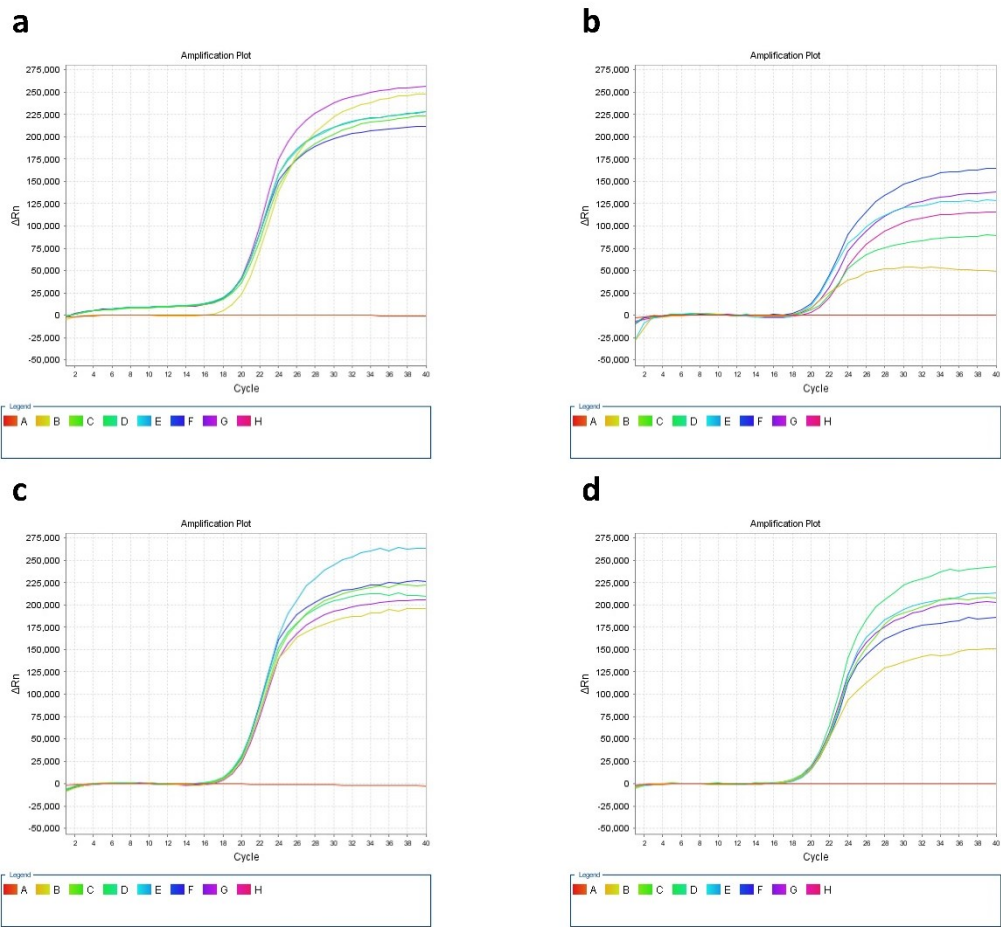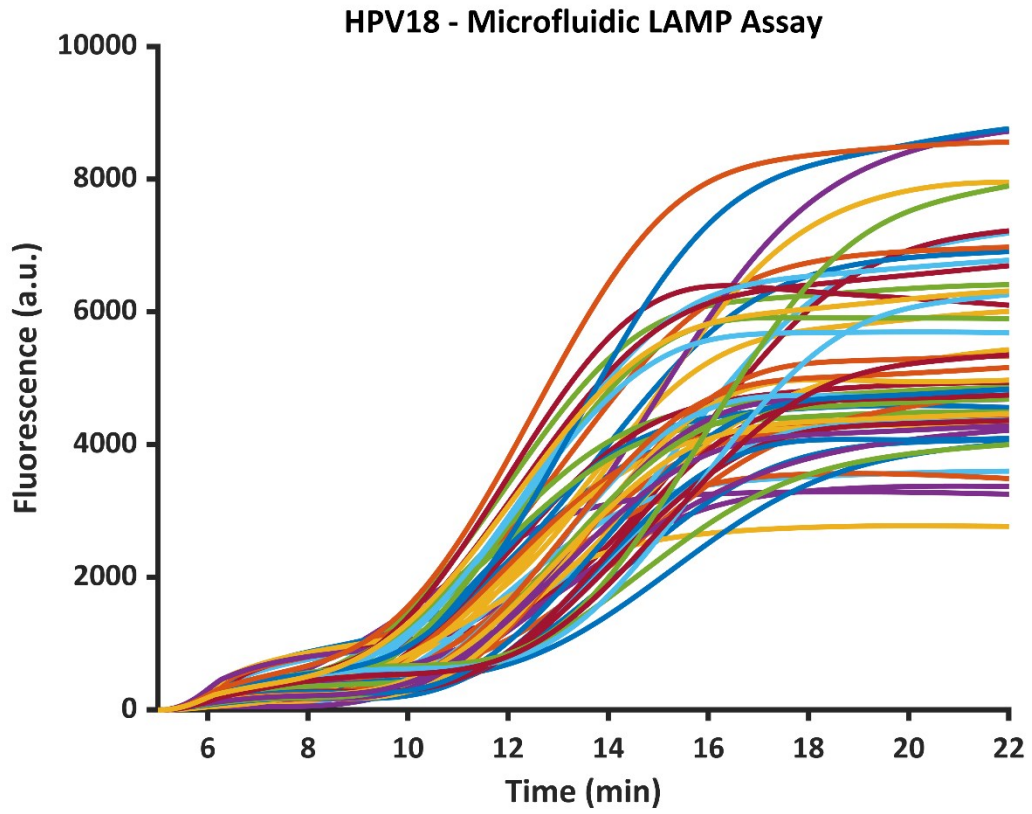
Supplemental Figure 2. The amplification curves of nucleic acid detection based on four purification methods, including (a) microfluidic Chelex-100, (b) pyrolysis, (c) commercial kit A and (d) commercial kit B.
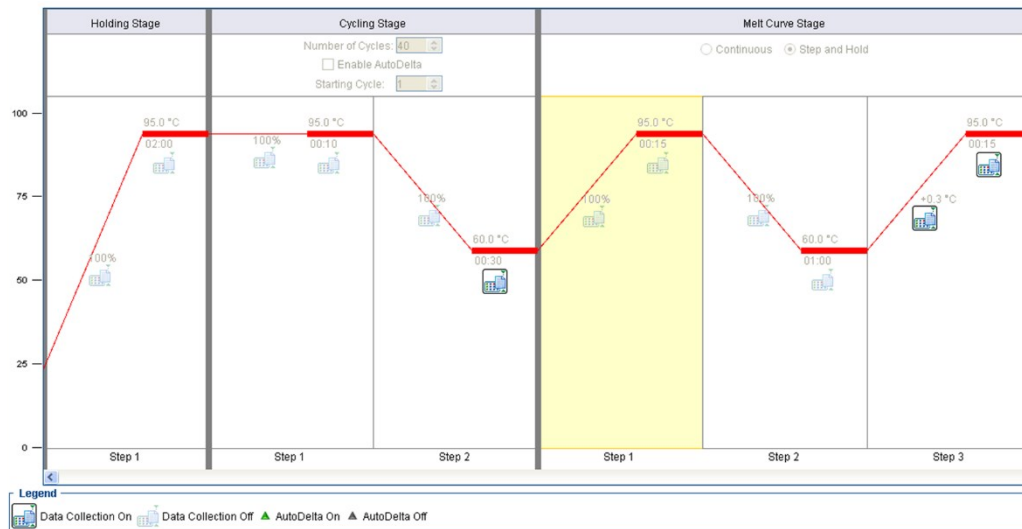
Supplemental Figure 3. The qPCR run methods, including holding stage, cycling stage and melt curve stage, used for conversion from Ct into Tt. The converting equation is Tt = Ct * 0.6667 + 2.
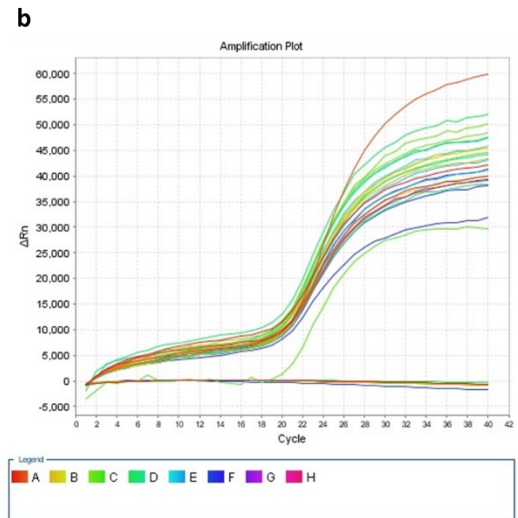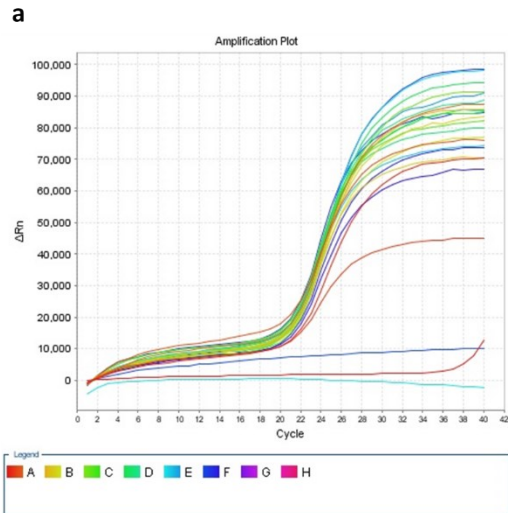
Supplemental Figure 4. The amplification curves of qPCR assay for HPV18 diagnosis. The results of 48 duplicated experiments are divided into two plots: (a) 1st to 24th results (24 positive and 3 negative controls), (b) 24th to 48th results (24 positive and 4 negative controls).

Supplemental Figure 5. The amplification curves of microfluidic LAMP assay for HPV18 diagnosis (n=52, 48 positive sample and 4 false positive). Replicate experiments are displayed in different colors with intention to observe easily.

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