Supplementary Information

Selenium atom on phosphate enhances specificity and sensitivity of DNA polymerization and detection

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Negative controls:

Since this method was initially developed for pathogenic bacteria detection, *E. coli* genomic DNA was selected. Later, we also tested our method with human genomic DNA, and no difference was observed between *E. coli* and human genomic DNAs, as the negative controls.

Table S1 Primer sequences of LAMP and PCR for HPV detection.

Primer name		Sequence (5′ → 3′)
Primer Set 1	A1F3	CAGACGATTTATACATTAAAGGC **(PCR primer F1)**
	A1B3	TGGCAGCACATAATGACAT **(PCR primer B1)**
	A1FIP	TCAGAGGTAACCATAGAACCACTAGTCTACTGCAAATTTAGCCAGTT
	A1BIP	CGAGCACAGGGCCACAATAATGCGTGTAGTATCAACAACA
Primer Set 2	A2F3	ACAGTTTATTTTTCAACTGTGC **(PCR primer F2)**
	A2B3	TTAGGTGCTGGAGGTGTA **(PCR primer B2)**
	A2FIP	AGTCCTCCAAAATAGTGGAATTCATAAATAACCTTAACTGCAGACGT
	A2BIP	GAATTTTGGTCTACAACCTCCCCTGACAAGCAATTGCCTGG
Primer Set 3	A3F3	GCCATATCTACTTCAGAAACTACA **(PCR primer F3)**

	A3B3	GCCTGGGATGTTACAAACC **(PCR primer B3)**
	A3FIP	ACGTCTGCAGTTAAGGTTATTTTGCACTTTAAGGAGTACCTACGAC
	A3BIP	TGAATTCCACTATTTTGGAGGACTGTTCTAGTGTGCCTCCTGG
Primer Set 4	A4F3	TGGCATTTGTTGGGGTAA **(PCR primer F4)**
	A4B3	ATGTATGTATGTCATAACGTCTG **(PCR primer B4)**
	A4FIP	TCTGAAGTAGATATGGCAGCACATAGTTACTGTTGTTGATACTACACG
	A4BIP	TAAGGAGTACCTACGACATGGGAGGTTATTTTGCACAGTTGAA

Table S2 LAMP Primer set for COVID-19 RNA detection.

Primer name	Sequence (5′ → 3′)
COV-F3	GCCAAAAGGCTTCTACGCA
COV-B3	TTGCTCTCAAGCTGGTTCAA
COV-LF	GCGACTACGTGATGAGGAACG
COV-LB	AATGGCGGTGATGCTGCTCT
COV-FIP	TCCCCTACTGCTGCCTGGAGCGGCAGTCAAGCCTCTTC
COV-BIP	TTCTCCTGCTAGAATGGCTGGCTCTGTCAAGCAGCAGCAAAG

Table S3 Analysis of dNTPαSe incorporation into DNA by ICP-MS

	Reaction 1	Reaction 2	Reaction 3	Reaction 4	Reaction 5	Average
Se in initial reaction mixture (ppb)	36.7	39.5	41.6	38.5	34.1	38.1
Se in purified products (ppb)	23.1	28.4	26.5	23.3	19.5	24.1
Se-Incorporation (%)	62.9	71.9	63.6	60.3	57.2	63.4

The SEA experiments with five HPV16 positive samples were performed with the buffer containing dNTPαSe (0.125 mM) in the total dNTP (1.125 mM). Before reaction, the Se concentration in the

original reaction mixture was analyzed by ICP-MS. After reaction, the SEA product was purified by TIANquick Mini Purification Kit, then the Se concentration in SEA products was analyzed by ICP-MS. This study has indicated the dNTPαSe incorporation up to 63.4%, relative to its composition in the total dNTPs.

Table S4 Quantitative analysis of the specificity enhancement (or background suppression) by SEA

Primer Sets	Normalized LAMP Background (Figure 3G)	Normalized SEA Background (Figure 3H)	Relative Background Suppression by SEA (folds)
Primer Set 1 (N1)	83900	666	126
Primer Set 2 (N2)	63100	3110	20
Primer Set 3 (N3)	50100	878	57
Primer Set 4 (N4)	56900	1440	40

The normalized background values were from the area grey values in the gel lanes of the negative controls from Figure 3G and 3H.

Table S5 Quantitative analysis of the sensitivity enhancement (signal facilitation) by SEA

Primer Sets	SEA SNR (Figure 3H)	LAMP SNR (Figure 3G)	Sensitivity Enhancement by SEA (folds)
Primer Set 1 (P1 *vs* N1)	80.7	1.2	69.1
Primer Set 2 (P2 *vs* N2)	42.5	1.0	43.8
Primer Set 3 (P3 *vs* N3)	89.6	0.9	103.4
Primer Set 4 (P4 *vs* N4)	50.9	0.8	61.0

SNR (signal to noise ratio) refers to ratios of the area grey values in the gel lanes between the positive samples and negative controls. The SNR values of SEA and LAMP were calculated from Figure 3H and 3G, respectively.

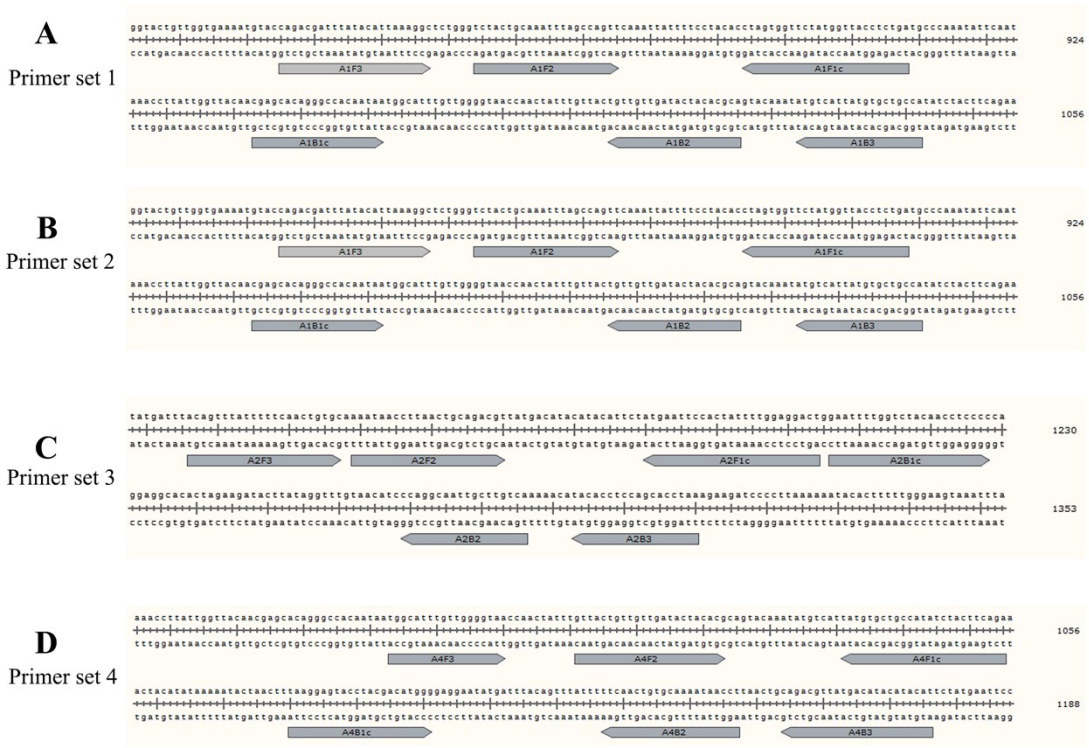

Figure S1. HPV16-L1 gene fragment labeled with the LAMP primers. (A) HPV16-L1 gene fragment labeled with Primer Set 1; (B) HPV16-L1 gene fragment labeled with Primer Set 2; (C) HPV16-L1 gene fragment labeled with Primer Set 3; (D) HPV16-L1 gene fragment labeled with Primer Set 4.

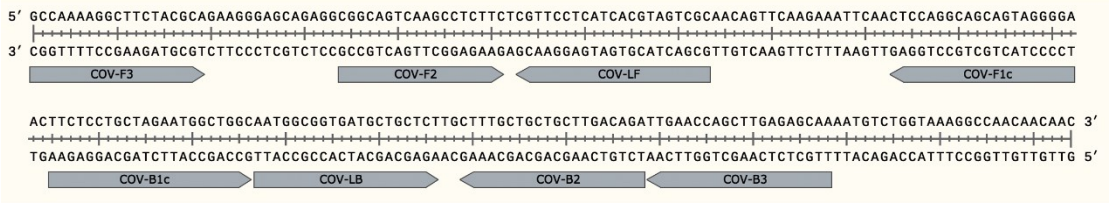

Figure S2. COVID-19 gene fragment labeled with the LAMP primers.

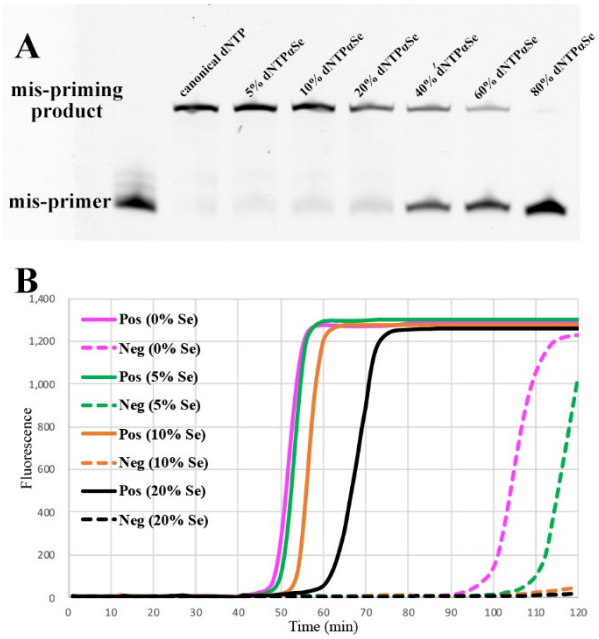

Figure S3. The dNTPαSe-dose dependence of SEA specificity. (A) dNTPαSe-dose dependence of mis-priming suppression. The template sequence (48 nt): 5'-tctgaagtagatatggcagcacatagttactgt-tgttgatactacacg-3'; The FAM-labeled mis-primer sequence (18 nt): 5'-FAM-cgtgtagtatcaacatga-3' (the boxed characters referred to mismatched bases). The nonspecific products were decreased with the increase of dNTPαSe concentration. (B) dNTPαSe-dose dependence of SEA specificity. The Ct values of negative controls increased with the growing of dNTPαSe concentration, suggesting that the SEA specificity enhancement with dNTPαSe.

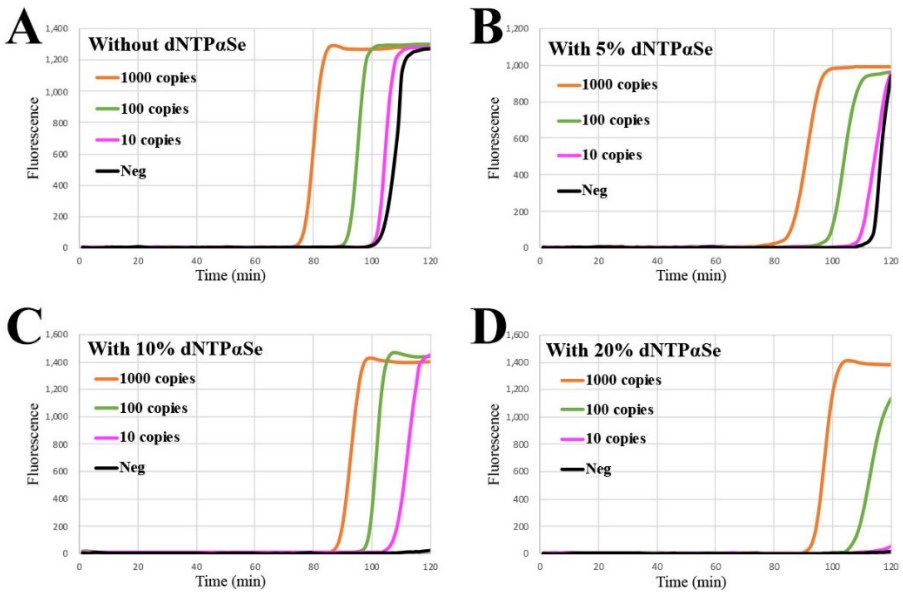

Figure S4. The optimization of dNTPαSe concentrations for SEA amplification. The SEA experiments were carried out with various concentrations of dNTPαSe. When 10% canonical dNTP was replaced by dNTPαSe, our SEA strategy achieved both highest specificity and sensitivity. (A) without dNTPαSe. (B, C and D) with 5, 10 and 20% dNTPαSe, respectively.