Supporting Information

Synergistically enhanced colorimetric molecular detection using smart cup: a case for instrument-free HPV-associated cancer screening

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Table S1 LAMP primers for HPV 16, 18, 31, *E. Coli* O157, *Salmonella* and λDNA

Target	Primer	Primer Sequence (5'-3')
HPV 16 [1]	F3	CAAATTATTTTCCTACACCTAGTGG
	B3	GTCATAACGTCTGCAGTTAAGG
	FIP	GTGGCCCTGTGCTCGTTGTCTATGGTTACCTCTGATGCC
	BIP	CACGCAGTACAAATATGTCACCCCATGTCGTAGGTACTCC
	Loop F	GCTGCCATATCTACTTCAGAAACTACA
HPV 18 [1]	F3	TGTATTCTCCCTCTCCAAGTG
	B3	GAATATAGGACATAACATCTGCAG
	FIP	GCCAGCAAACACCATTGTTACTCTATTGTTACCTCTGACTCCC
	BIP	ACCACTCGCAGTACCAATTTAACCCTCAACATGTCTGCTATACTGC
	Loop F	ACCCTGTGCCTTATGTAACC
HPV 31 [1]	F3	AACTCAACGCTTAGTTTGGGC
	B3	CCTTTACCCCAATGCTCTCC
	FIP	GGATGACCACTAATACCTACACCCTGTGTTGGTTTAGAGGTAGGTC
	BIP	CACTGAAAACTCTAATAGATATGCCGGTGCAACCAAGTAAACACAGTT GTG
	Loop F	TAATGGCTGCCCGCGA
E. Coli [2]	F3	TCGGTGTCTGTTATTAACCA
	B3	TGGAAACCGTTGTCACAC
	FIP	AGACGAAGATGGTCAAAACGCGCAGTTATTTTGCTGTGGA
	BIP	CCGGGTTCGTTAATACGGCACGGGCACTGATATATGTGT
	Loop F	TGATAGACATCAAGCCCTCGT
	Loop B	CAAATACTTTCTACCGTTTT
Salmonella [3]	F3	GGCGATATTGGTGTTTATGGGG
	B3	AACGATAAACTGGACCACGG
	FIP	GACGACTGGTACTGATCGATAGTTTTTCAACGTTTCCTGCGG
	BIP	CCGGTGAAATTATCGCCACACAAAACCCACCGCCAGG
	Loop F	GACGAAAGAGCGTGGTAATTAAC
	Loop B	GGGCAATTCGTTATTGGCGATAG
λDNA [4]	F3	GGCTTGGCTCTGCTAACACGTT
	B3	GGACGTTTGTAATGTCCGCTCC
	FIP	CAGCCAGCCGCAGCACGTTCGCTCATAGGAGATATGGTAGAGCCGC
	BIP	GAGAGAATTTGTACCACCTCCCACCGGGCACATAGCAGTCCTAGGGA CAGT
	Loop F	CTGCATACGACGTGTCT
	Loop B	ACCATCTATGACTGTACGCC

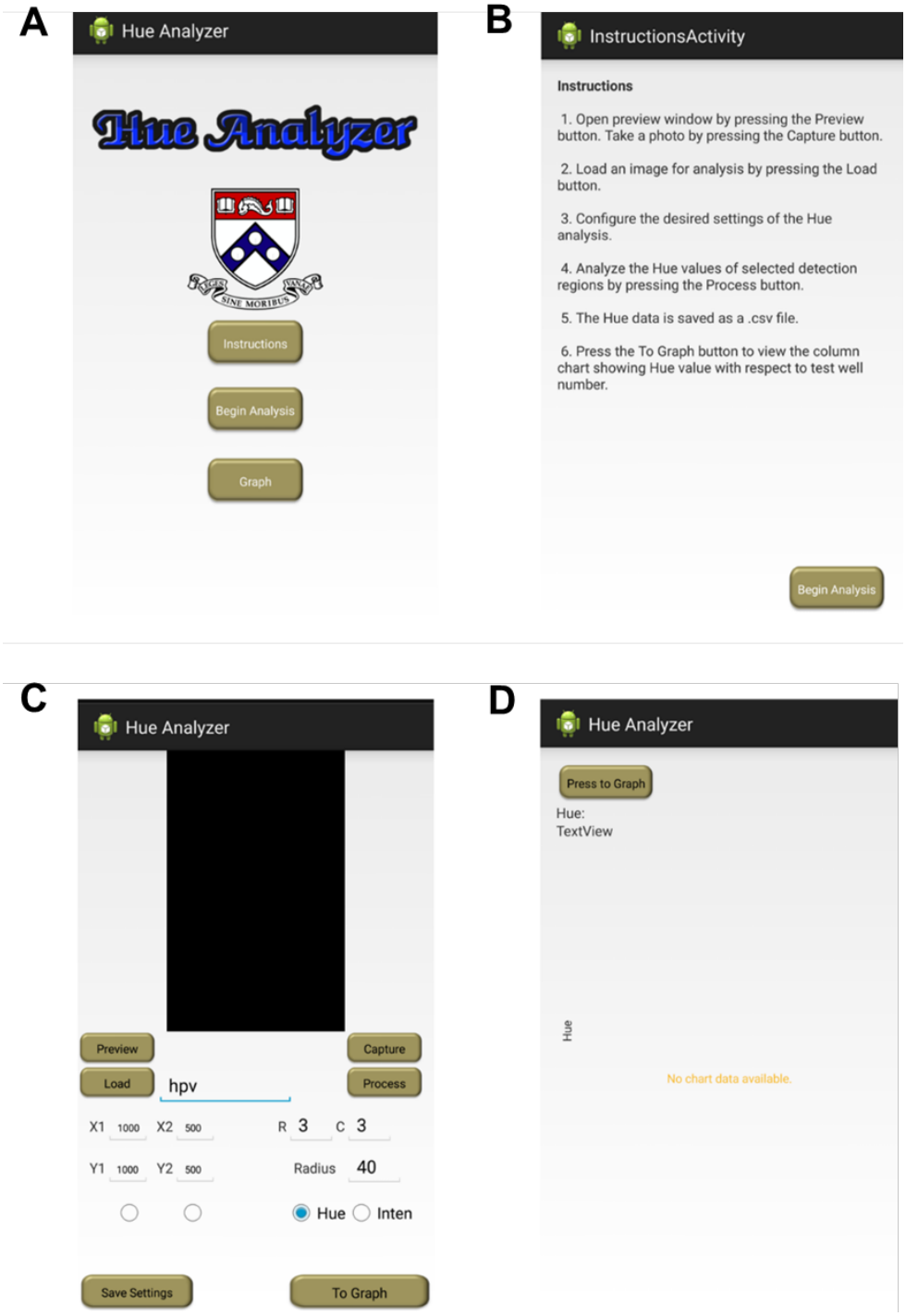

Figure S1 Screenshots of the interfaces of our custom Hue Analyzer app for hue value quantification and HPV-associated cancer screening. (A) Main menu, (B) Instructions, (C) Parameter settings and image capturing, and (D) Data analysis and result reporting.

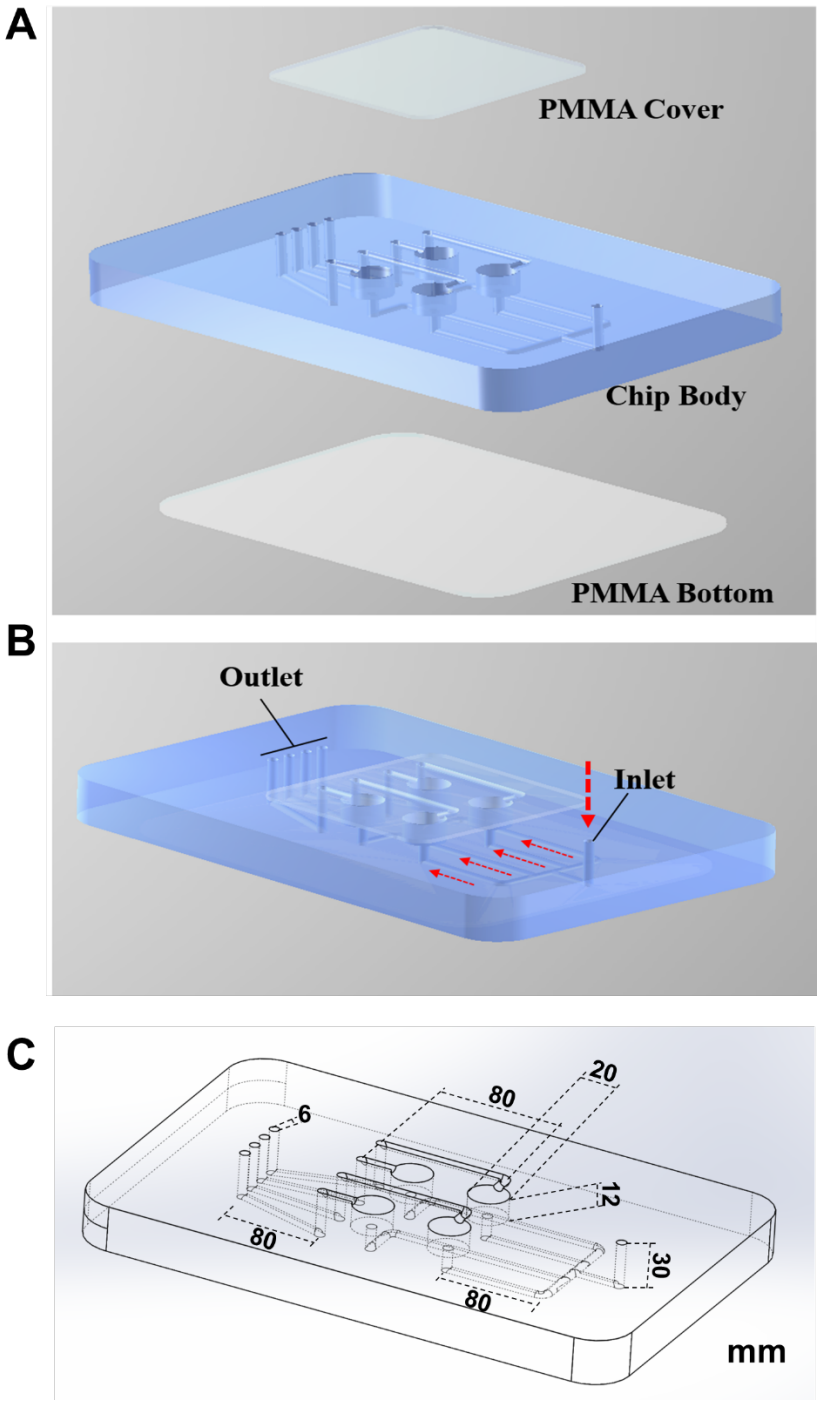

Figure S2 Schematic illustration of microfluidic chip housing four reaction chambers. (A) An exploded view of the chip used for detecting multiple HPV genotypes. The chip consists of three layers: one top PMMA film, one PMMA chip body, and one PMMA bottom. The various features of the chip body were milled with a computer numerical control (CNC) machine. (B) Assembled microfluidic chip. (C) CAD illustration of the chip.

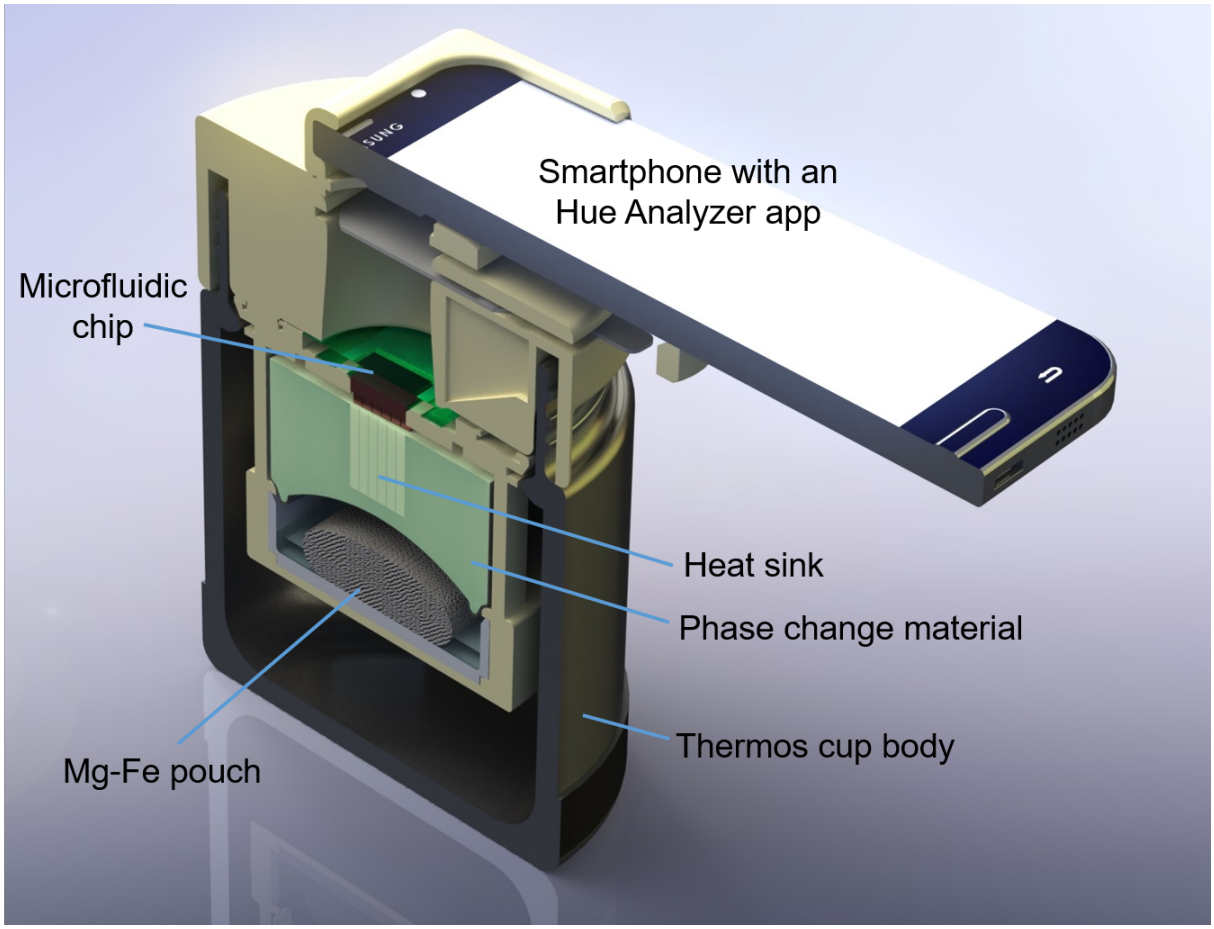

Figure S3 Cross-section of our smart cup-based POC diagnostic platform for HPV-associated cancer screening.

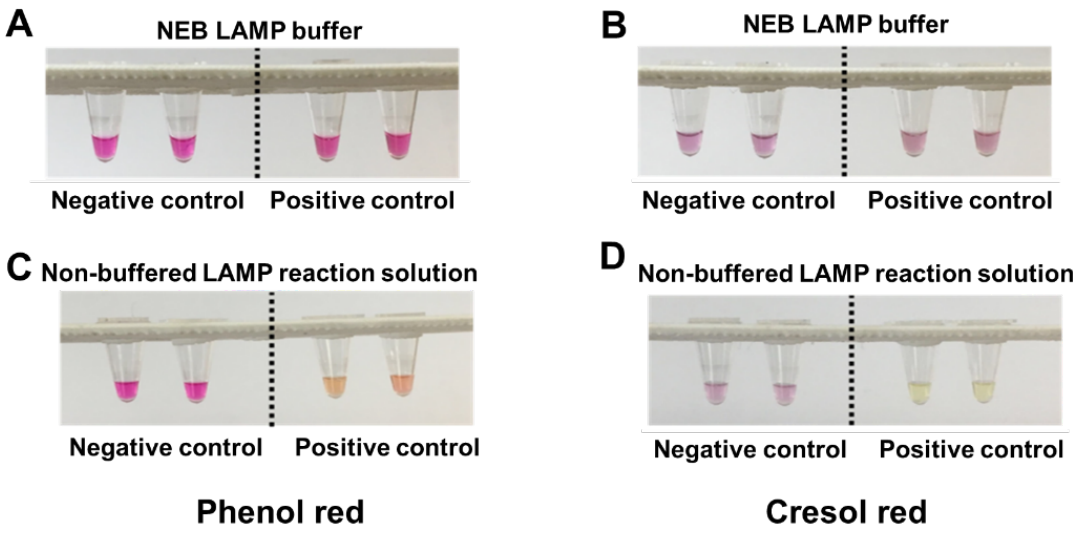

Figure S4 pH change after 60-min LAMP reaction (with/without 10^4 copies of HPV 16 DNA per reaction). (A, B) NEB LAMP buffer solution and (C,D) non-buffered LAMP solution. (A, C): 100 μM phenol red was used as the pH indicator. (B, D): 100 μM cresol red was used as the pH indicator.

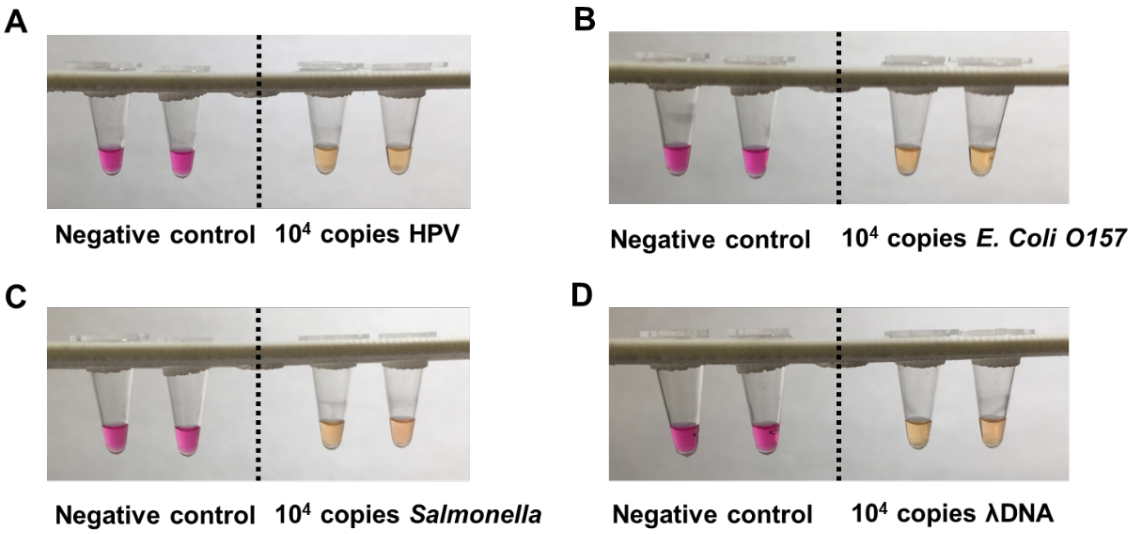

Figure S5 pH value change of different nucleic acid targets detection in non-buffered LAMP solution after 60-min LAMP reaction. (A) HPV 16, (B) *E. coli* O157, (C) *Salmonella,* and (D) λDNA. 100 μM phenol red was used as the pH indicator.

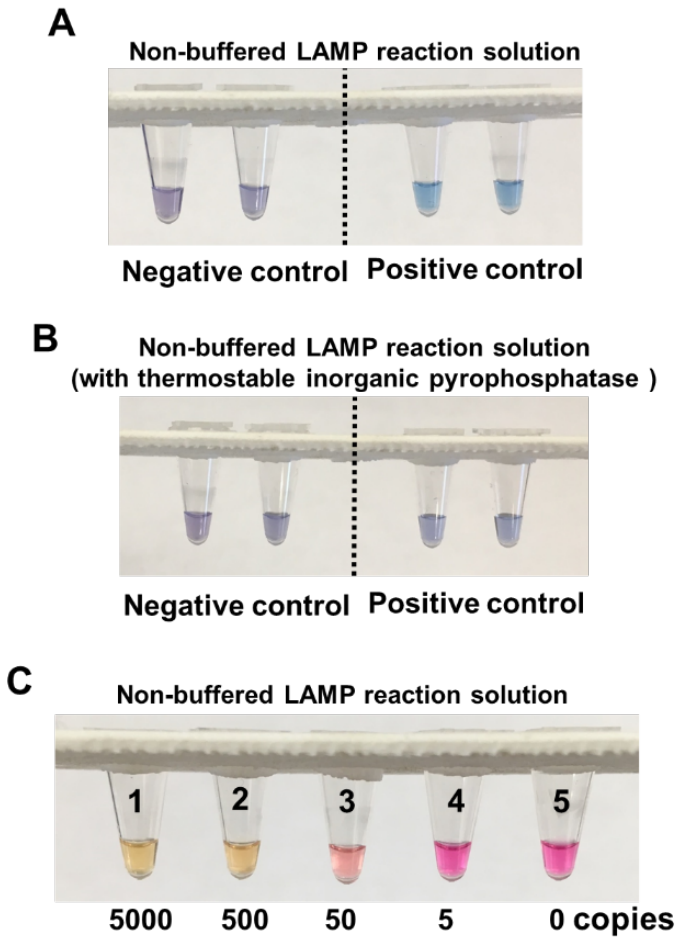

Figure S6 Comparison of colorimetric LAMP detection between synergistic effect and non-synergistic effect. (A) Colorimetric LAMP detection of HPV 16 DNA (10^4 copies) in non-buffered LAMP solution without pyrophosphatase. (B) Colorimetric LAMP detection of HPV 16 DNA (10^4 copies) in non-buffered LAMP solution with pyrophosphatase. (C) Colorimetric LAMP assay of 10-fold serially diluted HPV 16 DNA in non-buffered LAMP solution by using Phenol red as the pH indicator.

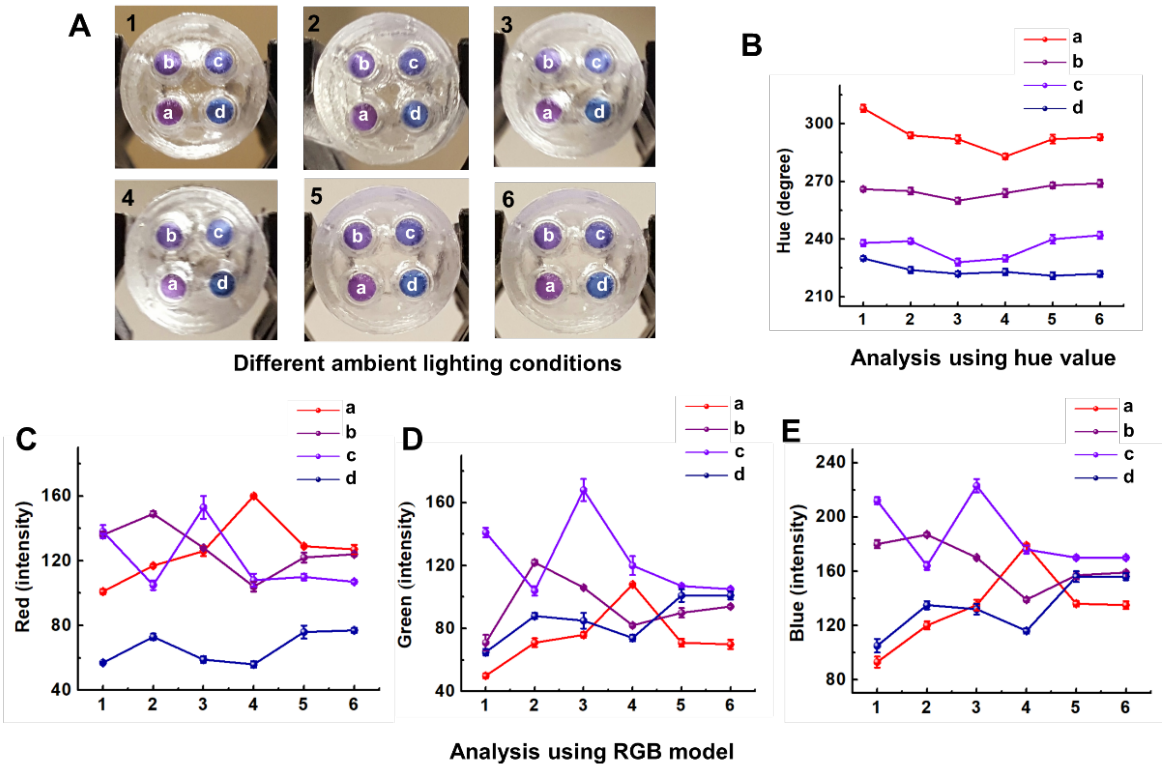

Figure S7 Reliability comparison of colorimetric detection between hue value analysis and RGB model. (A) Serial optical images of HNB indicator in the presence of Mg^{2+} ions (a: 16 mM, b: 12 mM, c: 8 mM, and d: 4 mM) under different ambient lighting conditions. (B) Hue values of HNB in chamber a, b, c and d under different ambient light. (C-E) Red, Green, and Blue values of HNB in chamber a, b, c and d under different ambient light. Error bars denote s.d. (n=3).

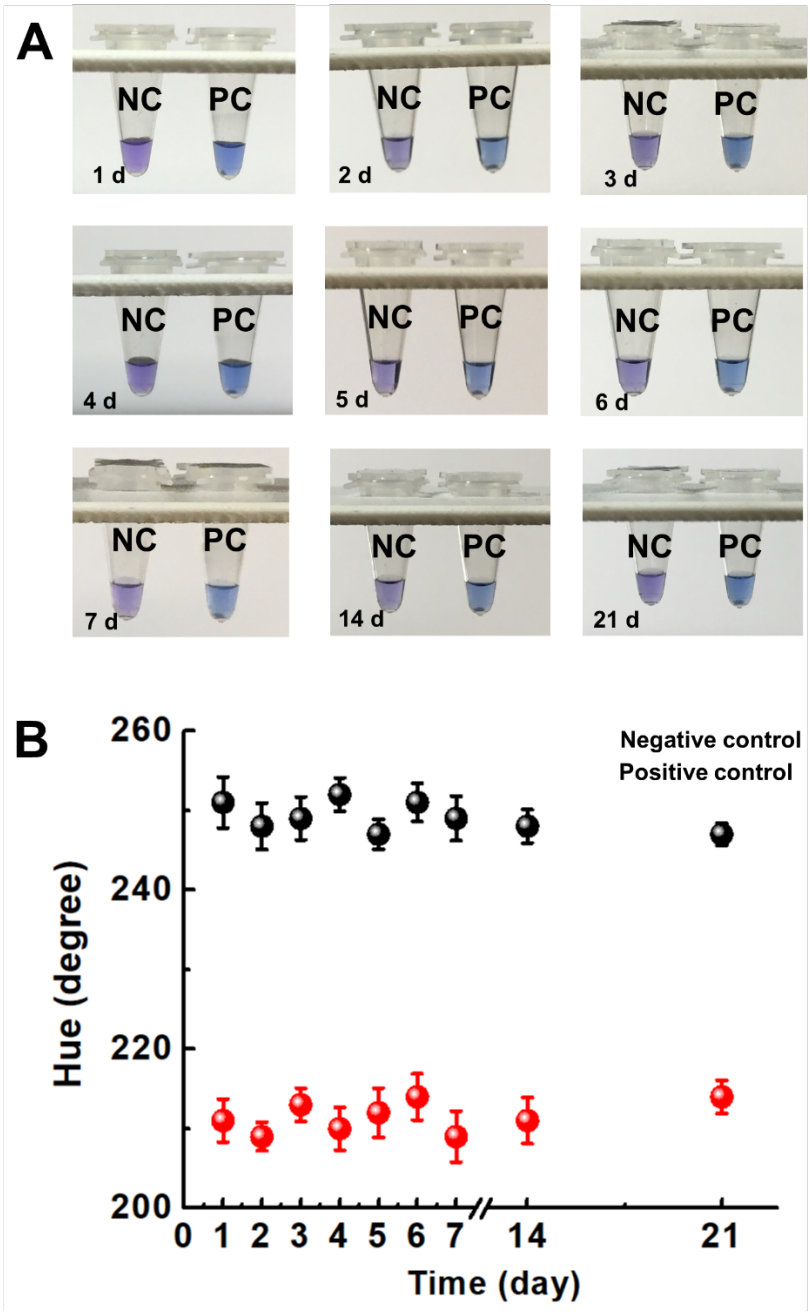

Figure S8 Stability evaluation of HNB indicator in non-buffered LAMP solution after LAMP reaction. (A) Serial optical images of LAMP amplification products including negative control (NC) and positive control (PC). (B) Hue value comparsion of LAMP amplification products stored at room temperature at different time (days). Error bars denote s.d. (n=3).

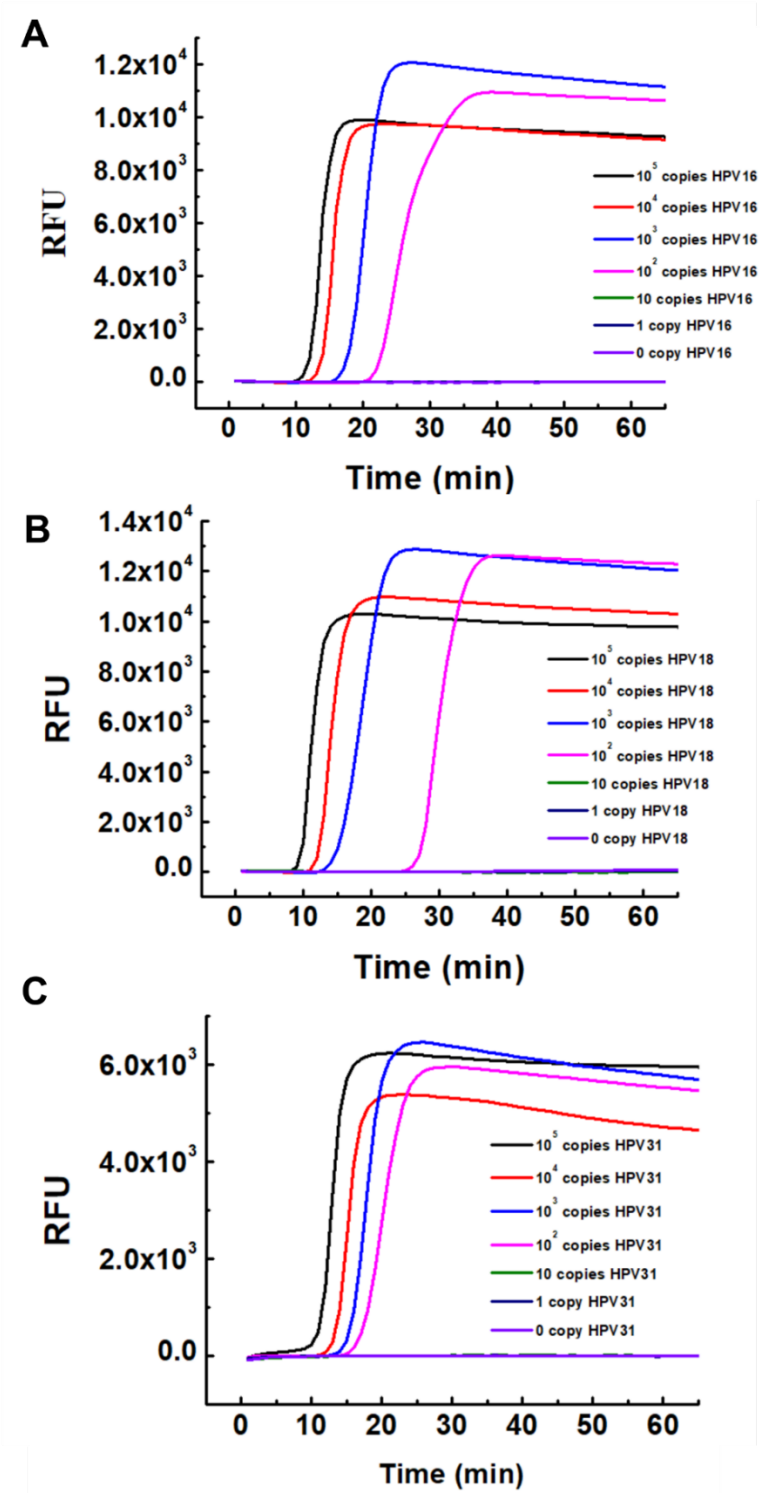

Figure S9 Real-time fluorescence LAMP amplification curves of 10-fold serially diluted HPV DNAs in Optigene GspSSD2.0 Isothermal Mastermix. (A) HPV 16 DNA, (B) HPV 18 DNA and (C) HPV 31 DNA.

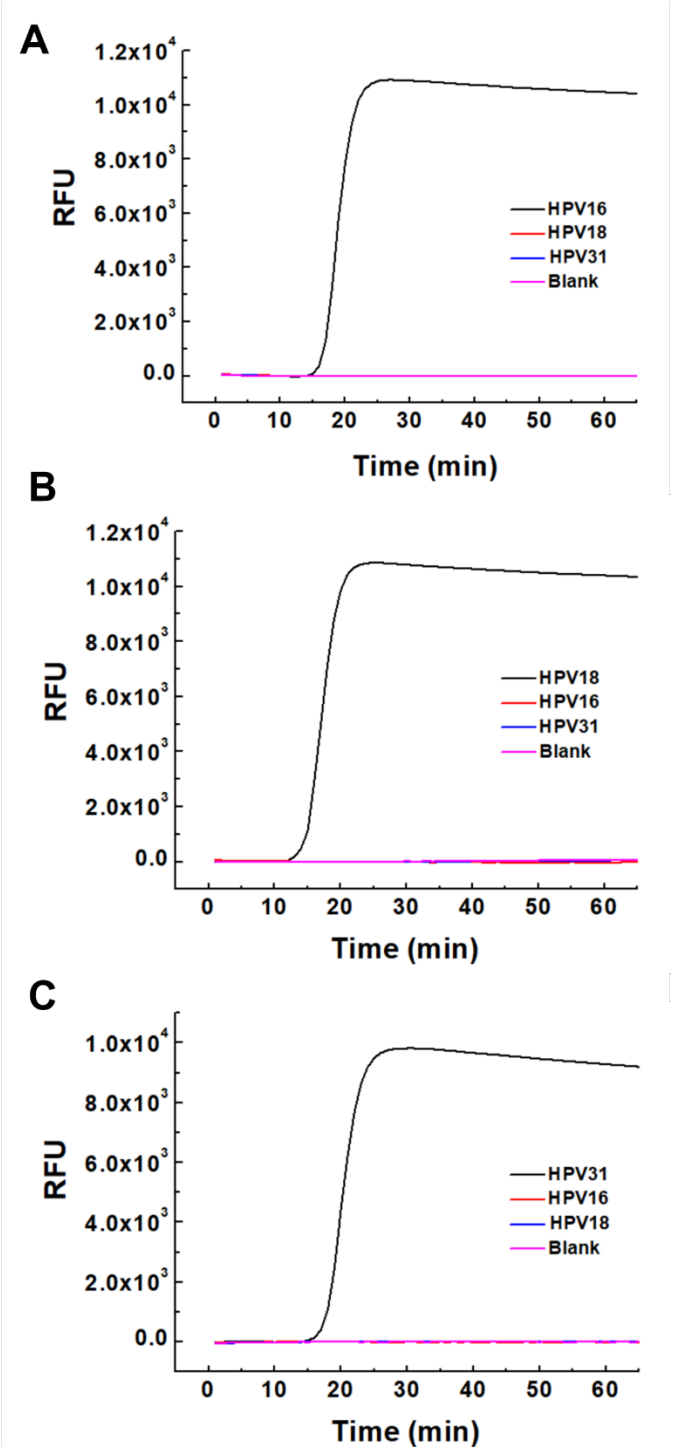

Figure S10 Specificity analysis of HPV DNA detection by real-time fluorescence LAMP amplification in Optigene GspSSD2.0 Isothermal Mastermix. (A) 10^3 copies of HPV 16 DNA template was added to LAMP reaction vials with HPV 16, 18 and 31 primers and without any primers (blank control), respectively. (B) 10^3 copies of HPV 18 DNA template was added to LAMP reaction vials with HPV 16, 18 and 31 primers and without any primers (blank control), respectively. (C) 10^3 copies of HPV 31 DNA template was added to LAMP reaction vials with HPV 16, 18 and 31 primers and without any primers (blank control), respectively.

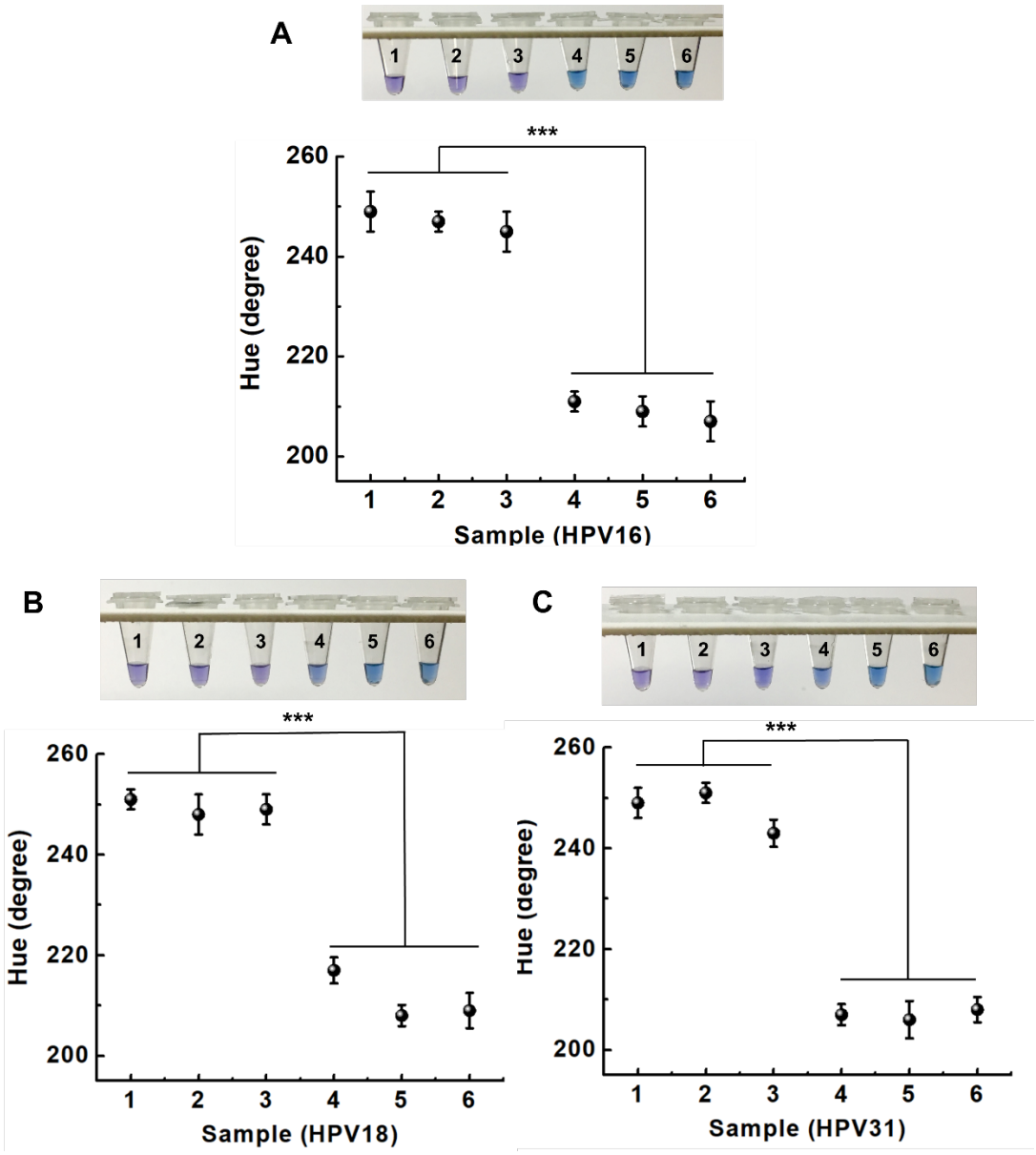

Figure S11 Colorimetric detection and hue value analysis of LAMP amplification products of 10-fold diluted HPV DNA. (A) HPV16 DNA, (B) HPV 18 DNA, and (C) HPV 31 DNA. The HPV DNA concentrations in 1-6 groups are, respectively, 0, 1, 10, 10^2, 10^3, and 10^4 copies per reaction. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among groups 4-6 (10^2 to 10^4 copies per reaction) and group 1 (negative control, 0 copy per reaction). Error bars denote s.d. (n=3).

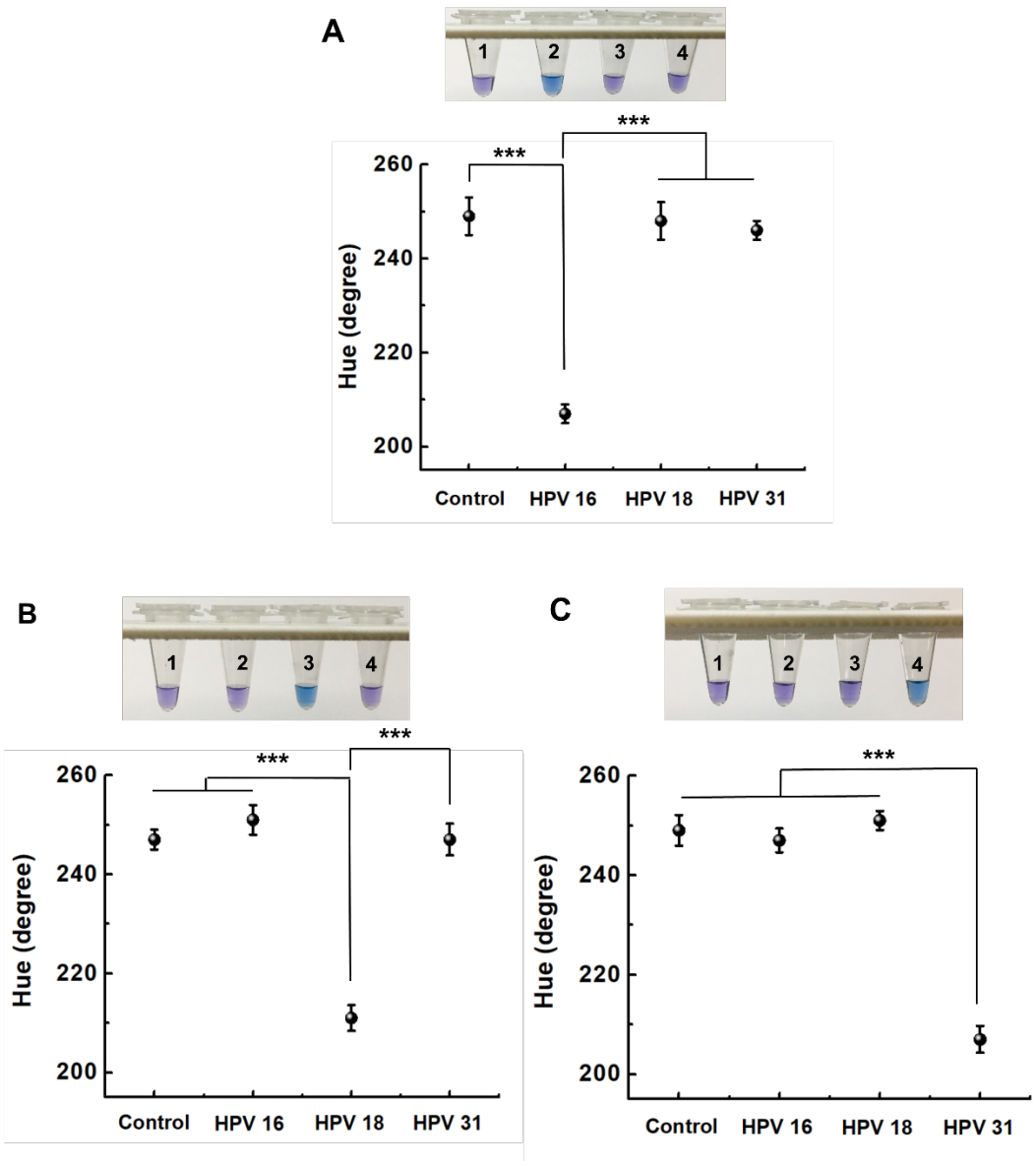

Figure S12 Specificity analysis of the colorimetric LAMP detection and hue value analysis. (A) HPV 16 positive, (B) HPV 18 positive and (C) HPV 31 positive. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV positive group and other groups. Error bars denote s.d. (n=3).

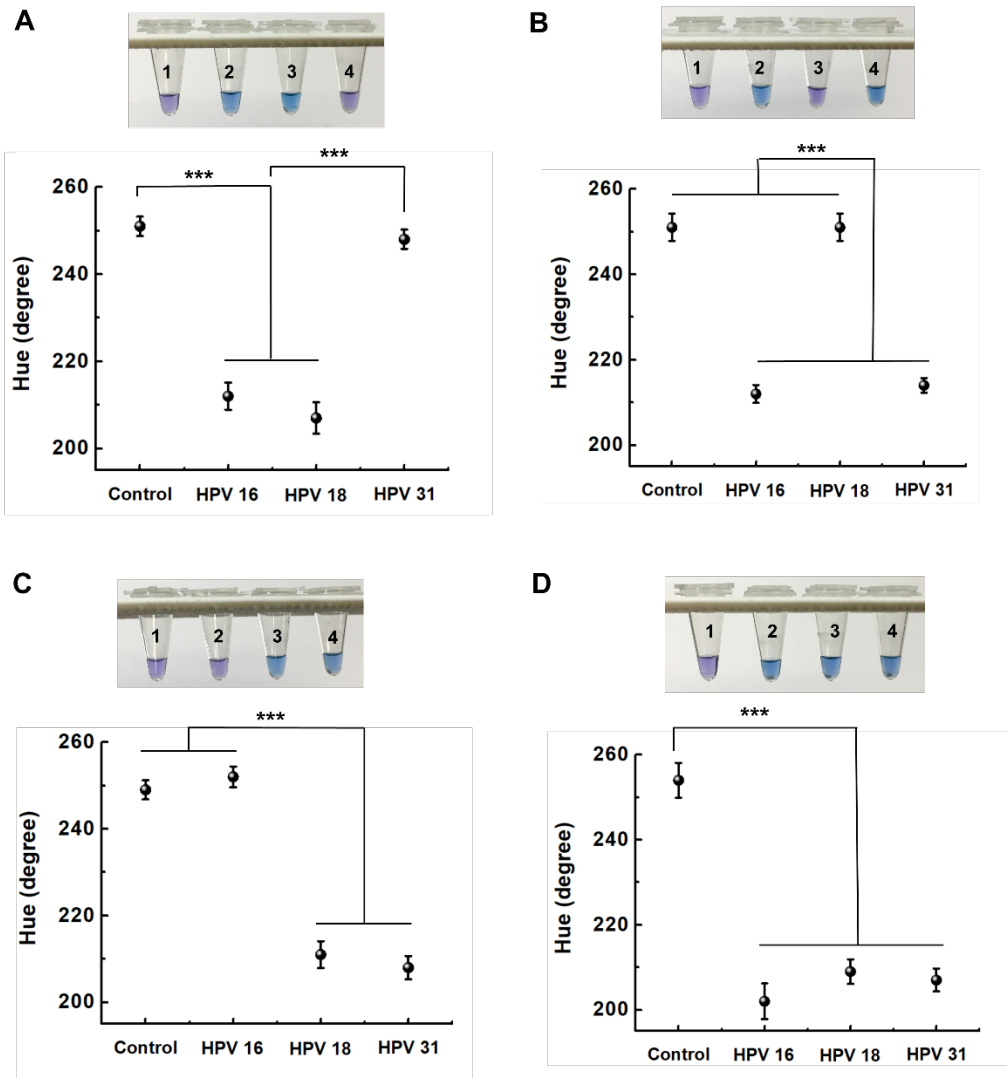

Figure S13 Colorimetric detection and hue value analysis of multiple HPV DNA in the same sample. (A) Simultaneous detection of HPV 16 and 18 in the same sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16/18 and other groups. (B) Simultaneous detection of HPV 16 and 31 in the same sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16/31 and other groups. (C) Simultaneous detection of HPV 18 and 31 in the same sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 18/31 and other groups. (D) Simultaneous detection of HPV 16, 18 and 31 in the same sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16, 18 and 31 and blank control group (no HPV primers inside). Error bars denote s.d. (n=3).

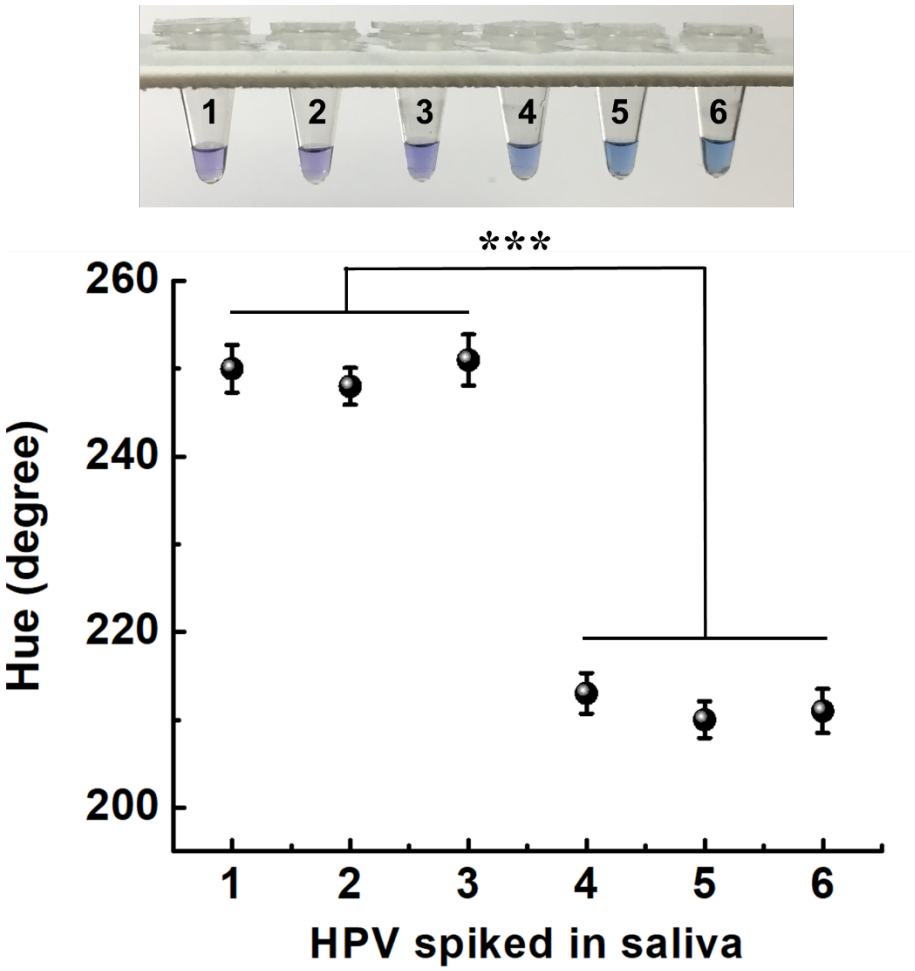

Figure S14 Colorimetric LAMP detection and hue value analysis of HPV 16 spiked in saliva. The concentration of HPV16 groups ranges from 10^4 to 0 copies per reaction. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among groups 4-6 (10^4 to 10^2 copies per reaction) and group 1 (negative control, 0 copy per reaction). Error bars denote s.d. (n=3).

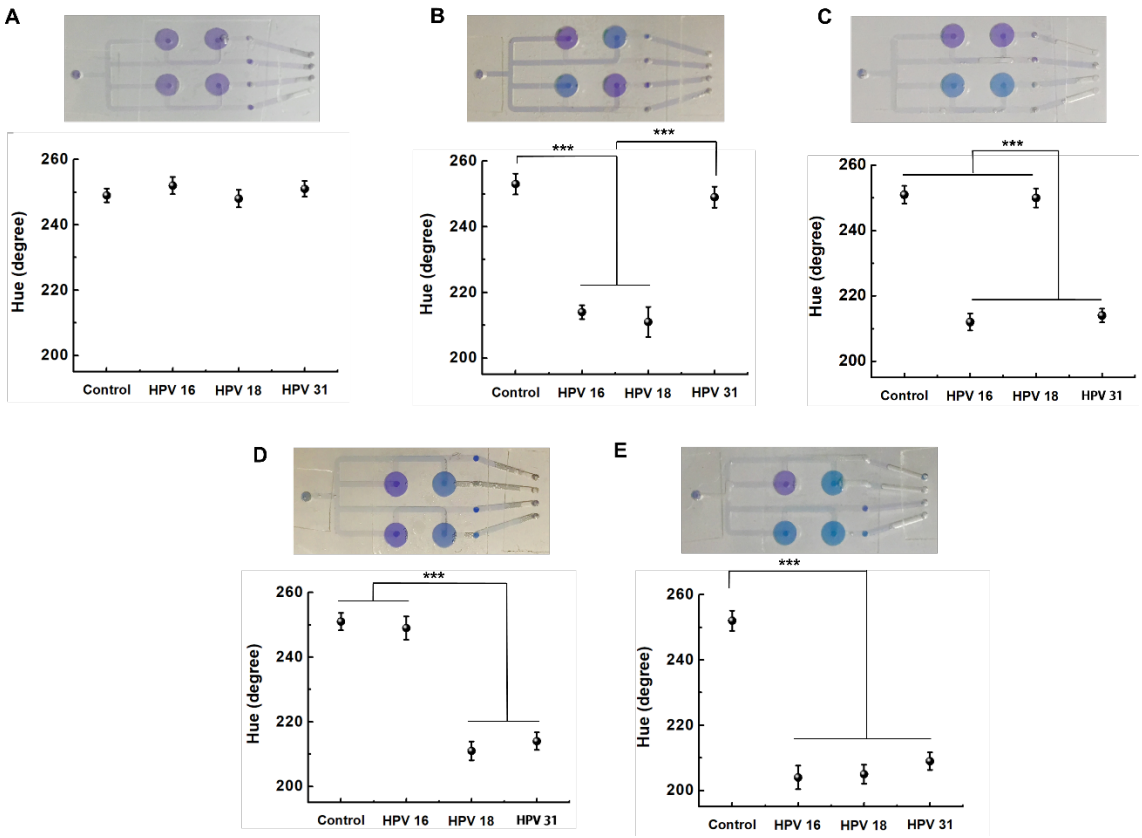

Figure S15 Simultaneous detection of multiple HPV DNAs using our POC diagnostic platform. (A) Negative saliva sample was tested. (B) Simultaneous detection of spiked HPV 16 and 18 in the saliva sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16/18 and other groups. (C) Simultaneous detection of spiked HPV 16 and 31 in the saliva sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16/31 and other groups. (D) Simultaneous detection of HPV 18 and 31 in the saliva sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test,) among group HPV 18/31 and other groups. (E) simultaneous detection of spiked HPV 16, 18 and 31 in the saliva sample. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16, 18, 31 and control group (blank control). Error bars denote s.d. (n=3).

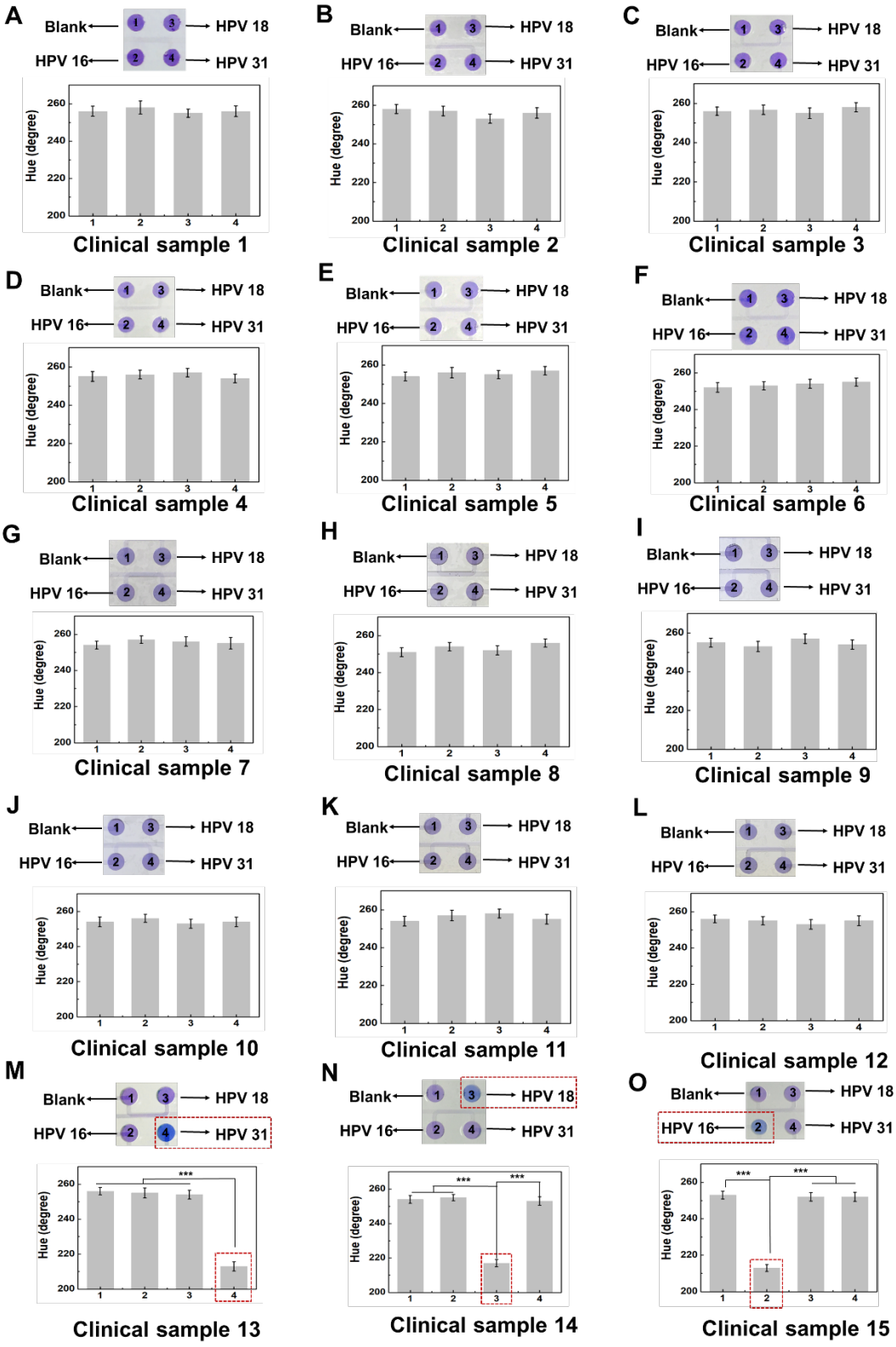

Figure S16 HPV-associated cervical cancer screening in clinical swab samples in our POC diagnostic platform. (A-L) negative clinical samples. (M-O) positive clinical samples. *** indicates a significant difference in the hue value ($p < 0.001$, t-test) among group HPV 16, 18, 31 and other groups, respectively. The red dash box indicates positive results. Error bars denote s.d. (n=3).

Reference

1. Satoh T, Matsumoto K, Fujii T, Sato O, Gemma N, Onuki M, et al. Rapid genotyping of carcinogenic human papillomavirus by loop-mediated isothermal amplification using a new automated DNA test (Clinichip HPV™). *J Virol Methods*. 2013; 188: 83-93.
2. Zhao X, Li Y, Wang L, You L, Xu Z, Li L, et al. Development and application of a loop-mediated isothermal amplification method on rapid detection Escherichia coli O157 strains from food samples. *Mol Biol Rep*. 2010; 37: 2183-8.
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4. Schlappi TS, McCalla SE, Schoepp NG, Ismagilov RF. Flow-through capture and in situ amplification can enable rapid detection of a few single molecules of nucleic acids from several milliliters of solution. *Anal Chem*. 2016; 88: 7647-53.