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

Supporting information for:

Smartphone-Based Multiplex 30-minute Nucleic Acid Test of Live Virus from Nasal Swab Extract

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Pathogens	Assay	Sequences
Streptococcus equi subsp. equi (S. equi)	LAMP	**F3**: AAA ACT AAG TGC CGG TGC
		B3: GAG GCG CCT TTT AGA AGA
		FIP: TAC GAC TAA CCT CAG AGT TCG CTA TCA GTA TTA GTT GCA ACA AGT G
		BIP: CGA CTC CAA GAT TAT CGC GTG ATT GAA CTT TTT GGG CTG ATG A
		Loop F: ACA GTT GTC CCT CCC AAC A
		Loop B: GCG ATA TAG CCA TAA GTG GAG ATG
Streptococcus equi subsp. zooepidemicus (S. zoo)	LAMP	**F3:** AAA GAC CCT CAT GGG AAA T
		B3: CCT TAG TTG CCG CAT AGG
		FIP: CCT GAC TAA CCA AAT ATA AGC CCT TGA GCT GGA CGA TAA GAC CT
		BIP: TGT TGG ACG TAT TTT GGT TGC TCT TCT GAG CCT TCT AAA CCT G
		Loop B: GGT GTC ATT ATT AAC ATG GCC TCT
Equine herpesvirus 1 (EHV1)	LAMP	**F3:** GGC ATT TAC GTG TGG TCC TT
		B3: TCG CGG GCA TTT TTG TAC C
		FIP: GTC CAG CAA CGG TGC GTT GTG GCA CGC TCG TTA ACA GT
		BIP: CGA GCC TGA AGG GGG AAA ACT GGA GCT GTG TGG AAA GTA GC
		Loop F: AGG TTG AGA CGG TAA CGC TG
		Loop B: CAC GTG CGT CGT CGC AA
	PCR	**F:** GCG CCA GCT GTT TAA CCT TC
		R: CGG GCA TTT TTG TAC CAC CG
Equine herpesvirus 4 (EHV4)	LAMP	**F3:** CAA GAC GTA ACA ACG GGA GT
		B3: CGC AAG TAA CGG CGA TGA
		FIP: CGC TCT CCG TTT TCT TCC GAC AAG CCA CCC AGG ATT AGT CAA
		BIP: TTA CCC GGA CGG CCT TCC AAC GGG CAT GTC CTC AAC AA
		Loop F: GCC TGC TAC TCC GCA TG
		Loop B: AGC GTT GTA TAT GAT GCA TCC CCT
Equine influenza virus (EIV)	LAMP	**F3:** ATG CAA TGC TAG GAG ACC
		B3: AGA AAC TAT CGG CTG ATC C
		FIP: TGT CAT ATG GGT AGC AAT TGC TGC AGT ATG AGA ATT GGG ACC T
		BIP: GCA TCC TCA GGA ACA TTR GAY TCC GTT TTG AGT GAC AC
		Loop F: AAA GCG CTG CTT CTT TCT
		Loop B: AGG GAT TCA CAT GGA CAG

Table S1. Sequences of the primers used for detecting five equine pathogens. LAMP primers were designed for S. equi and S. zoo, targeting the seM and sorD genes respectively.[1] The primers for EHV1,[2, 3] EHV4[2] and EIV[4] were obtained from literature.

Pathogens	Assay	Sequences
Escherichia coli (E. coli)	LAMP	**F3:** TGA CTA AAA TGT CCC CGG
		B3: CGT TCC ATA ATG TTG TAA CCA G
		FIP: GAA GCT GGC TAC CGA GAC TCC CAA AAG CAA CAT GAC CGA
		BIP: GCG ATC TCT GAA CGG CGA TTC CTG CAA CTG TGA CGA AG
		Loop F: GCC GCA TAA TTT AAT GCC TTG TCA
		Loop B: ACG CGA AAG ATA CCG CTC T
Equine herpesvirus 3 (EHV3)	LAMP	**F3:** AAA ACG GGG ATA ACG AGC
		B3: GCG AGA GTA GCT GTT GTG
		FIP: CTT CTG CTG TCC GCA CTC AGG ATC TCC GTG GTA CAC C
		BIP: GAC CAA CTA CGC GGG TAT CGG ACA ATG AGG CCG ATG AG
		Loop F: CTC ATC TTG GGA GTG TGT CTC
		Loop B: GGA CCG GAC ACG AGA ATG
Scrambled primers with no target (SC)	LAMP	**F3:** AGC TTC ATT ATT AGA CCA CTT GC
		B3: GTC TAT TAT TCC GAA TCA CG
		FIP: GAG TAT CAC ATG TAA GGA CTT CTA ATA CAA CTT CGT CTC AAT TCC
		BIP: GTT ACT GTT GGT ACT ACA ACT AGC TCC TGG TTC TTG TTA TAC AAT A
		Loop F: ACT CCG GCG TGA GTA GTA AT
		Loop B: CGA TGG GTG TGC AGG ACT CA

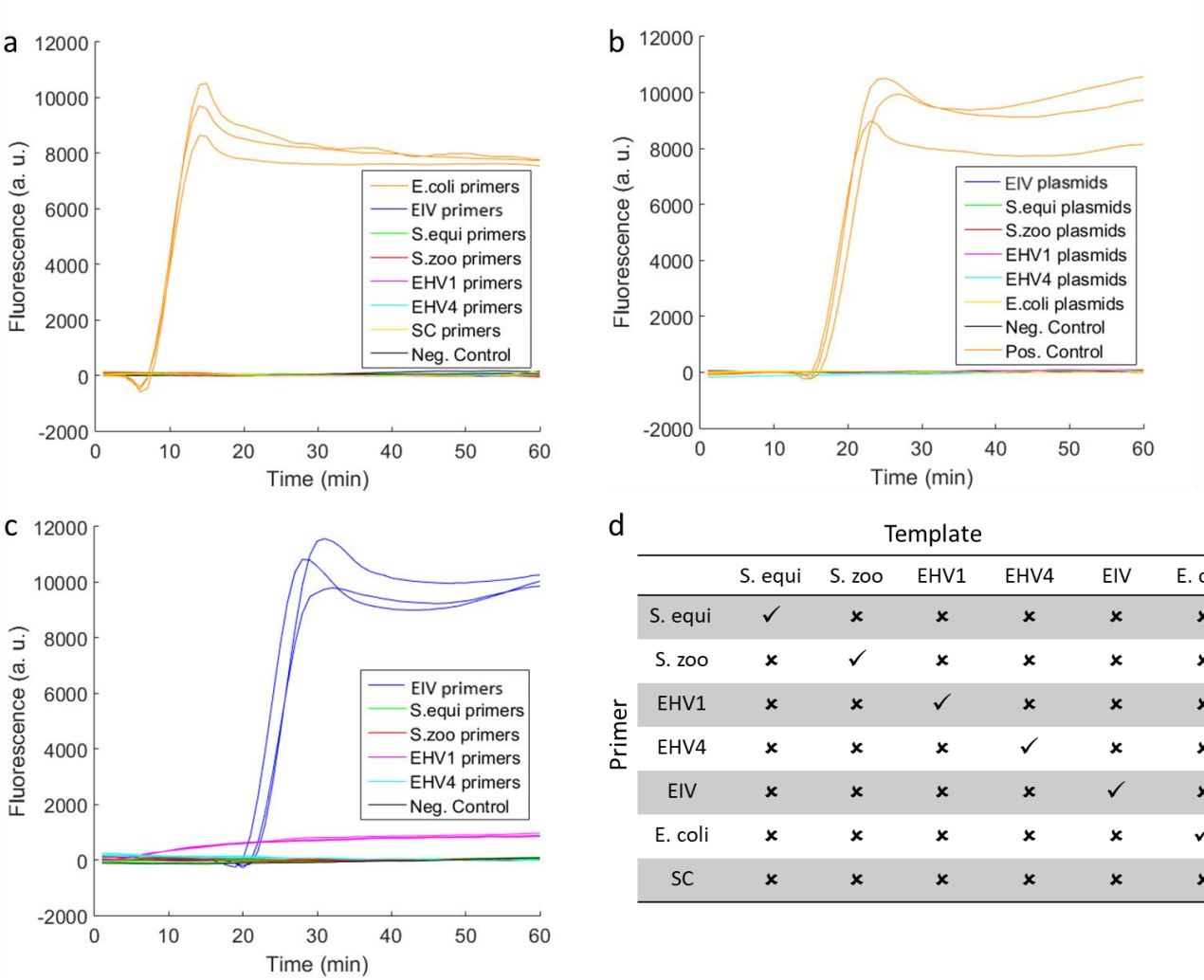
Table S2. Sequences of the primers used in control assays. The primers for E. coli[5] were from literature. The EHV3 primers were designed to target the glycoprotein G (gG) gene (GenBank: AF081188.1).

Component	Final Concentration	20 µL Reaction
10mM dNTPs	1.4 mM	2.8 µL
10X Isothermal Amplification Buffer	1X	2 µL
5M Betaine	0.4 M	1.6 µL
100mM Magnesium Sulfate Solution	6 mM	1.2 µL
12.5X LAMP Primer Mix (2.5 µM of F3 and B3, 20 µM of FIP and BIP, and 10 µM of Loop F and Loop B primers)	1X	1.6 µL
8000 units/mL Bst 2.0 Warmstart DNA Polymerase	640 units/mL	1.6 µL
20X Evagreen Dye	1X	1 µL
DEPC-treated nuclease-free water	-	1.8 µL
Template DNA	-	6.4 µL

Table S3. Recipe for LAMP reaction mix. For on-chip tests, primer mix was deposited on chips separately, and it was replaced by nuclease-free water in the reaction mix. Betaine was obtained from Sigma Aldrich. Evagreen dye was from Biotium. Primers were synthesized by Integrated DNA Technologies (IDT). All the other reagents were from New England Biolabs.

Component	Final Concentration	20 µL Reaction
2X Luna Universal qPCR Master Mix	1X	10 µL
Forward primer (10 µM)	0.25 µM	0.5 µL
Reverse primer (10 µM)	0.25 µM	0.5 µL
DEPC-treated nuclease-free water	-	4 µL
Template DNA	-	5 µL

Table S4. Recipe for PCR reaction mix. Primers were synthesized by Integrated DNA Technologies (IDT). The qPCR Master Mix is from New England Biolabs.



Primer \ Template	S. equi	S. zoo	EHV1	EHV4	EIV	E. coli
S. equi	✓	×	×	×	×	×
S. zoo	×	✓	×	×	×	×
EHV1	×	×	✓	×	×	×
EHV4	×	×	×	✓	×	×
EIV	×	×	×	×	✓	×
E. coli	×	×	×	×	×	✓
SC	×	×	×	×	×	×

Fig. S1: Off-chip verification of the LAMP assay specificity. The real-time reaction curves of (a) the E. coli DNA (a positive control for on-chip tests) against all the other primers to confirm that it won't interact with other assays; (b) the SC primers (a set of scrambled primers as a negative control for on-chip tests) against all the other templates to confirm that it will not amplify under good conditions; (c) the EIV plasmids against the other four equine assay primers. (d) Summary of the specificity of the LAMP assays.

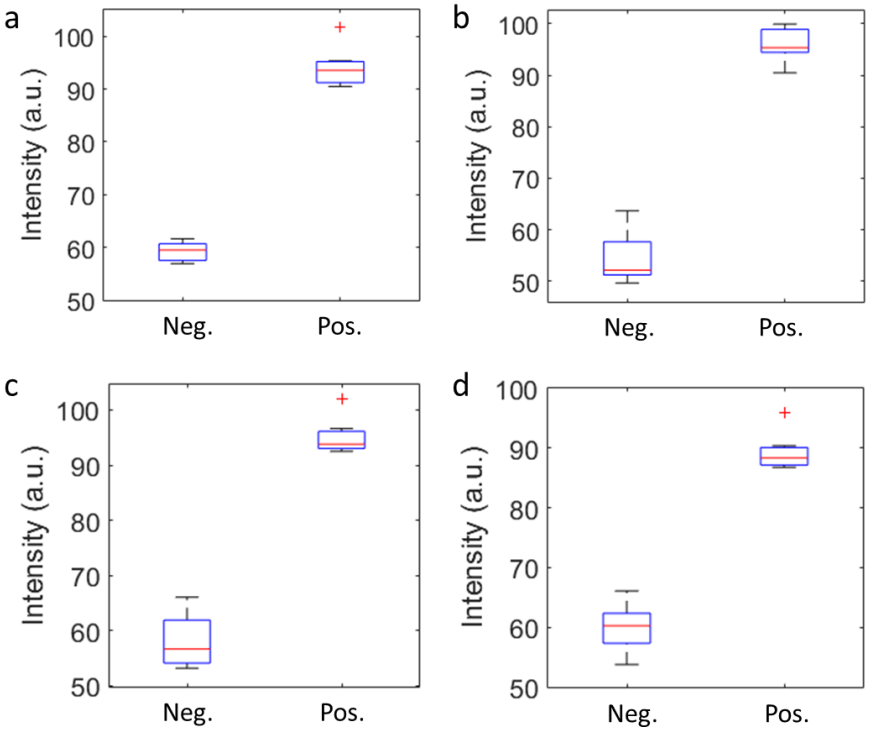


Fig. S2: On-chip characterization of four LAMP assays using plasmid DNA templates. The boxplots of the average channel intensities of positive and negative samples are plotted for a) S. equi, b) S. zoo, c) EHV4, and d) EIV at 1000 copies/µL. Each boxplot is based on eight experimental duplicates.

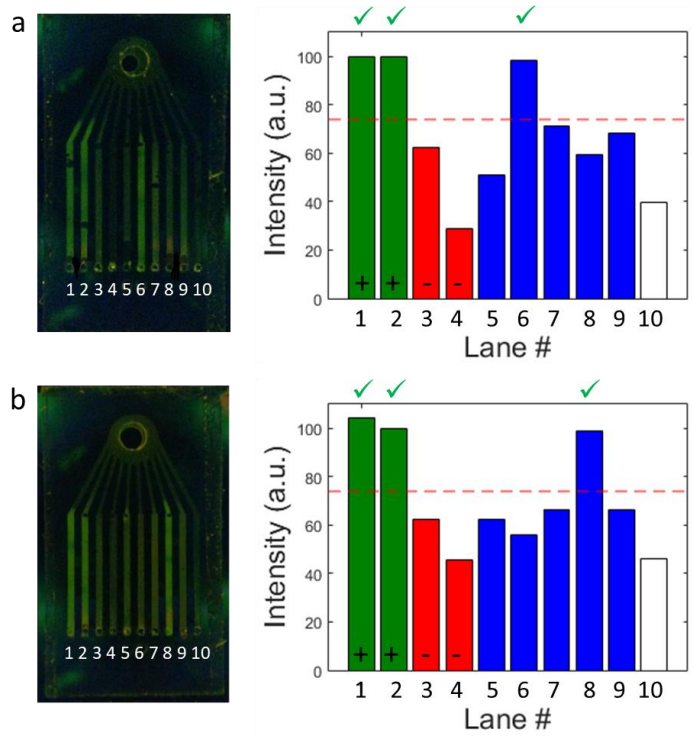


Fig. S3: On-chip multiplex detection of equine pathogen DNA. The amplified chips and the corresponding channel intensities are shown for a) S. zoo and b) EHV4 at 1000 copies/µL.

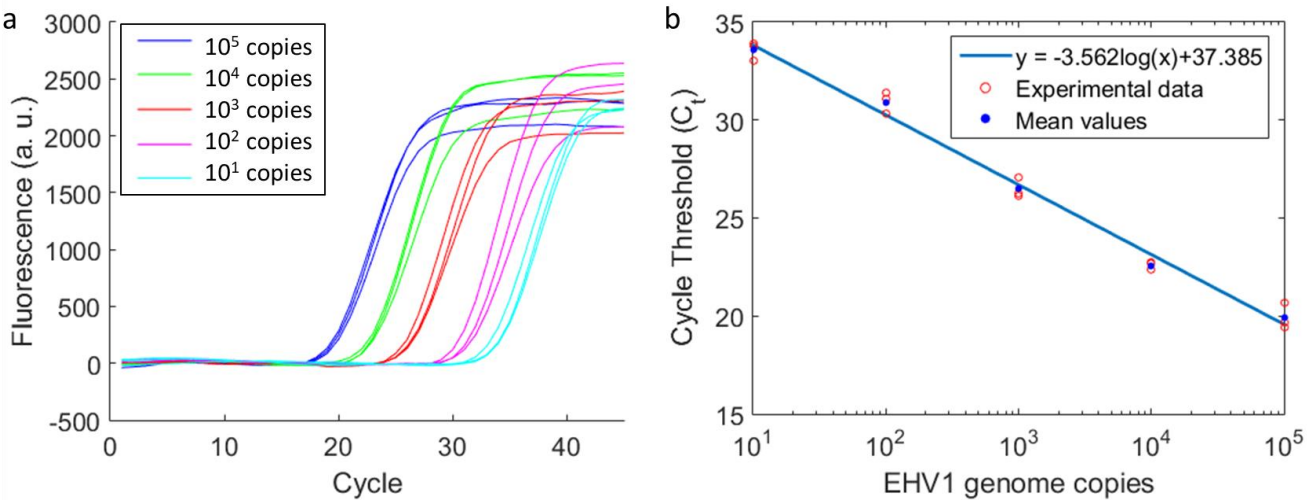


Fig. S4: Off-chip PCR for quantification of EHV1 DNA. a) The real-time PCR reaction curves of EHV1 plasmid at log concentrations; b) The PCR standard curve for quantification of EHV1 genome copies.

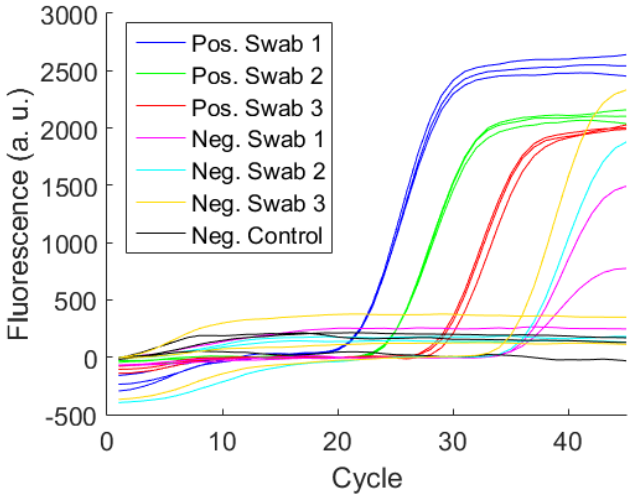


Fig. S5: Real-time PCR reactions of six horse swab samples. All nasal swabs were collected from healthy horses and were negative samples originally. Positive samples (Pos. Swab 1~3) have EHV1 viruses spiked in at decreasing concentrations. Calculated concentrations of EHV1 genome copies in the samples are shown in Table S5.

Sample	C_t	Copies	Avg. Copies	Avg. Conc. (copies/mL)
Pos. Swab 1	21.43	30146	31629	$6.33X10^6$
	21.30	32788		
	21.34	31952		
Pos. Swab 2	24.45	4279	3977	$7.95X10^5$
	25.33	2423		
	24.14	5229		
Pos. Swab 3	29.32	184	274	$5.48X10^4$
	28.63	287		
	28.32	351		
Neg. Swab 1	36.35	2	1	200
	36.9	1		
	-	0		
Neg. Swab 2	-	0	1	200
	36.05	2		
	-	0		
Neg. Swab 3	-	0	2	400
	-	0		
	34.81	5		

Table S5: PCR quantification results of the horse nasal swab samples. C_t: threshold cycle. The numbers of EHV1 genome copies were computed using the standard curve in Fig. S4:

$$\frac{\#\ of\ EHV1\ copies}{reaction} = exp\left(\frac{C_t - 37.385}{-3.562}\right)$$

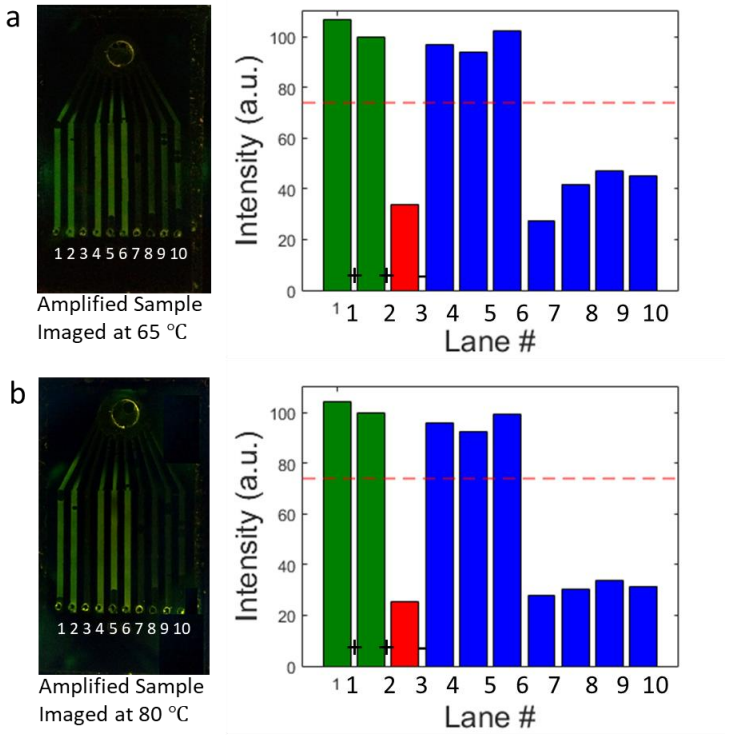


Fig. S6: On-chip detection of the positive horse swab sample 1. The smartphone pictures taken at a) 65 ℃ and b) 80 ℃ of the amplified chip and corresponding average channel intensities.

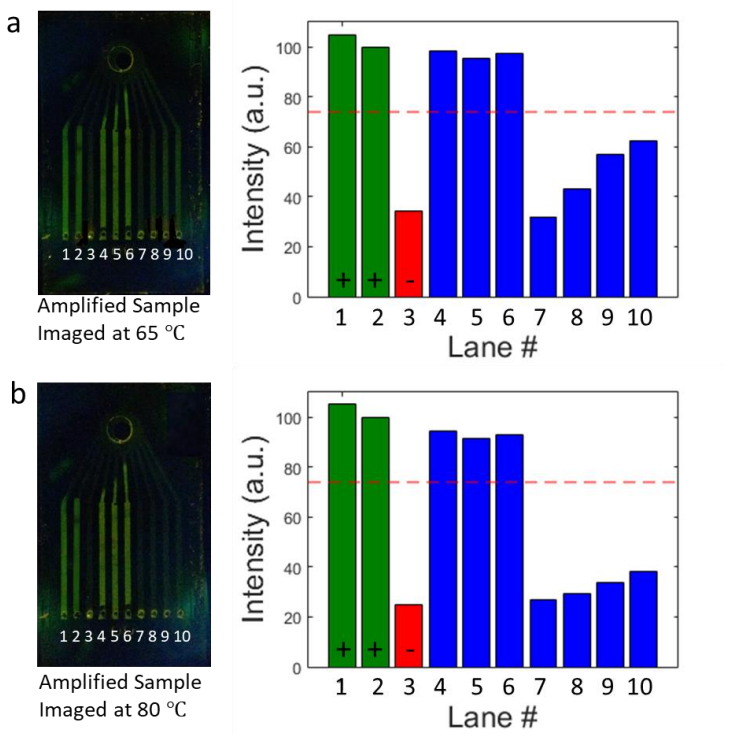


Fig. S7: On-chip detection of the positive horse swab sample 2. The smartphone pictures taken at a) 65 ℃ and b) 80 ℃ of the amplified chip and corresponding average channel intensities.

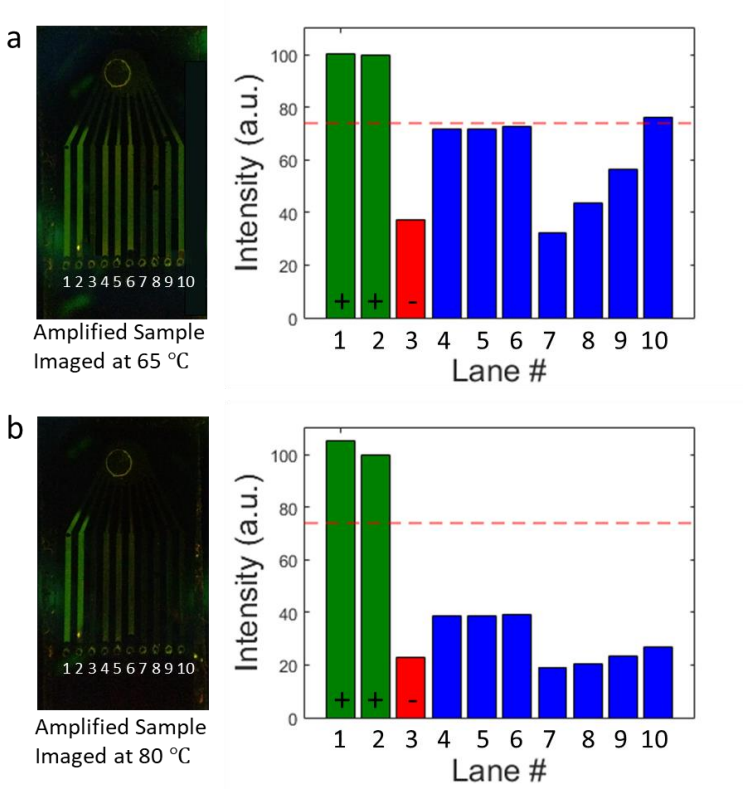


Fig. S8: On-chip detection of the negative horse swab sample 1. The smartphone pictures taken at a) 65 ℃ and b) 80 ℃ of the amplified chip and corresponding average channel intensities.

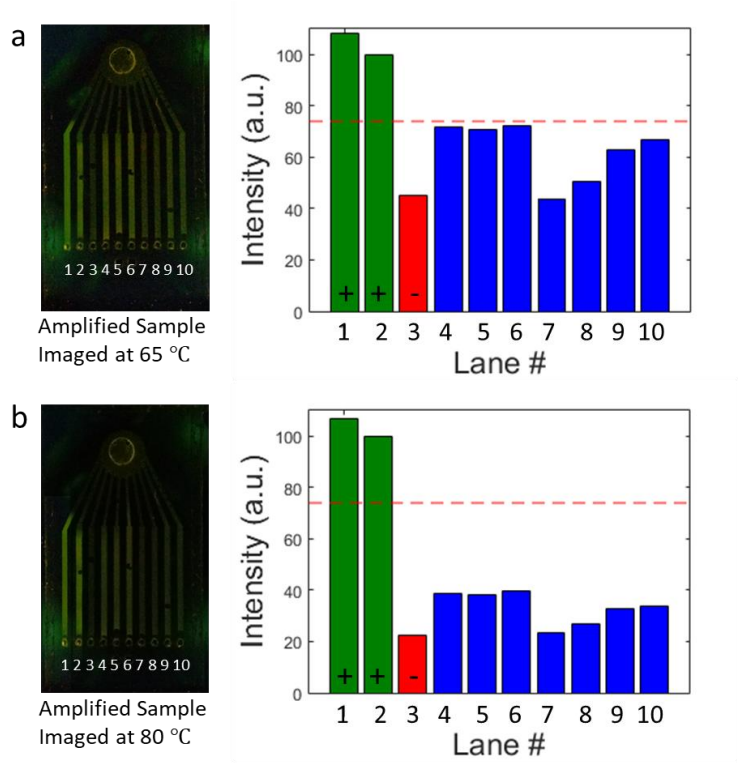


Fig. S9: On-chip detection of the negative horse swab sample 2. The smartphone pictures taken at a) 65 ℃ and b) 80 ℃ of the amplified chip and corresponding average channel intensities.

References

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