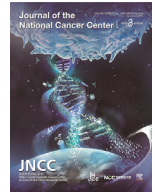




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Full Length Article

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



Jian Yin^{1,2,†}, Shuqian Cheng^{3,4,†}, Daokuan Liu¹, Yabin Tian³, Fangfang Hu^{1,5}, Zhigao Zhang^{3,6}, Tiancen Zhu¹, Zheng Su¹, Yujing Liu¹, Sumeng Wang¹, Yiwei Liu^{1,7}, Siying Peng^{1,5}, Linlin Li¹, Sihong Xu³, Chuntao Zhang^{3,*}, Youlin Qiao², Wen Chen^{1,*}

¹ Department of Cancer Epidemiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

² School of Population Medicine and Public Health, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

³ Division II of In Vitro Diagnostics for Infectious Diseases, National Institutes for Food and Drug Control, Beijing, China

⁴ National Vaccine and Serum Institute (NVSI), Beijing, China

⁵ The State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, National Institute of Diagnostics and Vaccine Development in Infectious Diseases, Collaborative Innovation Center of Biologic Products, School of Public Health, Xiamen University, Xiamen, China

⁶ Institute of Antibody Engineering, School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou, China

⁷ College of Life Sciences, Hebei University, Baoding, China

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ABSTRACT

Background: The SPF10 LiPA-25 system for human papillomavirus (HPV) detection with high analytical performance is widely used in HPV vaccine clinical trials. To develop and evaluate more valent HPV vaccines, other comparable methods with simpler operations are needed.

Methods: The performance of the LiPA-25 against that of other 7 assays, including 4 systems based on reverse hybridization (Bohui-24, Yaneng-23, Tellgen-27, and HybriBio-16) and 3 real-time polymerase chain reaction (PCR) assays (HybriBio-23, Bioperfectus-21, and Sansure-26), was evaluated in selected 1726 cervical swab and 56 biopsy samples. A total of 15 HPV genotypes (HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) were considered for comparison for each HPV type.

Results: Among the swab samples, compared to LiPA-25, compatible genotypes were observed in 94.1% of samples for HybriBio-23, 92.8% for Yaneng-23, 92.6% for Bioperfectus-21, 92.4% for HybriBio-16, 91.3% for Sansure-26, 89.7% for Bohui-24, and 88.0% for Tellgen-27. The highest overall agreement of the 15 HPV genotypes combined was noted for HybriBio-23 ($\kappa = 0.879$, McNemar's test: $P = 0.136$), followed closely by HybriBio-16 ($\kappa = 0.877$, $P < 0.001$), Yaneng-23 ($\kappa = 0.871$, $P < 0.001$), Bioperfectus-21 ($\kappa = 0.848$, $P < 0.001$), Bohui-24 ($\kappa = 0.847$, $P < 0.001$), Tellgen-27 ($\kappa = 0.831$, $P < 0.001$), and Sansure-26 ($\kappa = 0.826$, $P < 0.001$). Additionally, these systems were also highly consistent with LiPA-25 for biopsy specimens (all, $\kappa > 0.897$).

Conclusions: The levels of agreement for the detection of 15 HPV types between other 7 assays and LiPA-25 were all good, and HybriBio-23 was most comparable to LiPA-25. The testing operation of HPV genotyping should also be considered for vaccine and epidemiological studies.

1. Introduction

The incidence rate of cervical cancer has decreased in many countries for decades, while it has increased in China.¹ In 2018, World Health Organization (WHO) called for action toward the elimination of cervical cancer. Human papillomavirus (HPV) vaccine is one of two powerful weapons against HPV infection.² HPV infection is associated with

benign and malignant lesions of the cutaneous and mucosal epithelia. Persistent infection with high-risk HPV (HR-HPV) genotypes is the leading cause of the development of cervical cancer.^{3,4} Epidemiologic research studies have classified 14 HPV genotypes as high risk, based on their association with cervical cancer, i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.^{5,6} Among them, two HR-HPVs (HPV 16 and 18) are the causative factors for about 70% of cervical cancers. Another two low-risk (LR) HPV genotypes (HPV 6 and 11) cause 90% of

* Corresponding authors.

E-mail addresses: zhangct2@126.com (C. Zhang), chenwen@cicams.ac.cn (W. Chen).

† These authors contributed equally to this work.

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genital warts, and most of them require treatment.⁷ HPV vaccination and screening programs are effective strategies in disease prevention and elimination.^{2,8}

To evaluate the effects of type-specific preventive or therapeutic vaccination in the population, sensitive and specific HPV genotyping methods are critical for the selection and monitoring of study subjects. Although several HPV detection methods could monitor the oncogenic vaccine types,^{9–11} the original SPF10 Line Probe Assay (LiPA-25) system has good analytical sensitivity and specificity for HPV genotyping in clinical specimens^{12,13} and was widely applied in HPV vaccine as a golden standard for determining bivalent vaccine efficacy^{14–20} and epidemiologic HPV studies in the world.^{21,22} The system is based on broad-spectrum polymerase chain reaction (PCR) assay for the amplification of a 65-bp fragment in the L1 region, and a reverse hybridization assay, which allows the detection of 13 individual HR-HPV genotypes (i.e., HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66), 11 individual LR-HPV genotypes (i.e., HPV 6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74), and a compound genotype (i.e., HPV 68/73).²³

In the last several years, advances have been made in HPV detection methods, and various PCR-based genotyping methods with primers aimed at the late region (L1 or L2) or the early region (E1, E2, E4, E6, or E7) of the viral genome have been reported.^{24–26} These products mainly consist of real-time fluorescence PCR assays with type-specific primers,^{24–27} or using tagging oligonucleotide cleavage and extension (TOCE) technology,^{28,29} and the general amplification reactions with consensus PCR primer sets that subsequently are detected by type-specific probe hybridization^{24,30–32} or sequencing.^{33,34} However, limited studies with representative samples were conducted to evaluate the performance of these methods.

We performed a blind and head-to-head study by using selected cervical samples, with a focus on HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, to compare seven multiplex HPV tests with SPF10 LiPA-25 for finding the eligible method to apply for HPV vaccine efficacy and epidemiologic studies.

2. Materials and methods

2.1. Subjects

Cervical swab and formalin-fixed, paraffin-embedded (FFPE) biopsy specimens were provided by the Department of Cancer Epidemiology in Cancer Hospital, Chinese Academy of Medical Sciences. A total of 1,897 cervical swab specimens were collected in Thinprep® Pap Test PreservCyt® solution and stored at -80°C, of which the HPV-status was determined with the SPF10 LiPA-25, were selected and prepared 500 µl aliquots for 7 other assays. In addition, we collected 56 FFPE samples diagnosed with CIN+, including cervical intraepithelial neoplasia grade 3 (CIN3; n = 3), cervical micro-invasive carcinoma (n = 2), squamous-cell carcinoma (SCC; n = 49), and adenocarcinoma (n = 2). To confirm the presence of CIN or worse in the FFPE samples used for HPV DNA analysis, a sandwich sectioning method was used.³⁵ The outer sections were stained with hematoxylin and eosin for histological diagnosis, while the inner sections were used for HPV detection by the 8 systems. Seven systems of HPV DNA detection were selected for this study and named with “company name” plus “the number of detected HPV genotypes,” i.e., Bohui-24, Yaneng-23, Tellgen-27, HybriBio-16, HybriBio-23, Bioperfectus-21, and Sansure-26.

2.2. Plasmids

To compare the sensitivities of these assays in the limit of detection analysis, a 10-fold dilution series of the HPV L1, HPV E6, and HPV E7 plasmids purified in a background of human genomic DNA (human placenta, SIGMA-ALDRICH®) for 15 HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66), were tested in 8 assays. The original concentration of HPV DNA was 10⁷ copies/ml. These plasmids

were provided by the National Institutes for Food and Drug Control of China. The limit of detection for 15 HPV genotypes represented either 10 to 1000 copies/test for LiPA-25, 60 to 600 copies/test for Bohui-24, 5 to 500 copies/test for Yaneng-23, 20 to 500 copies/test for Tellgen-27, 10 to 1000 copies/test for HybriBio-16, 20 to 2000 copies/test for HybriBio-23, 20 to 2000 copies/test for Bioperfectus-21, and 5 to 500 copies/test for Sansure-26 (Supplementary Table 1).

2.3. HPV genotyping tests

These methods have different procedures for DNA extraction, PCR, and genotyping test, which were performed according to the manufacturer's instructions. Detailed methods are provided in the Supplementary Materials. In this study, the 8 genotyping systems were divided into two methods: the hybridization method and the real-time fluorescence PCR method.

2.3.1. Hybridization methods by LiPA-25, Bohui-24, Yaneng-23, HybriBio-16, and Tellgen-27

Magnetic bead-based DNA extraction for swab samples or DNA extraction by the boiling method for FFPE samples was performed. Broad-spectrum primers target on L1 region were applied for PCR amplification, and amplicons were genotyped by reverse hybridization with linear/two-dimension probe arrays on membrane of LiPA-25, Bohui-24, Yaneng-23, and HybriBio-16, or with multiplex Luminex bead-based probe array of Tellgen-27 (Table 1 and Supplementary Table 2).

2.3.2. Real-time PCR detection by HybriBio-23, Bioperfectus-21, and Sansure-26

For HybriBio-23 and Bioperfectus-21, magnetic bead-based DNA extraction was used for swab samples, which is different from that of Sansure-26's one-step technology with lysis buffer for the direct and rapid release of DNA. Boiling method or magnetic bead-based DNA extraction was used for FFPE samples. Real-time PCR with type-specific primers targeted on L1/L2/E1/E2/E4/E6/E7 was used for HPV genotyping (Table 1 and Supplementary Table 2).

2.4. Statistical analysis

Only the 15 HPV genotypes (i.e., HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) jointly detected by 8 methods were included for analysis. LiPA-25 was used as a reference for comparison with the other 7 methods. Chi-square test was used to evaluate the difference between LiPA25 and other 7 assays by infection status. When the specimens were analyzed by the two methods (LiPA-25 and other system), genotyping results of specimens were categorized into 3 groups: concordant (100% identical), compatible (at least one genotype of multiple infection was detected by both assays), and discordant.³⁶ Regarding HPV types, the proportion of positive agreement (Ppos) and the proportion of negative agreement (Pneg) were calculated as reported previously,²⁵ and two-tailed McNemar's test was used for mutual comparisons. The level of agreement was determined using Cohen's kappa statistics. The level of statistical significance was set at 0.05. All analyses were performed using R language software (R version 3.5.3).

3. Results

3.1. Genotyping agreement on cervical swab and biopsy samples

The data of 15 genotypes jointly detected by 8 systems were used for analysis. For swab samples, 1,897 women were selected for this study, 171 samples were not included for analysis due to the defects of samples, and finally, 1,726 samples were included for data analysis. The number of invalid detections was 0 for LiPA-25, 1 for Bohui-24, 7 for Yaneng-23, 27 for Tellgen-27, 15 for HybriBio-16, 11 for HybriBio-23, 0 for Bioperfectus-21, and 0 for Sansure-26, and HPV DNA positivity was 58.0% (1,001/1,726), 50.9% (878/1,725), 54.0% (929/1,719),

Table 1
Comparison of the main characteristics of 8 HPV genotyping systems.

System	Methodology of DNA extraction		PCR and HPV genotyping method				Internal control (human DNA)
	Swab	Biopsy	Primer	Targeted region	Amplimer length (bp)	Detection method	
LiPA-25	Magnetic bead-based	Boiling	Broad-spectrum	L1: SPF10	~ 65	Reverse hybridization	No
Bohui-24	Magnetic bead-based	Boiling	Broad-spectrum	L1: 6280~6540	~ 260	Reverse hybridization	Yes
Yaneng-23	Magnetic bead-based	Boiling	Broad-spectrum	L1: GP5+/6+	~ 135	Reverse hybridization	Yes
HybriBio-16	Magnetic bead-based	Boiling	Broad-spectrum	L1: MY09/11	~ 450	Flow cytometry hybridization	Yes
Tellgen-27	Chelex®100-based	Boiling + Chelex®100-based	Broad-spectrum	L1: MY09/11	~ 450	Real-time PCR	Yes
HybriBio-23	Magnetic bead-based	Boiling	Multiplex type-specific	L1/L2/E1/E2/E4/E6/E7	~ 150	Real-time PCR	Yes
Bioperfectus-21	Magnetic bead-based	Boiling + Magnetic bead-based	Multiplex type-specific	L1/E1/E2/E7	~ 120	Real-time PCR	Yes
Sansure-26	One-step	Boiling + Magnetic bead-based	Multiplex type-specific	L1/L2/E1/E6/E7	~ 200	Real-time PCR	Yes

HybriBio-23: HPV types, CT≤40; Internal control, CT≤40; Positive control, CT≤36; Negative control, undetected.
 Bioperfectus-21: HPV types (HPV6, CT<37.4; HPV11, CT<37.9; HPV16, CT<38.0; HPV18, CT<37.8; HPV31, CT<36.2; HPV33, CT<37.2; HPV35, CT<37.2; HPV39, CT<37.6; HPV45, CT<36.4; HPV51, CT<38.1; HPV52, CT<37.8; HPV56, CT<37.7; HPV58, CT<37.9; HPV59, CT<37.5; HPV66, CT<38.0); Internal control, CT≤36.7; Positive control, CT≤30; Negative control, undetected.
 Sansure-26: HPV types, CT≤39; Internal control, CT≤40; Positive control; CT≤36, Negative control, undetected.
 Abbreviation: HPV, human papillomavirus; PCR, polymerase chain reaction.

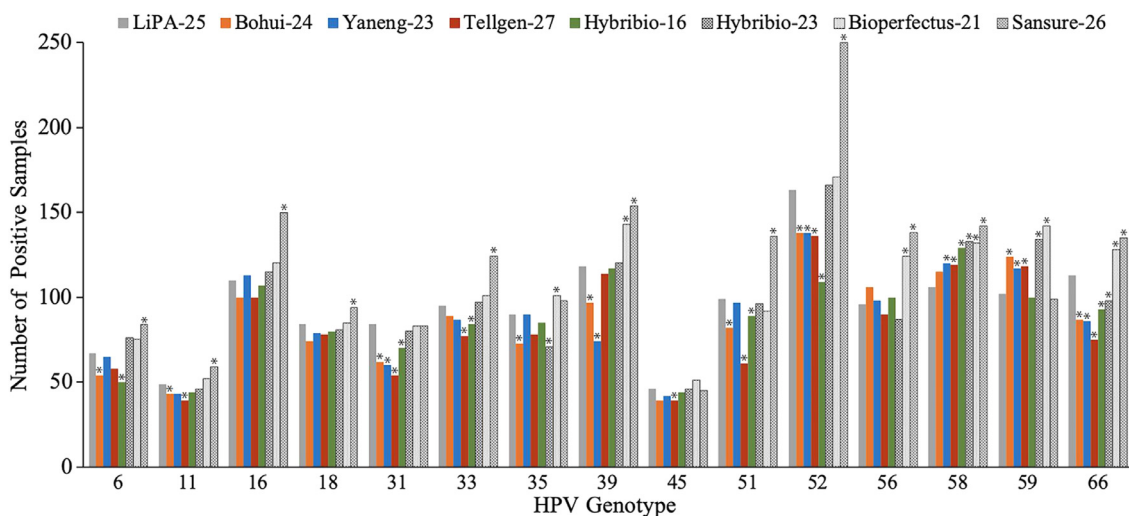


Fig. 1. Positives by HPV genotype from LiPA-25 and other 7 assays for cervical swab specimens. Significant differences ($P < 0.05$, McNemar's test) of other assays (Sansure-26, Bioperfectus-21, Bohui-24, Yaneng-23, Tellgen-27, HybriBio-23, and HybriBio-16) and LiPA-25 are indicated by asterisks.

50.1% (851/1,687), 53.3% (911/1,710), 56.4% (967/1,715), 58.9% (1,016/1,726), and 62.9% (1,086/1,726), correspondingly (Fig. 1 and Table 2).

The majority was single HPV genotype infection that ranged from 32.8% to 38.5%, while multiple genotypes infection ranged from 17.3% to 27.0% among the 8 methods, and the detection of single versus multiple genotypes was different only between Sansure-26, Bioperfectus-21, and LiPA-25 (chi-square test: both $P < 0.001$) (Table 2). For 56 FFPE samples included in the study, HPV DNA was detected in 53, 50, 52, 53, 53, 53, 52, and 54 samples. All HPV DNA-positive samples were a single HPV genotype for LiPA-25 and Bohui-24. The other systems contained more or less multiple HPV genotypes (Table 2).

As stated in materials and methods, HPV genotyping results of specimens were defined as concordant, compatible, and discordant; the results are summarized in Table 3. Compared to LiPA-25, 83.8% of swab samples contained concordant genotypes and 10.3% contained compatible genotypes for HybriBio-23, 83.3% and 9.4% for Yaneng-23, 79.7% and 12.8% for Bioperfectus-21, 83.9% and 8.5% for HybriBio-

16, 77.3% and 14.0% for Sansure-26, 81.2% and 8.5% for Bohui-24, and 79.5% and 8.5% for Tellgen-27, respectively. For the FFPE samples, the samples of concordant genotypes and compatible genotypes varied little (94.6%-100%) between the other 7 methods and LiPA-25.

Subsequently, we made a comparison of individual HPV genotypes by each of the two methods. The agreement among these selected cervical swab or biopsy samples for identification by LiPA-25 and the other 7 methods is summarized in Fig. 1, Table 3, and Supplementary Tables 3-16. For most genotypes from swab specimens, including HPV types 16 and 18, the most important two genotypes, the results obtained by the two methods, including Bioperfectus-21 and LiPA-25, Yaneng-23 and LiPA-25, HybriBio-23 and LiPA-25, and HybriBio-16 and LiPA-25, were not significantly different (all, $P > 0.05$). To be specific, Bioperfectus-21 was more sensitive than LiPA-25 for HPV types 35, 39, 56, 58, 59, and 66; Yaneng-23, HybriBio-23, and HybriBio-16 were all more sensitive than LiPA-25 for HPV types 58 and 59; whereas LiPA-25 was more sensitive for HPV types 31, 39, 52, and 66 than Yaneng-23, for HPV

Table 2
Distribution of the number of 15 HPV genotypes^a detected by cervical swab and biopsy specimens using LiPA-25 and other 7 assays.

Methods	No. of samples	0 type (%)	All positive (%)	Single type (%)	Multiple types (%)				P ^b
					≥ 2 types	2 types (%)	3 types (%)	≥ 4 types (%)	
Swab									
LiPA-25	1,726	725 (42.0)	1,001 (58.0)	664 (38.5)	337 (19.5)	269 (15.6)	55 (3.2)	13 (0.7)	
Bohui-24	1,725	847 (49.1)	878 (50.9)	571 (33.1)	307 (17.8)	224 (13.0)	70 (4.1)	13 (0.7)	0.587
Yaneng-23	1,719	790 (46.0)	929 (54.0)	631 (36.7)	298 (17.3)	228 (13.2)	60 (3.5)	10 (0.6)	0.490
Tellgen-27	1,697	846 (49.9)	851 (50.1)	557 (32.8)	294 (17.3)	222 (13.1)	56 (3.3)	16 (0.9)	0.706
HybriBio-16	1,710	799 (46.7)	911 (53.3)	605 (35.4)	306 (17.9)	235 (13.8)	60 (3.5)	11 (0.6)	1.000
HybriBio-23	1,715	748 (43.6)	967 (56.4)	615 (35.9)	352 (20.5)	254 (14.8)	77 (4.5)	21 (1.2)	0.221
Bioperfectus-21	1,726	710 (41.1)	1,016 (58.9)	596 (34.6)	420 (24.3)	287 (16.6)	107 (6.2)	26 (1.5)	< 0.001
Sansure-26	1,726	640 (37.1)	1,086 (62.9)	620 (35.9)	466 (27.0)	298 (17.3)	121 (7.0)	47 (2.7)	< 0.001
Biopsy									
LiPA-25	56	4 (7.1)	52 (92.9)	52 (92.9)	0 (0)	0 (0)	0	0	
Bohui-24	56	6 (10.7)	50 (89.3)	50 (89.3)	0 (0)	0 (0)	0	0	-
Yaneng-23	56	4 (7.1)	52 (92.9)	49 (87.5)	3 (5.4)	3 (5.4)	0	0	0.118
Tellgen-27	56	3 (5.4)	53 (94.6)	51 (91.0)	2 (3.6)	2 (3.6)	0	0	0.495
HybriBio-16	56	3 (5.4)	53 (94.6)	49 (87.5)	4 (7.1)	4 (7.1)	0	0	0.118
HybriBio-23	56	3 (5.4)	53 (94.6)	47 (83.9)	6 (10.7)	5 (8.9)	1 (1.8)	0	0.027
Bioperfectus-21	56	4 (7.1)	52 (92.9)	49 (87.5)	3 (5.4)	3 (5.4)	0	0	0.118
Sansure-26	56	2 (3.6)	54 (96.4)	45 (80.3)	9 (16.1)	9 (16.1)	0	0	0.003

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

^b Chi-square test or Fisher’s exact test, the difference between LiPA-25 and other 7 assays for the detection of single versus multiple genotypes (≥ 2 types).
Abbreviation: HPV, human papillomavirus.

Table 3
Agreement between 15 HPV genotypes^a detected by both LiPA-25 and other 7 assays.

Type	Bohui-24 (%)	Yaneng-23 (%)	Tellgen-27 (%)	HybriBio-16 (%)	HybriBio-23 (%)	Bioperfectus-21 (%)	Sansure-26 (%)
Swab							
	n = 1,725	n = 1,719	n = 1,697	n = 1,710	n = 1,715	n = 1,726	n = 1,726
Concordant	1,401 (81.2)	1,432 (83.3)	1,349 (79.5)	1,434 (83.9)	1,438 (83.8)	1,376 (79.7)	1,334 (77.3)
Both negative	700 (40.6)	695 (40.4)	677 (39.9)	692 (40.5)	682 (39.7)	658 (38.1)	609 (35.3)
Single types	489 (28.3)	530 (30.8)	474 (27.9)	522 (30.5)	533 (31.1)	496 (28.7)	498 (28.9)
Multiple types	212 (12.3)	207 (12.1)	198 (11.7)	220 (12.9)	223 (13.0)	222 (12.9)	227 (13.1)
Compatible	147 (8.5)	162 (9.4)	144 (8.5)	146 (8.5)	176 (10.3)	220 (12.8)	241 (14.0)
LiPA-25 additional types	70 (4.1)	87 (5.1)	64 (3.8)	78 (4.5)	59 (3.4)	36 (2.1)	28 (1.6)
Other ^b additional types	71 (4.1)	71 (4.1)	72 (4.2)	61 (3.6)	109 (6.4)	179 (10.4)	208 (12.1)
Both additional types	6 (0.3)	4 (0.2)	8 (0.5)	7 (0.4)	8 (0.5)	5 (0.3)	5 (0.3)
Discordant	177 (10.3)	125 (7.3)	204 (12.0)	130 (7.6)	101 (5.9)	130 (7.5)	151 (8.7)
LiPA-25 additional types	147 (8.5)	95 (5.5)	169 (10.0)	107 (6.2)	66 (3.9)	53 (3.0)	31 (1.8)
Other ^b additional types	24 (1.4)	26 (1.5)	29 (1.7)	20 (1.2)	33 (1.9)	67 (3.9)	116 (6.7)
Both additional types	6 (0.4)	4 (0.3)	6 (0.3)	3 (0.2)	2 (0.1)	10 (0.6)	4 (0.2)
Biopsy							
	n = 56	n = 56	n = 56	n = 56	n = 56	n = 56	n = 56
Concordant	53 (94.6)	52 (92.8)	54 (96.4)	50 (89.3)	48 (85.7)	52 (92.8)	45 (80.4)
Both negative	3 (5.4)	3 (5.4)	3 (5.4)	2 (3.6)	2 (3.6)	3 (5.4)	2 (3.6)
Single types	50 (89.2)	49 (87.4)	51 (91.0)	48 (85.7)	46 (82.1)	49 (87.4)	43 (76.8)
Multiple types	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Compatible	0 (0.0)	3 (5.4)	2 (3.6)	4 (7.1)	6 (10.7)	3 (5.4)	9 (16.0)
LiPA-25 additional types	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other ^b additional types	0 (0.0)	3 (5.4)	2 (3.6)	4 (7.1)	6 (10.7)	3 (5.4)	9 (16.0)
Both additional types	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)
Discordant	3 (5.4)	1 (1.8)	0 (0.0)	2 (3.6)	2 (3.6)	1 (1.8)	2 (3.6)
LiPA-25 additional types	3 (5.4)	1 (1.8)	0 (0.0)	1 (1.8)	1 (1.8)	1 (1.8)	0 (0.0)
Other ^b additional types	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	1 (1.8)	0 (0.0)	1 (1.8)
Both additional types	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)

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

^b Other indicated HPV only detected by Sansure-26, Bioperfectus-21, Bohui-24, Yaneng-23, Tellgen-27, HybriBio-23, and HybriBio-16, respectively.
Abbreviation: HPV, human papillomavirus.

types 35 and 66 than HybriBio-23, and for HPV types 6, 31, 33, and 66 than HybriBio-16. When we compared Sansure-26 and LiPA-25, for most genotypes, including HPV types 16 and 18, Sansure-26 was more sensitive than LiPA25. The results for the HPV types 31, 35, 45, and 59 were not significantly different. Regarding Bohui-24 and LiPA-25, LiPA-25 was more sensitive for most genotypes, while Bohui-24 was more sensitive for only HPV type 59. The HPV types 16, 18, 33, 45, 56, and 58 were no statistical evidence of an imbalance. As for Tellgen-27 and LiPA-25, LiPA-25 was more sensitive for HPV types 11, 31, 33, 45, 51, 52, and 66, while Tellgen-27 was more sensitive for HPV types 58 and

59. The results for the remaining HPV types were not significantly different. In 56 biopsy samples, we did not detect all of the 15 individual HPV types, and HPV 11, 33, 39, and 66 were detected by the 8 systems. Specifically, over 40 samples were detected HPV type 16 or 18 by the 8 systems, respectively, and the other HPV types were all present in less than 4 samples.

Almost perfect agreement for the 15 HPV-combined detection in swab specimens was found in LiPA-25 and the other 7 systems (Table 4), i.e., Sansure-26 (Cohen’s $\kappa = 0.826$), Bioperfectus-21 ($\kappa = 0.848$), Bohui-24 ($\kappa = 0.847$), Yaneng-23 ($\kappa = 0.871$), Tellgen-27 ($\kappa = 0.831$),

Table 4
Overall agreement between SPF10 LiPA-25 and other 7 assays in detecting 15 HPV genotypes^a in cervical swab and biopsy specimens.

Methods	No. of samples with LiPA-25 and other assay ^b result of:				Ppos	Pneg	Kappa value (95% CI)	P ^c
	+/+	+/-	-/+	-/-				
Swab								
Bohui-24	1,156	266	127	24,326	0.855	0.992	0.847 (0.839-0.855)	< 0.001
Yaneng-23	1,197	222	112	24,254	0.878	0.993	0.871 (0.864-0.878)	< 0.001
Tellgen-27	1,110	298	126	23,921	0.840	0.991	0.831 (0.823-0.839)	< 0.001
HybriBio-16	1,201	216	100	24,133	0.884	0.993	0.877 (0.870-0.884)	< 0.001
HybriBio-23	1,268	150	178	24,129	0.885	0.993	0.879 (0.872-0.886)	0.136
Bioperfectus-21	1,294	128	306	24,162	0.856	0.991	0.848 (0.841-0.855)	< 0.001
Sansure-26	1,344	78	447	24,021	0.837	0.989	0.826 (0.819-0.833)	< 0.001
Biopsy								
Bohui-24	50	2	0	788	0.980	0.999	0.979 (0.964-0.994)	0.500
Yaneng-23	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250
Tellgen-27	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250
HybriBio-16	52	0	5	783	0.954	0.997	0.951 (0.929-0.973)	0.063
HybriBio-23	51	1	9	779	0.911	0.994	0.904 (0.874-0.934)	0.021
Bioperfectus-21	52	0	3	785	0.972	0.998	0.970 (0.953-0.987)	0.250
Sansure-26	52	0	11	777	0.904	0.993	0.897 (0.867-0.928)	0.001

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

^b Other assay indicated LiPA-25, Sansure-26, Bioperfectus-21, Bohui-24, Yaneng-23, Tellgen-27, HybriBio-23 and HybriBio-16, respectively.

^c McNemar test.

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

HybriBio-23 ($\kappa = 0.879$), and HybriBio-16 ($\kappa = 0.877$). To be specific, the lowest kappa value was HPV 52 for Sansure-26, and HybriBio-16, and HPV31 for other 5 systems (Supplementary Tables 3-9). In biopsy specimens for 15 HPV combined detection, similar results were obtained (all, $\kappa > 0.897$).

The highest Ppos for the 15 types combined in swab specimens was HybriBio-23 with 0.885 (ranged from 0.805 for HPV31 to 0.939 for HPV18), and followed by HybriBio-16 with 0.884 (0.790 for HPV52 to 0.927 for HPV18), Yaneng-23 with 0.878 (0.729 for HPV39 to 0.989 for HPV35), Bioperfectus-21 with 0.856 (0.766 for HPV31 to 0.950 for HPV11), Bohui-24 with 0.855 (0.795 for HPV31 to 0.935 for HPV11), Tellgen-27 with 0.840 (0.739 for HPV31 to 0.894 for HPV45), and Sansure-26 with 0.837 (0.754 for HPV31 to 0.933 for HPV18). In contrast, the variation of Pneg between the two assays (other 7 methods and LiPA-25) was very small (ranged from 0.968 to 0.999), and the least of Pneg was all HPV 52 for the 7 systems. In biopsy specimens, the range of Ppos between the two assays (other 7 methods and LiPA-25) was 0.904 for Sansure-26 to 0.980 for Bohui-24, and the range of Pneg was 0.993 for Sansure-26 to 0.999 for Bohui-24 as well.

As mentioned above, overall agreement rates for the detection of 15 HPV types were high (all, $\kappa > 0.826$); however, it is clear that this agreement resulted from the agreement of HPV-negative specimens, as shown through the high Pneg rates. The observed lower proportions of positive agreement index that there are discrepancies in the different assays' abilities to determine type-specific HPV positives, which suggests differences in assay sensitivity.

In total, LiPA-25 detected 1,422 HPV genotypes (15 types-combined) in swab specimens, which was significantly lower than 1,791 and 1,600 genotypes found by sansure-26 ($P < 0.001$) and Bioperfectus-21 ($P < 0.001$) and was significantly higher than 1,283 by Bohui-24 ($P < 0.001$), 1,309 by Yaneng-23 ($P < 0.001$), 1,236 by Tellgen-27 ($P < 0.001$), and 1,301 by HybriBio-16 ($P < 0.001$), respectively (Table 4). Although the total of HPV genotypes (1,446) detected by HybriBio-23 was higher than those of LiPA-25, there was no significant difference ($P = 0.136$, Table 4). Regarding biopsy, multiple HPV infection was not detected by LiPA-25, and the number of specimens was relatively few. A significant difference was found between Sansure-26 and LiPA-25, and between HybriBio-23 and LiPA-25.

3.2. Detection by TS16 and TS18 PCR/DEIA

For improving the detection rates, we conducted the detection of TS16 and TS18 PCR/DEIA for the positive SPF10 DEIA samples. For

swab samples, the number of HPV16 positive sample detected by SPF10 LiPA-25 was 100, and the union of two sets (HPV16 positive of LiPA-25 and TS16 DEIA positive) was 117 (Supplementary Tables 3-9). The number of HPV18-positive sample was 84, which is only 2 less than the union of two sets (HPV18 positive for LiPA-25 and TS18 DEIA positive) (Supplementary Tables 3-9). Regarding biopsy, the result of LiPA-25 was identical with that of TS16 or TS18 DEIA.

4. Discussion

Since the HPV persistent infection is a surrogate virological endpoint in vaccine trials, it is vital to use reliable and precise testing methods for HPV genotypes to evaluate the efficacy of candidate HPV vaccines.²⁷ The accuracy and precision of HPV genotypes detection on cervical specimens (cervical swab and biopsy specimens) depend on the type of the molecular technology platform used. In this study, 4 systems of hybridization (2 membrane-based, 1 microfluidics + membrane-based, and 1 bead-based) and 3 systems of real-time fluorescence PCR were compared with the LiPA-25 system for identification of the commonly targeted 15 HPV genotypes. The results indicated a high concordance, although the other 7 systems were executed under suboptimal conditions. Briefly, in 1,726 swab specimens, compared with LiPA-25, 88.0% for Sansure-26 to 94.1% for HybriBio-23 of swab samples contained concordant and compatible HPV genotypes. Similar results were obtained for 56 biopsy specimens, for which >94.6% of samples for all 7 systems contained concordant and compatible genotypes. Moreover, these systems showed excellent strength of overall agreements for 15 genotypes detection in swab (all, $\kappa > 0.829$) or biopsy specimens (all, $\kappa > 0.897$).

For broad-spectrum and multiplex type-specific PCR assays, a competition can occur between multiple HPV genotypes within a test, especially, when some HPV genotypes are present at relatively low concentrations, while other types are present at high levels. Due to this competition effect, these approaches may underestimate the presence of HPV genotypes. It is also likely that the competition from the multiplex type-specific PCR approach is weaker than that from broad-spectrum PCR assays. In both swab and biopsy specimens, the total amount of multiplex type-specific PCR assays (e.g., Sansure-26, Bioperfectus-21, and HybriBio-23) for 15 HPV genotypes was more than that of the broad-spectrum PCR assay (e.g., LiPA-25), which suggested the former was more sensitive. Moreover, the three systems could detect more multiple genotypes in individual samples than LiPA-25. Another explanation is that based on the real-time PCR method, the sample is divided into

multi-reaction tubes, which could reduce competitive inhibition of detection of multiple HPV infections. These results demonstrated using a broad-spectrum PCR-based approach can be circumvented by using a multiplex type-specific PCR assay for PCR competition among multiple HPV genotypes.

In contrast, although LiPA-25 is a broad-spectrum PCR assay as HybriBio-16, Yaneng-23, Bohui-24, and Tellgen-27, LiPA-25 was more sensitive than the others. One probable reason is that the SPF10 primer set amplifying a 65-bp region is shorter than that of other primer sets, such as GP5+/6+ and MY09/11, due to the fact that a shorter amplification product was thought to be more analytically sensitive for HPV detection. There might also be some false-positive results due to lack of an internal control in LiPA-25 system, while all other systems have an internal control with human DNA (housekeeping gene).

It is known that each PCR primer set has a marginal preference for different HPV types, which is more likely to be amplified. LiPA-25 can detect more HPV 31 and 52 and less HPV 58 or 59 (Fig 1 and Supplementary Table 3-9) than most of the other 7 methods, although no statistical evidence of an imbalance between Bioperfectus-21, HybriBio-23, and LiPA-25 was found for HPV 31 and 52. Previous studies reported similar results.^{27,36} For example, *van Alewijk et al.*³⁶ tested 400 cervical scraping specimens using LiPA-25 and PGMY Line Blot Assay (LBA). LiPA-25 significantly appeared more sensitive for the detection of HPV 31 ($P < 0.05$) and 52 ($P < 0.05$), less sensitive for HPV 59 ($P < 0.05$) than PGMY LBA. In another study on the comparison between LiPA-25 and MPTS123 system, a E6-based multiplex type-specific system, for 860 swab samples, MPTS123 system was significantly more sensitive for HPV 58 ($P < 0.05$) and HPV 59 ($P < 0.05$).²⁷ Multiple HPV vaccines, which can prevent cervical cancer that results from different HPV types, have been launched and approved for use in individuals with different age in the world. Studies have shown that HPV subtypes have significant age and regional specificities.³⁷⁻⁴⁰ In 2013, the working group at the IARC/United States National Cancer Institute (NCI) Expert Meeting recommended using a virological end-point (persistent HPV infection for 6 months or longer), rather than a disease end-point such as CIN2+, as the primary end-point for some future clinical efficacy trials of vaccine.⁴¹ In United States or Europe, the prevalent HPV types were 16, 18, 45, 31, and 33. The 5 common high-risk types with infection rates were 16, 52, 58, 53, and 18 in Chinese mainland.³⁹ In addition, in China, the most five common HPV types in CIN1 were HPV 52, 16, 58, 18, and 53,⁴² in CN2/3 were HPV 16, 58, 52, 33, and 31.⁴² Considering that the high prevalence of HPV 52 and 58 is just below HPV 16, both in the general population and in patients with CIN2+, the HybriBio-23 and bioperfectus-21 with high-sensitive 52 and 58 are suitable for the novel vaccine, especially applicable to the Chinese population.

As described in a previous study,⁴³ FFPE biopsy tissue specimens pose a critical challenge for DNA extraction and subsequent HPV identification. The efficiency of PCR amplification is inversely correlated to the length of the amplicon produced. The amplicon lengths of the other 7 systems area from 120 bp to 450 bp, longer than that of SPF10 (65 bp). However, the other 7 systems detected more types, except Bohui-24. Moreover, the use of type-specific primer pairs increased the detection of HPV DNA from FFPE biopsy specimens.⁴³ The HybriBio-23, Sansure-26, and Bioperfectus-21, three systems with multiplex type-specific primer-added multi-detection tubes, were more sensitive for the 15 HPV types than LiPA-25.

Either system could be used as a stand-alone system to detect and identify HPV genotypes. To improve the detection rate of types, the combination of SPF10 LiPA-25 and the type-specific PCR DEIA systems for HPV16 and HPV18 is a well-established methodology for HPV16 & 18 vaccine studies.^{14,15,19} Consequently, it also increased the workload and the cost associated with HPV genotyping. In our study, we compared the combination of LiPA-25 plus TS16 or TS18 PCR/DEIA with other 7 systems, respectively, and the combined agreement rate of HPV16 moderately increased for all 7 systems. In contrast, the combined agreement

rate of HPV 18 for Bohui-24 or Tellgen-27 decreased slightly, while for the other systems, there was a marginal rise, comparing the results of HPV 18 for only LiPA-25.

There are also disadvantages and advantages of these HPV genotyping systems. In general, the sample size is challenging for HPV genotyping because more participants need to be recruited to evaluate the effects for more values vaccine. Therefore, it is necessary to use a high-throughput and automatic or semi-automatic method for HPV genotyping. Although the real-time PCR methods are more sensitive for HPV genotyping, the workload is 6-8 folds as much as hybridization methods because the samples need to be parallelly divided into 6-8 detection tubes. The microarray hybridization from HybriBio-16 utilized a flow-through hybridization technique by actively directing the targeting molecules toward the immobilized probes within the membrane fibers. Compared with conventional reverse dot hybridization, the method makes the DNA into the pore of hybrid membrane by diversion, which greatly improves the speed and efficiency of hybridization. The nucleic acid chip detector (BHF-VI) from Bohui-24 was fully automatic, and the swab samples were only added to the wells from HPV detection chip. The three processes, including target DNA extraction, PCR amplification, and reverse dot hybridization, are carried out under the drive of a micropump. After about 4 h, the software in instrument can automatically report the results of detected sample. Therefore, besides the accuracy of HPV genotypes detection, workload, reaction time, and other factors should be taken into consideration.

There are several strengths and limitations in the study. The most important strength was that this was a head-to-head and large-scale study covering detection of all the high-risk HPV and the most important two low-risk HPV 6 and HPV 11 in two specimens, including exfoliated cervical cells and FFPE samples. The primer targets regions from the 8 selected systems not only include the later region but also the early region. In addition, all these systems except LiPA-25 included the housekeeping gene as an internal control, which could reduce sampling errors and false negative detection. There are also limitations. First, the panel size of FFPE biopsy specimens was much smaller than that of swabs, and not every HPV genotype was included. Moreover, the composition of the panel of 1,726 swab samples was based on the results of SPF10 LiPA-25 system obtained 3 to 5 years ago. Although all the samples were stored at -80°C , it might have a negative influence on the detection of HPV genotypes due to DNA degeneration.

In conclusion, analytically sensitive and type-specific measures of HPV infections are vital for continually monitoring of HPV infection and determination of the causality of precancer. Detection of the 15 HPV types common to LiPA-25 without a human control primer and other 7 systems with housekeeping gene primers was similar in swab and biopsy specimens, although there were more or less different in the 15 HPV types in comparison. Considering the prevalence of different types, e.g., HPV 31, 52, and 58, in population, HybriBio-23 is highly suitable for studies on multiple-value vaccine efficacy, genotype surveillance, and disease association. We also need to consider the operating procedure and workload of the HPV genotyping method for vaccine and epidemiological studies. This study contributes to our understanding of the performance characteristics, practicability, and comparability of the 8 systems of HPV genotyping.

Declaration of competing interest

The authors declare that they have no conflict of interests.

Ethics statement

This study was performed in accordance with the recommendations of the Declaration of Helsinki and approved by the Institutional Review Board of Cancer Hospital, Chinese Academy of Medical Sciences (No. 20/243-2439).

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Author contributions

W.C., J.Y., Y.Q., C.Z., and S.X. designed the study. J.Y., S.C., D.L., Y.T., F.H., Z.Z., T.Z., Z.S., Y.L., S.W., Y.L., S.P., and L.L. managed the data. J.Y. conducted the statistical analysis and drafted the paper. W.C., Y.T., S.X., C.Z., and Y.Q. reviewed the paper. All of the authors commented on the draft and decided to submission.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jncc.2022.06.003.

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