

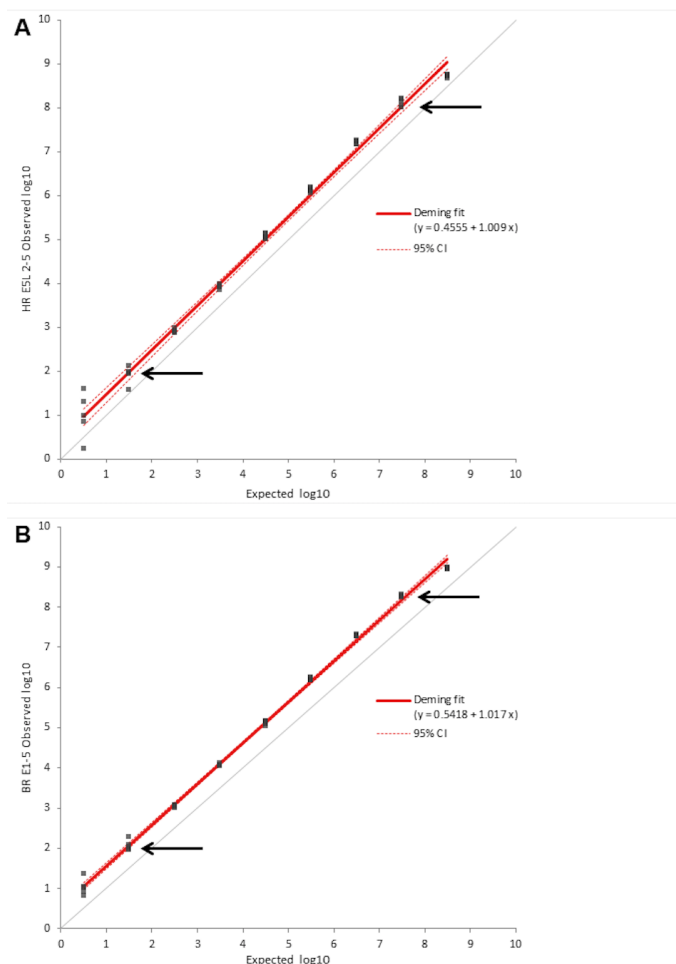
### Supplementary Figure S1

**Figure S1** Average read counts at each HPV-16 read count in TCGA samples (N = 94). Only 2 out of the 94 samples have >10 average read counts across the HPV-16 genome.

Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E6	150.0	30.0	6.0	150.0	30.0	150.0	30.0	15.0	60.0	30.0	60.0	15.0	6.0	6.0	6.0	6.0
E7	60.0	30.0	30.0	150.0	6.0	6.0	30.0	15.0	30.0	6.0	6.0	30.0	6.0	6.0	6.0	6.0
BR E1-4	150.0	150.0	1.2	6.0	30.0	30.0	30.0	50.0	1.2	1.2	1.2	3.0	6.0	6.0	6.0	6.0
BR E1-5	60.0	150.0	30.0	6.0	6.0	15.0	30.0	30.0	1.2	6.0	30.0	30.0	6.0	6.0	6.0	6.0
BR E1-6	150.0	150.0	30.0	30.0	30.0	50.0	150.0	15.0	30.0	6.0	6.0	30.0	6.0	6.0	6.0	30.0
HR E5L2-4	30.0	30.0	150.0	60.0	150.0	30.0	30.0	15.0	30.0	30.0	6.0	30.0	30.0	30.0	30.0	6.0
BR E6-1	150.0	30.0	150.0	60.0	30.0	60.0	30.0	150.0	30.0	30.0	30.0	15.0	60.0	60.0	30.0	6.0
BR E6-3	60.0	30.0	150.0	150.0	150.0	50.0	30.0	15.0	30.0	150.0	30.0	15.0	30.0	30.0	6.0	6.0

Sample #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
E6	30.0	6.0	1.2	0.6	1.2	0.3	1.2	1.2	0.3	0.6	15.0	3.0	3.0	15.0	3.0	15.0
E7	1.2	6.0	1.2	1.2	1.2	1.2	1.2	1.2	0.3	0.3	1.0	1.0	3.0	15.0	0.6	15.0
BR E1-4	6.0	1.2	0.3	1.2	0.6	0.3	1.2	0.6	1.2	0.6	0.6	1.0	1.0	30.0	0.6	9.0
BR E1-5	1.2	0.2	1.2	3.0	0.6	0.3	1.2	0.6	0.3	0.6	0.6	0.6	1.0	15.0	0.6	15.0
BR E1-6	6.0	1.2	1.2	6.0	1.2	0.3	1.2	1.2	1.2	0.3	0.6	0.6	3.0	150.0	0.6	9.0
HR E5L2-4	6.0	6.0	1.2	1.2	6.0	0.3	1.2	1.2	0.6	0.6	0.6	1.0	3.0	30.0	0.6	30.0
BR E6-1	6.0	1.2	6.0	1.2	1.2	6.0	1.2	1.2	1.2	1.2	1.0	1.0	1.0	150.0	0.6	15.0
BR E6-3	1.2	6.0	6.0	1.2	1.2	1.2	1.2	0.6	0.3	0.6	0.6	1.0	1.0	150.0	0.6	15.0

**Figure S2** Primer/probe design in the three specific regions and PCR amplification. (A) In the specific region of E1, we designed six candidate primer/probe sets (BR E1-1, 2, 3, 4, 5, and 6) of amplicon lengths 75 bp, 75 bp, 76 bp, 80 bp, 79 bp, and 78 bp, respectively. (B) PCR shows that the six designed primers appropriately reacted against DNA derived from CaSki cell lines and did not react against DNA derived from lymphocyte of a healthy individual. (C) In the region of junction between E5 and L2, we designed four candidate primer/probe sets (HR E5L2-1,2,3, and 4) of amplicon lengths 70 bp, 70 bp, 75 bp, and 67 bp, respectively. (D) PCR shows that the four designed primers appropriately reacted against DNA derived from CaSki cell lines and did not react against DNA derived from lymphocyte of a healthy individual. (E) In the region of E6, we designed five candidate primer/probe sets (BR E6-1,2,3,4, and 5) of amplicon lengths 80 bp, 81 bp, 85 bp, 85 bp, and 80 bp, respectively. F, PCR shows that the five designed primers appropriately reacted against DNA derived from CaSki cell lines and did not react against DNA derived from lymphocyte of a healthy individual. Each arrow indicates the primer location and the bold line shows the probe location.



**Supplementary Figure S3**

**Figure S3** Measurement range of HPV 16 CLIA test reagents in saliva. HPV-16 whole genome plasmid spiked into saliva. Arrows indicate upper and lower limits of quantification, accounting for precision and linearity. Data are plotted in copies/reaction (conversion to copies/mL, add 0.7 per replicate). Y axis = measured copies, X axis = expected copies a) HR E5L 2-4 assay, b) BR E1-5 assay. Similar results with E7 assay and SiHA DNA (not shown).

Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E6	150.0	30.0	6.0	150.0	30.0	150.0	30.0	15.0	60.0	30.0	60.0	15.0	6.0	6.0	6.0	6.0
E7	60.0	30.0	30.0	150.0	6.0	6.0	30.0	15.0	30.0	6.0	6.0	30.0	6.0	6.0	6.0	6.0
BR E1-4	150.0	150.0	1.2	6.0	30.0	30.0	30.0	50.0	1.2	1.2	1.2	3.0	6.0	6.0	6.0	6.0
BR E1-5	60.0	150.0	30.0	6.0	6.0	15.0	30.0	30.0	1.2	6.0	30.0	30.0	6.0	6.0	6.0	6.0
BR E1-6	150.0	150.0	30.0	30.0	30.0	50.0	150.0	15.0	30.0	6.0	6.0	30.0	6.0	6.0	6.0	30.0
HR E5L2-4	30.0	30.0	150.0	60.0	150.0	30.0	30.0	15.0	30.0	30.0	6.0	30.0	30.0	30.0	30.0	6.0
BR E6-1	150.0	30.0	150.0	60.0	30.0	60.0	30.0	150.0	30.0	30.0	30.0	15.0	60.0	60.0	30.0	6.0
BR E6-3	60.0	30.0	150.0	150.0	150.0	50.0	30.0	15.0	30.0	150.0	30.0	15.0	30.0	30.0	6.0	6.0

Sample #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
E6	30.0	6.0	1.2	0.6	1.2	0.3	1.2	1.2	0.3	0.6	15.0	3.0	3.0	15.0	3.0	15.0
E7	1.2	6.0	1.2	1.2	1.2	1.2	1.2	1.2	0.3	0.3	1.0	1.0	3.0	15.0	0.6	15.0
BR E1-4	6.0	1.2	0.3	1.2	0.6	0.3	1.2	0.6	1.2	0.6	0.6	1.0	1.0	30.0	0.6	9.0
BR E1-5	1.2	0.2	1.2	3.0	0.6	0.3	1.2	0.6	0.3	0.6	0.6	0.6	1.0	15.0	0.6	15.0
BR E1-6	6.0	1.2	1.2	6.0	1.2	0.3	1.2	1.2	1.2	0.3	0.6	0.6	3.0	150.0	0.6	9.0
HR E5L2-4	6.0	6.0	1.2	1.2	6.0	0.3	1.2	1.2	0.6	0.6	0.6	1.0	3.0	30.0	0.6	30.0
BR E6-1	6.0	1.2	6.0	1.2	1.2	6.0	1.2	1.2	1.2	1.2	1.0	1.0	1.0	150.0	0.6	15.0
BR E6-3	1.2	6.0	6.0	1.2	1.2	1.2	1.2	0.6	0.3	0.6	0.6	1.0	1.0	150.0	0.6	15.0

**Table S1** Threshold of tumor cell numbers for HPV-16 detection. The column shown is the lowest tumor cell counts for the primer/probe for each sample set.

Primer/probe set	Sensitivity				
	100 cells	30 cells	10 cells	3 cells	1 cell
E6	0.906	0.844	0.531	0.250	0.125
E7	0.969*	0.938*	0.688	0.375	0.094
BR E1-4	0.938	0.875	0.781*	0.531*	0.219
BR E1-5	0.969*	0.938*	0.688	0.438	0.281*
BR E1-6	0.875	0.844	0.625	0.344	0.156
HR E5L2-4	0.938	0.844	0.500	0.313	0.156
BR E6-1	0.875	0.750	0.469	0.344	0.031
BR E6-3	0.844	0.781	0.500	0.375	0.156

\* showed the highest sensitivity

**Table S2** Sensitivity from each tumor DNA dilution according to the prime/probe set.