

Supplementary materials

Head-to-head comparison of 7 high-sensitive human papillomavirus nucleic acid detection technologies with SPF10 LiPA-25 system among 1782 cervical specimens

Jian Yin, Shuqian Cheng, Daokuan Liu, Yabin Tian, Fangfang Hu, Zhigao Zhang, Tiancen Zhu, Zheng Su, Yujing Liu, Sumeng Wang, Yiwei Liu, Siying Peng, Linlin Li, Sihong Xu, Chuntao Zhang, Youlin Qiao, and Wen Chen

HPV genotyping tests

1. LiPA-25

LiPA-25 system are produced by Lab Biomedical Products, Rijswijk, The Netherlands (based on licensed Innogenetics technology)^{19,30}, which was regarded as the golden standard in this study. Briefly, HPV DNA was extracted from 200 µl cervical cytological specimen by the MagNAPure LC DNA isolation procedure (Roche Diagnostics, Switzerland). DNA was isolated from FFPE cervical biopsy specimens by incubating three 4 µm sections in proteinase K solution, as described previously¹⁹.

The SPF10 primer sets detect a broad spectrum of HPV genotypes by amplification of a 65bp fragment from the L1 open reading frame of HPV. Subsequently, we use a cocktail of universal probes in a DNA enzyme immunoassay (DEIA) to detect HPV DNA positivity (over 65 genotypes). Finally, the positive samples are analyzed for presence of individual HPV genotypes by the LiPA-25, which can identify 25 different HPV genotypes (HPV6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73 (LiPA-25 cannot distinguish between HPV68 and 73, 70 and 74) by reverse hybridization technology.

Earlier studies indicated that comparing with the SPF10 LiPA-25 system alone, combining this system with the type-specific PCR DEIA systems for HPV16 and HPV18 led to higher HPV detection rates for these types, which diminished the rate of false-negative diagnosis³¹. HPV18 type-specific (TS18) PCR primers generate a 126 bp fragment of the L1 gene as well, while HPV16 type-specific (TS16) PCR primers amplified a 92 bp segment targeting the E6/E7 region^{31,32}. In

this study, the positive samples detected by SPF10 DEIA were also tested by TS16 and TS18 PCR/DEIA.

2. Bohui-24

Bohui-24 system can qualitatively detect 24 HPV genotypes (HPV 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, 82 and 83) using HPV genotyping kit (Microfluidic Chip, BOHUI[®], Beijing, China) and directly indicate the genotypes of HPV infection. This product is used in conjunction with the nucleic acid chip detector (BHF-VI) manufactured by BOHUI company (BOHUI[®], Beijing, China). The three areas, target DNA extraction, PCR amplification and reverse dot hybridization, are carried out respectively under the drive of micropump. We only need to add swab samples to the wells from HPV detection chip. The FFPE samples was pretreated using sample extraction kit (BOHUI[®], Beijing, China) before adding to wells. Nucleic acid is extracted by magnetic bead method, specific primers are specially designed for the L1 region of HPV genome, and the hybridization membrane of the detection area is coated with detection probe for 24 HPV types.

The amplification products with the target gene could be captured by the probe on the chip. The blue spot in specific position of the hybridization membrane identifies the positive of the individual HPV genotype. The specific HPV genotype is negative, if the spot doesn't turn into blue. Finally, the hybrid zone is scanned with a CCD camera after the completion of reaction. When the detection signal value of the probe is greater than or equal to the cut-off value, the corresponding type of the probe is judged to be positive by the software, otherwise, it's negative. The software in instrument can automatically report the results of detected samples.

3. Yaneng-23

Yaneng-23 system utilizes the PCR-based reverse dot blot (RDB) hybridization technique, which was designed for the qualitative detection and identification of 23 HPV genotypes, including HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81 and 82²⁴. Its methodology can be briefly described as followed.

HPV DNA from swab specimens was extracted using YN-E192 automated nucleic acid extraction system (Yaneng BioSciences (Shenzhen) Co., Ltd, China) by magnetic bead-based DNA

purification technology. The FFPE sections were incubated at 100 °C for 10 min with 30 µl lysis buffer, and the supernatant was reserved after centrifugation at 13,000 rpm for 5 min. Subsequently, the isolated DNA was amplified by PCR using biotin-labelled primer pool which targets the HPV L1 gene region, as well as an additional pair of biotin-labelled primers which amplify the human β-actin gene to monitor the original sample adequacy and DNA extraction quality.

The primer pool, as well as multiple PCR amplification reaction system and procedure were optimized to efficiently amplify all target genotypes at the same time. After PCR amplification, the resulting biotinylated amplicons were denatured and hybridized with specific oligonucleotide probes which were immobilized as dots on the membrane strip. All probes were elaborate designed so that under the hybridization condition, each one of them can only hybridize with the PCR amplicon of specific genotype, but does not display any non-specific hybridization with the PCR amplicon of any other genotype. As needed, multiple probes were used sometimes for individual genotype to reduce missed detection caused by subtypes and variants within that genotype.

Following a stringent washing step to wash away unbound PCR products, the streptavidin-conjugated horseradish peroxidase is added, and bound to any biotinylated hybridizing product previously formed. Finally, the membrane strip was incubated with TMB chromogen to generate blue precipitate, and the result can be visually interpreted by the naked eye or Biochip Scanner. The hybridization, washing, and color development process can be completed on the automatic molecular hybridization instrument.

4. Tellgen-27

Tellgen-27 system (TELLGEN Life Science Co. Ltd., Shanghai, China) targets 27 HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 55, 56, 58, 59, 61, 66, 68, 81, 82 and 83) and human DNA (β-globin), and uses L1 consensus PCR followed by use of the Luminex's xMAP-based assay that combines flow cytometry with color-coded microspheres^{25,33}.

HPV DNA from swab was extracted using TELLGEN nucleic acid extraction kit (TELLGEN Life Science Co. Ltd., Shanghai, China), and from FFPE was extracted using FFPE DNA kit (CoWin Biosciences Co. Ltd., Beijing, China). The HPV is amplified by PCR using biotin-labeled

consensus PCR primers targeting the HPV L1 gene region. The thermocycler was programmed as follows: 95 °C for 5 min; 5 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s; 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; and 72 °C for 3 min. Amplified PCR products are hybridized to sets of color-coded beads with coated HPV type-specific probes, and are subsequently incubated with phycoerythrin-conjugated streptavidin. The incubation conditions are as follows: 5min at 95 °C; 40 cycles of 1 min at 95 °C; 1 min at 55 °C; and 1 min at 72 °C. Finally, beads connected the reaction products are read by a Luminex 200 analyzer instrument (Luminex Corporation, Texas, USA). HPV genotypes can be determined by the unique fluorescent dye signature on set of beads.

5. HybriBio-16

HybriBio 16 system (HybriBio Limited Corp., Chaozhou, China) is a PCR-based reverse blot hybridization assay, which can detect 16 HPV genotypes: 14 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 2 LR-HPV (HPV6 and 11).

The DNA from swab samples was extracted by HybriBio automated nucleic acid extraction system. DNA extraction from FFPE biopsy specimens was performed using HybriBio cell lysis kit (Ref: HBCL). Extracted DNA was amplified by PCR using HPV L1 consensus PCR primers, and was specifically determined by flow-through hybridization with immobilized genotype-specific probes to a gene chip as described previously³⁴. The thermocycler was programmed as follows: 20 °C for 10 min; 95 °C for 9 min; 40 cycles of 94 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s; and 72 °C for 5 min.

Microarray hybridization was performed to detect specific HPV genotype using a nylon membrane on which HPV genotype-specific oligonucleotide probes were immobilized. Besides, an HPV consensus probe was designed to capture the other HPV genotypes except above-mentioned 16 HPV genotypes. The results were manually interpreted using the provided guide from the 16 HPV genoarray diagnostic kit.

6. HybriBio-23

HybriBio-23 HPV genotyping was performed with real-time fluorescence PCR (HybriBio Limited Corp., Chaozhou, China), which can discriminate 23 HPV genotypes (HPV6, 11, 16, 18, 31, 33,

35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81 and 82).

Briefly, DNA extraction is similar with HybriBio-16. The amplified reaction was carried out with a 20 µl container including PCR primers which targeting the HPV L1, L2, E1, E2, E4, E6 and E7 gene regions, DNA polymerase, template DNA, and the TaqMan probe. There were 6 reaction tubes (6 multiplex PCRs) in the kit and 4 fluorescence probe detection channels (FAM, HEX/JOE, ROX/Red 610, and Cy5) in the sequence detector. The reaction cycle was programmed as follows: 95 °C for 10 min; 45 cycles of 95 °C for 15 s, and 60 °C for 1 min; and finally, 38 °C for 5 s. A 178 bp fragment of the beta-globin gene was introduced to assess the quality of the extracted sample DNA.

7. Bioperfectus-21

Bioperfectus-21 system (Bioperfectus Limited Corp., Jiangsu, China) targets 21 HPV genotype and uses the BioPerfectus multiplex real time (BMRT) HPV assay²⁰.

The DNA from the cervical swab or FFPE specimens was extracted by magnetic bead adsorption and purified using a viral nucleic acid isolation kit (magnetic beads) (Bioperfectus Limited Corp., Jiangsu, China), and using a FFPE DNA isolation kit (magnetic beads) (Bioperfectus Limited Corp., Jiangsu, China). The enriched nucleic acid was detected by real-time HPV assay, which can measure type-specific HPV viral load and genotyping simultaneously aimed at multiple regions: L1, E1, E2 and E7 region. The system was performed with the fluorescence-based multiplex real-time PCR to distinguish 21 HPV genotypes (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81 and 82) via 8 reaction tubes (8 multiplex PCRs) per sample. Meanwhile, human TOP3, a single-copy gene encoding DNA topoisomerase III, was amplified as a control for determining relative number of viral copies in a given sample.

PCR amplification was conducted in a total reaction volume of 20 µl, which comprised 2 µl DNA samples, 10 µl PCR reaction mix, and 8 µl reaction mixture. To prevent reamplification of carry-over PCR products, all reactions with uracil-DNA-glycosylase (UDG) were pre-incubated at 50 °C for 5 min, followed by an initial denaturation at 95 °C for 10 min, which also inactivates UDG but activates the DNA polymerase, and 45 cycles at 95 °C for 10 s, 58 °C for 40 s.

Perfectus software v1.0, which was used for genotyping and quantitative analysis of HPV nucleic acid (Bioperfectus Limited Corp., China), was applied for quantitative analyses of HPV viral loads.

8. Sansure-26

Sansure-26 system (Sansure biotech inc., Hunan, China) uses multiplex PCRs technology and Taqman technology. Briefly, this approach uses a nucleic acid lysis buffer to allow rapid lysis and release of HPV DNA, and utilizes pairs of specific primers targeting multiple regions: L1, L2, E1, E6 and E7 region, and specific fluorescence probes of the 26 HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 67, 68 and 73) via 7 reaction tubes (7 multiplex PCRs).

Sansure HPV diagnostic kits are based on self-developed and global unique one-step technology platform, using unique formula of lysis buffer for a direct and rapid release of target DNA, which don't need heating, high-speed centrifugation, and to remove supernatant. Comparing with traditional HPV diagnostic method with 2-3 hours for pretreatment. Sansure one-step technology just need 3 simple steps: adding lysis buffer, specimen and PCR-mix, which only takes 10 minutes to complete DNA extraction. The DNA from the cervical FFPE specimens was extracted by multi-type sample DNA/RNA extraction-purification kit (magnetic-bead method) (Sansure Biotech Inc., Hunan, China). The PCR detection system utilizes uracil-N-glycosylase (UNG) + dUTP contamination-proof system to fully degrade possible PCR amplified products in order to avoid a false positive result, and uses a positive control to monitor β -globin in human epidermal cells for the evaluation of amplification reaction of specimens and monitor the presence of PCR inhibitors, as well as to evaluate the nucleic acid extraction efficiency, in order to avoid a false negative result. The reaction cycle was programmed as follows: 50 °C, 2 min; 94 °C for 5 min; 45 cycles of 94 °C for 15 s, and 57 °C for 30 s; and finally, 25 °C for 10 s.

Supplementary Table 1

The Limit of detection of 15 HPV genotypes for 8 HPV genotyping systems.

Types	Limit of detection, copies/test							
	LiPA-25	Bohui-24	Yaneng-23	Tellgen-27	HybriBio-16	HybriBio-23	Bioperfectus-21	Sansure-26
HPV6	10	60	50	50	10	20	2,000	50
HPV11	10	600	50	50	10	20	20	50
HPV16	10	600	50	50	10	200	200	50
HPV18	10	60	50	50	10	200	20	50
HPV31	10	600	500	500	10	20	200	50
HPV33	10	60	50	500	100	20	200	50
HPV35	10	600	5	50	100	2,000	20	50
HPV39	10	600	500	50	10	2,000	200	50
HPV45	100	600	500	500	100	200	2,000	500
HPV51	10	600	50	500	100	2,000	2,000	50
HPV52	10	600	50	50	100	2,000	2,000	50
HPV56	10	60	50	20	100	2,000	20	5
HPV58	1000	600	50	500	1,000	20	200	500
HPV59	100	600	50	50	100	20	200	500
HPV66	10	60	50	500	10	2,000	20	50

Supplementary Table 2

Comparison of the main characteristics of 8 HPV genotyping systems.

System	DNA extraction		Polymerase chain reaction		
	Swab specimen volume (μ l) or 4 μ m biopsy thin section	Elution volume (μ l)	Sample DNA volume (μ l)	Relative amount ^a	Amplification reaction volume (μ l)
Swab					
LiPA-25	200	100	10	1	50
Bohui-24	200	60	3	0.5	15
Yaneng-23	250	100	10	1.25	25
Tellgen-27	200	200	5	0.25	20.8
HybriBio-16	200	70	1	0.14	25
HybriBio-23	200	70	2	0.29	20
Bioperfectus-21	500	80	2	0.63	20
Sansure-26	500	100	5	1.25	50
Biopsy					
LiPA-25	3	100	10	1	50
Bohui-24	3	60	3	0.5	15
Yaneng-23	3	30	5	1.67	25
Tellgen-27	3	50	5	1	20.8
HybriBio-16	3	60	1	0.17	25
HybriBio-23	3	60	1	0.17	20
Bioperfectus-21	3	80	2	0.25	20
Sansure-26	3	100	10	1	50

^aLiPA was regarded as the reference.

Supplementary Table 3

Agreement between Bohui-24 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,725 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	51	16	3	1,655	0.843	0.994	0.837 (0.765-0.910)	0.006
11	43	6	0	1,676	0.935	0.998	0.933 (0.879-0.987)	0.041
16	87	23	13	1,602	0.829	0.989	0.817 (0.758-0.876)	0.134
16 ^b	91	26	9	1,599	0.839	0.989	0.828 (0.772-0.884)	0.007
18	66	18	8	1,633	0.835	0.992	0.828 (0.762-0.893)	0.078
18 ^c	66	20	8	1,631	0.825	0.991	0.817 (0.749-0.884)	0.038
31	58	26	4	1,637	0.795	0.991	0.786 (0.710-0.862)	< 0.001
33	84	11	5	1,625	0.913	0.995	0.908 (0.863-0.953)	0.211
35	72	18	1	1,634	0.883	0.994	0.878 (0.823-0.932)	< 0.001
39	89	29	8	1,599	0.828	0.989	0.817 (0.758-0.875)	0.001
45	37	9	2	1,677	0.871	0.997	0.867 (0.789-0.945)	0.070
51	78	21	4	1,622	0.862	0.992	0.854 (0.798-0.911)	0.001
52	130	33	8	1,554	0.864	0.987	0.851 (0.806-0.896)	< 0.001
56	85	11	21	1,608	0.842	0.990	0.832 (0.774-0.890)	0.112
58	98	8	17	1,602	0.887	0.992	0.879 (0.832-0.926)	0.110
59	95	7	29	1,594	0.841	0.989	0.830 (0.775-0.885)	< 0.001
66	83	30	4	1,608	0.830	0.990	0.820 (0.760-0.880)	< 0.001
Overall ^d	1,156	266	127	24,326	0.855	0.992	0.847 (0.839-0.855)	< 0.001
Overall ^f	1,160	271	123	24,321	0.855	0.992	0.847 (0.839-0.855)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 4

Agreement between Yaneng-23 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,719 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	59	8	6	1,646	0.894	0.996	0.890 (0.832-0.947)	0.789
11	43	5	0	1,671	0.945	0.999	0.944 (0.894-0.993)	0.074
16	100	9	13	1,597	0.901	0.993	0.894 (0.850-0.938)	0.522
16 ^b	106	10	7	1,596	0.926	0.995	0.920 (0.883-0.958)	0.628
18	75	9	4	1,631	0.920	0.996	0.916 (0.871-0.962)	0.267
18 ^c	77	9	2	1,631	0.933	0.997	0.930 (0.889-0.971)	0.070
31	55	29	5	1,630	0.764	0.990	0.754 (0.672-0.836)	< 0.001
33	84	11	3	1,621	0.923	0.996	0.919 (0.876-0.961)	0.061
35	89	1	1	1,628	0.989	0.999	0.988 (0.972-1.005)	1.000
39	70	48	4	1,597	0.729	0.984	0.714 (0.637-0.791)	< 0.001
45	40	6	2	1,671	0.909	0.998	0.907 (0.842-0.971)	0.289
51	89	10	8	1,612	0.908	0.994	0.903 (0.858-0.947)	0.814
52	125	38	13	1,543	0.831	0.984	0.814 (0.764-0.865)	0.001
56	86	10	12	1,611	0.887	0.993	0.880 (0.830-0.930)	0.831
58	100	5	20	1,594	0.889	0.992	0.881 (0.835-0.927)	0.005
59	98	4	19	1,598	0.895	0.993	0.888 (0.842-0.933)	0.004
66	84	29	2	1,604	0.844	0.990	0.835 (0.777-0.892)	< 0.001
Overall ^d	1197	222	112	24,254	0.878	0.993	0.871 (0.864-0.878)	< 0.001
Overall ^f	1205	223	104	24,253	0.881	0.993	0.874 (0.867-0.881)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 5

Agreement between Tellgen-27 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,697 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	52	15	6	1,624	0.832	0.994	0.826 (0.751-0.900)	0.081
11	38	10	1	1,648	0.874	0.997	0.870 (0.794-0.947)	0.016
16	93	17	7	1,580	0.886	0.992	0.878 (0.830-0.927)	0.066
16 ^b	97	20	3	1,577	0.894	0.993	0.887 (0.841-0.933)	0.001
18	69	15	9	1,604	0.852	0.993	0.844 (0.783-0.906)	0.307
18 ^c	69	17	9	1,602	0.841	0.992	0.833 (0.770-0.897)	0.170
31	51	33	3	1,610	0.739	0.989	0.729 (0.641-0.816)	< 0.001
33	75	20	2	1,600	0.872	0.993	0.865 (0.809-0.921)	< 0.001
35	71	17	7	1,602	0.855	0.993	0.848 (0.788-0.908)	0.066
39	99	19	15	1,564	0.853	0.989	0.843 (0.790-0.895)	0.607
45	38	8	1	1,650	0.894	0.997	0.891 (0.821-0.962)	0.046
51	60	37	1	1,599	0.759	0.988	0.748 (0.669-0.827)	< 0.001
52	124	37	12	1,524	0.835	0.984	0.819 (0.769-0.869)	0.001
56	79	17	11	1,590	0.849	0.991	0.841 (0.782-0.899)	0.345
58	95	10	24	1,568	0.848	0.989	0.838 (0.783-0.892)	0.026
59	93	7	25	1,572	0.853	0.990	0.843 (0.789-0.897)	0.003
66	73	36	2	1,586	0.793	0.988	0.782 (0.714-0.851)	< 0.001
Overall ^d	1110	298	126	23,921	0.840	0.991	0.831 (0.823-0.839)	< 0.001
Overall ^f	1114	303	122	23,916	0.840	0.991	0.831 (0.823-0.839)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 6

Agreement between HybriBio-16 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,710 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	49	18	1	1,642	0.838	0.994	0.832 (0.757-0.907)	< 0.001
11	42	7	2	1,659	0.903	0.997	0.901 (0.836-0.965)	0.182
16	100	9	7	1,594	0.926	0.995	0.921 (0.882-0.959)	0.803
16 ^b	106	10	1	1,593	0.951	0.997	0.947 (0.916-0.978)	0.016
18	76	8	4	1,622	0.927	0.996	0.923 (0.880-0.966)	0.387
18 ^c	77	9	3	1,621	0.928	0.996	0.924 (0.881-0.967)	0.149
31	63	21	7	1,619	0.818	0.991	0.810 (0.740-0.880)	0.014
33	82	12	2	1,614	0.921	0.996	0.917 (0.874-0.960)	0.016
35	80	10	5	1,615	0.914	0.995	0.910 (0.864-0.955)	0.302
39	104	14	13	1,579	0.885	0.992	0.877 (0.830-0.923)	1.000
45	40	6	4	1,660	0.889	0.997	0.886 (0.815-0.956)	0.752
51	87	12	2	1,609	0.926	0.996	0.921 (0.880-0.962)	0.016
52	107	55	2	1,546	0.790	0.982	0.772 (0.714-0.830)	< 0.001
56	87	8	13	1,602	0.892	0.993	0.886 (0.837-0.934)	0.383
58	102	4	27	1,577	0.868	0.990	0.858 (0.809-0.908)	< 0.001
59	90	12	10	1,598	0.891	0.993	0.884 (0.836-0.932)	0.831
66	92	20	1	1,597	0.898	0.993	0.891 (0.845-0.937)	< 0.001
Overall ^d	1201	216	100	24,133	0.884	0.993	0.877 (0.870-0.884)	< 0.001
Overall ^f	1208	218	93	24,131	0.886	0.994	0.880 (0.873-0.887)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 7

Agreement between HybriBio-23 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,715 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	63	4	13	1635	0.881	0.995	0.876 (0.817-0.935)	0.052
11	43	6	3	1663	0.905	0.997	0.903 (0.839-0.966)	0.505
16	104	5	11	1595	0.929	0.995	0.924 (0.886-0.961)	0.211
16 ^b	110	6	5	1594	0.952	0.997	0.949 (0.919-0.979)	1.000
18	77	6	4	1628	0.939	0.997	0.936 (0.896-0.976)	0.752
18 ^c	78	7	3	1627	0.940	0.997	0.937 (0.898-0.976)	0.343
31	66	18	14	1617	0.805	0.990	0.795 (0.725-0.865)	0.596
33	90	5	7	1613	0.938	0.996	0.934 (0.896-0.971)	0.773
35	69	21	2	1623	0.857	0.993	0.850 (0.789-0.911)	< 0.001
39	103	15	17	1580	0.866	0.990	0.856 (0.806-0.905)	0.860
45	41	5	5	1664	0.891	0.997	0.888 (0.819-0.957)	1.000
51	88	11	8	1608	0.903	0.994	0.897 (0.850-0.943)	0.646
52	144	19	22	1530	0.875	0.987	0.862 (0.820-0.904)	0.755
56	81	14	6	1614	0.890	0.994	0.884 (0.833-0.935)	0.118
58	103	3	30	1579	0.862	0.990	0.852 (0.802-0.902)	< 0.001
59	100	1	34	1580	0.851	0.989	0.840 (0.788-0.893)	< 0.001
66	96	17	2	1600	0.910	0.994	0.904 (0.861-0.947)	0.001
Overall ^d	1268	150	178	24129	0.885	0.993	0.879 (0.872-0.886)	0.136
Overall ^f	1275	152	171	24127	0.888	0.993	0.881 (0.874-0.888)	0.317

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 8

Agreement between Bioperfectus-21 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,726 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	64	3	11	1,648	0.901	0.996	0.897 (0.844-0.951)	0.061
11	48	1	4	1,673	0.950	0.999	0.949 (0.904-0.994)	0.371
16	103	7	17	1,599	0.896	0.993	0.888 (0.844-0.933)	0.066
16 ^b	110	7	10	1,599	0.928	0.995	0.923 (0.887-0.959)	0.628
18	79	5	6	1,636	0.935	0.997	0.932 (0.891-0.972)	1.000
18 ^c	80	6	5	1,635	0.936	0.997	0.932 (0.892-0.972)	1.000
31	64	20	19	1,623	0.766	0.988	0.755 (0.678-0.831)	1.000
33	90	5	11	1,620	0.918	0.995	0.913 (0.871-0.956)	0.211
35	85	5	16	1,620	0.890	0.994	0.884 (0.834-0.933)	0.029
39	104	14	39	1,569	0.797	0.983	0.780 (0.722-0.839)	0.001
45	43	3	8	1,672	0.887	0.997	0.883 (0.815-0.952)	0.228
51	83	16	9	1,618	0.869	0.992	0.861 (0.808-0.915)	0.230
52	141	22	30	1,533	0.844	0.983	0.828 (0.782-0.874)	0.332
56	85	11	39	1,591	0.773	0.985	0.758 (0.691-0.824)	< 0.001
58	101	5	31	1,589	0.849	0.989	0.838 (0.785-0.890)	< 0.001
59	98	4	44	1,580	0.803	0.985	0.789 (0.730-0.848)	< 0.001
66	106	7	22	1,591	0.880	0.991	0.871 (0.824-0.917)	0.009
Overall ^d	1,294	128	306	24,162	0.856	0.991	0.848 (0.841-0.855)	< 0.001
Overall ^f	1,302	129	298	24,161	0.859	0.991	0.850 (0.843-0.857)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 9

Agreement between Sansure-26 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 1,726 cervical swab specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	62	5	22	1,637	0.821	0.992	0.813 (0.743-0.883)	0.002
11	47	2	12	1,665	0.870	0.996	0.866 (0.796-0.936)	0.016
16	110	0	40	1,576	0.846	0.987	0.834 (0.783-0.885)	< 0.001
16 ^b	117	0	33	1,576	0.876	0.990	0.866 (0.821-0.911)	< 0.001
18	83	1	11	1,631	0.933	0.996	0.929 (0.889-0.969)	0.009
18 ^c	85	1	9	1,631	0.944	0.997	0.941 (0.905-0.978)	0.027
31	63	21	20	1,622	0.754	0.988	0.742 (0.664-0.820)	1.000
33	94	1	30	1,601	0.858	0.990	0.849 (0.796-0.902)	< 0.001
35	87	3	11	1,625	0.926	0.996	0.921 (0.880-0.962)	0.061
39	114	4	40	1,568	0.838	0.986	0.825 (0.774-0.876)	< 0.001
45	42	4	3	1,677	0.923	0.998	0.921 (0.863-0.979)	1.000
51	95	4	41	1,586	0.809	0.986	0.795 (0.736-0.854)	< 0.001
52	158	5	92	1,471	0.765	0.968	0.735 (0.684-0.786)	< 0.001
56	91	5	47	1,583	0.778	0.984	0.762 (0.699-0.826)	< 0.001
58	104	2	38	1,582	0.839	0.988	0.827 (0.773-0.880)	< 0.001
59	84	18	15	1,609	0.836	0.990	0.826 (0.767-0.885)	0.728
66	110	3	25	1,588	0.887	0.991	0.878 (0.834-0.923)	< 0.001
Overall ^d	1,344	78	447	24,021	0.837	0.989	0.826 (0.819-0.833)	< 0.001
Overall ^f	1,353	78	438	24,021	0.840	0.989	0.829 (0.822-0.836)	< 0.001

^a McNemar's test.

^b The union of LiPA-25 and TS16 DEIA.

^c The union of LiPA-25 and TS18 DEIA.

^d Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

^f Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, TS16 and TS18.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 10

Agreement between Bohui-24 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	40	1	0	15	0.988	0.968	0.955 (0.869-1.042)	1.000
18	7	0	0	49	1	1	1	1.000
31	0	0	0	56	-	-	-	-
33	0	0	0	56	-	-	-	-
35	0	0	0	56	-	-	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	0	1	0	55	-	0.991	1	-
52	1	0	0	55	1	1	1	1.000
56	0	0	0	56	-	-	-	-
58	1	0	0	55	1	1	1	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	50	2	0	788	0.980	0.999	0.979 (0.964-0.994)	0.500

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 11

Agreement between Yaneng-23 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	41	0	0	15	1	1	1	1.000
18	7	0	0	49	1	1	1	1.000
31	0	0	0	56	-	-	-	-
33	0	0	0	56	-	-	-	-
35	0	0	1	55	-	0.991	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	1	0	0	55	1	1	1	1.000
52	1	0	0	55	1	1	1	1.000
56	0	0	1	55	-	0.991	-	-
58	1	0	1	54	0.667	0.991	0.659 (0-1)	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 12

Agreement between Tellgen-27 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	41	0	2	13	0.976	0.929	0.905 (0.776-1)	0.480
18	7	0	1	48	0.933	0.990	0.923 (0.774-1)	1.000
31	0	0	0	56	-	-	-	-
33	0	0	0	56	-	-	-	-
35	0	0	0	56	-	-	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	1	0	0	55	1	1	1	1.000
52	1	0	0	55	1	1	1	1.000
56	0	0	0	56	-	-	-	-
58	1	0	0	55	1	1	1	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 13

Agreement between HybriBio-16 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	41	0	1	14	0.988	0.966	0.953 (0.863-1)	1.000
18	7	0	1	48	0.933	0.990	0.923 (0.774-1)	1.000
31	0	0	1	55	-	0.991	-	-
33	0	0	0	56	-	-	-	-
35	0	0	0	56	-	-	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	1	0	0	55	1	1	1	1.000
52	1	0	0	55	1	1	1	1.000
56	0	0	1	55	-	0.991	-	-
58	1	0	1	54	0.667	0.991	0.659 (0-1)	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	52	0	5	783	0.954	0.997	0.951 (0.929-0.973)	0.063

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 14

Agreement between HybriBio-23 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	1	55	-	0.991	-	-
11	0	0	0	56	-	-	-	-
16	41	0	2	13	0.976	0.929	0.905 (0.776-1)	0.480
18	7	0	2	47	0.875	0.979	0.855 (0.657-1)	0.480
31	0	0	1	55	-	0.991	-	-
33	0	0	0	56	-	-	-	-
35	0	0	0	56	-	-	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	0	1	0	55	-	0.991	-	-
52	1	0	0	55	1	1	1	1.000
56	0	0	1	55	-	0.991	-	-
58	1	0	2	53	0.500	0.981	0.486 (0-1)	0.480
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	51	1	9	779	0.911	0.994	0.904 (0.874-0.934)	0.021

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 15

Agreement between Bioperfectus-21 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	41	0	2	13	0.976	0.929	0.905 (0.776-1.034)	0.480
18	7	0	1	48	0.933	0.990	0.923 (0.774-1.072)	1.000
31	0	0	0	56	-	-	-	-
33	0	0	0	56	-	-	-	-
35	0	0	0	56	-	-	-	-
39	0	0	0	56	-	-	-	-
45	0	0	0	56	-	-	-	-
51	1	0	0	55	1	1	1	1.000
52	1	0	0	55	1	1	1	1.000
56	0	0	0	56	-	-	-	-
58	1	0	0	55	1	1	1	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250

^a McNemar's test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.

Supplementary Table 16

Agreement between Sansure-26 and LiPA-25 for 2 low-risk and 13 high-risk HPV types among 56 cervical biopsy specimens.

HPV type	No. of samples with LiPA-25 and Bohui-24 result of:				Ppos	Pneg	Kappa value (95% CI)	P ^a
	+/+	+/-	-/+	-/-				
6	0	0	0	56	-	-	-	-
11	0	0	0	56	-	-	-	-
16	41	0	2	13	0.976	0.929	0.905 (0.776-1.034)	0.480
18	7	0	3	46	0.824	0.968	0.793 (0.565-1.021)	0.248
31	0	0	3	53	-	0.972	-	-
33	0	0	0	56	-	-	-	-
35	0	0	1	55	-	0.991	-	-
39	0	0	0	56	-	-	-	-
45	0	0	1	55	-	0.991	-	-
51	1	0	0	55	1	1	1	1.000
52	1	0	0	55	1	1	1	1.000
56	0	0	1	55	-	0.991	-	-
58	1	0	0	55	1	1	1	1.000
59	1	0	0	55	1	1	1	1.000
66	0	0	0	56	-	-	-	-
Overall ^b	52	0	11	777	0.904	0.993	0.897 (0.867-0.928)	0.001

^a McNemar² s test.

^b Overall included HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Abbreviation: CI, confidence interval; HPV, human papillomavirus; Ppos, proportion of positive agreement; Pneg, proportion of negative agreement.