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RESEARCH ARTICLE

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A direct comparison of four high-risk human papillomavirus tests versus the cobas test: Detecting CIN2+ in low-resource settings

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Abstract

Low-cost, accurate high-risk human papillomavirus (HR-HPV) tests are needed for cervical cancer screening in limited-resource settings. More than 200 cervical cytological specimens from hospital patients were collected and analyzed for a real-world study. We evaluated the analytical and clinical performance of four widely used HR-HPV test (Tellgen, Hybribio, Liferiver, and Sansure) based on real-time polymerase chain reaction technology platforms, compared with the cobas test. Cervical intraepithelial neoplasia grade 2 or worse lesions (CIN2+) were set as the disease endpoint, and all the five HPV tests were performed with equal sensitivity (McNemar's test; $P = 0.971$) and specificity (McNemar's test; $P = 0.953$). All genotyping using the INNO-LiPA HPV test showed that HPV-16, -52, and -54 were the most common types among CIN2+ cases. Overall, the four HR-HPV tests analyzed appear to be as effective as the cobas HPV test in both agreement and clinical performance. Therefore, each of these low-cost HPV test kits could be implemented in limited-resource settings to accelerate the control of cervical cancer. However, we suggest that there is a need to further standardize and optimize testing around clinical sensitivity and specificity.

KEYWORDS

cervical screening, cobas HPV test (cobas) , human papillomavirus (HPV), INNO-LiPA, real-time polymerase chain reaction (PCR)

1 | INTRODUCTION

Cervical cancer is associated with a substantial burden of disease and is the cause of a substantial number of deaths among women in low-resource settings.[1] A wealth of emerging evidence confirms that persistent infection with high-risk human papillomavirus (HR-HPV) is associated with more than 99% of all cervical cancers.[2,3] In particular,

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; C_t, cycle threshold; CI, confidence interval; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; NEG, negative; NPV, negative predictive value; PCR, polymerase chain reaction; POS, positive; PPV, positive predictive value; SCC, squamous cell carcinoma.

*Xue, Gao and Yin have contributed equally to this study.

HPV-16 and -18 are known to be the most common HPV types, leading to an estimated 70% of all cervical cancers.[4,5] The World Health Organization recently announced a global call for action to accelerate cervical cancer control.[6] However, costs can be prohibitive, and a number of HPV tests are readily available. Therefore, it is necessary to evaluate the cost-effectiveness of HPV screening methods.

HPV testing, due to its higher sensitivity compared with the traditional Pap test for detecting cervical intraepithelial neoplasia with grade 2 or worse lesions (CIN2+), has been investigated as a primary screening tool.[7-9] The hybrid capture II (HC2) test, which is based on a signal-amplified hybridization method, has received approval from the Food and Drug Administration (FDA).[10-12] Yet, this test is expensive at $71+ per test and can only determine whether HPV infection is present; it neither determines specific genotypes, such as HPV-16 and-18, nor it is capable of identifying between single and multiple HPV infections.[13]

Recently, various tests based on real-time polymerase chain reaction (PCR) have emerged. Compared with HC2 tests, the real-time PCR method has several advantages, such as simplicity, high throughput, and less time. The most frequently administered PCR test in trials is the cobas HPV test (Roche Systems Inc, Branchburg, NJ). The advantage of the cobas test is that it is a fully automated real-time PCR DNA amplification test that has been approved for screening by the FDA in 2014.[14] The cobas test was initially developed with clinical cut-off values, which enhanced the level of specificity thereby maximizing the predictive value of the oncogenic risk of CIN2+.[15] Unfortunately, the cobas test is not flawless, because it requires access to highly specialized, bulky instrumentation for sample pretreatment and detection purposes. The cobas HPV system weighs more than 150 kg and is 166 cm wide.[13] Also, each detection is comparatively costly at $35+ per test. These issues make the cobas HPV test impractical and inhibitive for low-resource settings with less developed infrastructures.

Until recently in China, numerous commercially available HR-HPV tests based on real-time PCR have been made available in hospitals and laboratories. Unfortunately, before 2015, China did not regulate HPV testing based on clinical sensitivity or specificity thresholds,[16] and only recently, researchers have begun to investigate performances of the most commonly used HR-HPV tests. Therefore, it would seem necessary to conduct a more comprehensive investigation into the most accessible and affordable HPV test kits to promote the best screening practice for health services in low-resource settings.

This study focused on a preliminary analytical evaluation of four widely used HR-HPV test kits that had been approved by the China Food and Drug Administration (CFDA) for HPV detection (Tellgen, Hybribio, Liferiver, and Sansure) to appraise and compare the efficacy of each test against the existing approved cobas HPV test for the detection of high-grade cervical lesions. The overarching aim was to determine whether more cost-effective and more easily administered HR-HPV tests can be used for cervical cancer screening campaigns in low-resource settings.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

To evaluate the analytical and clinical performances of the four HR-HPV tests, we collected a total of 214 cytology samples with cervical lesions results from December 2016 to April 2017. After informed consent was requested and approved by all participants, sampling was conducted. Two hundred and fourteen samples were selected from women aged 23 to 65 years, who had visited Beijing Obstetrics and Gynecology Hospital for a routine examination. Cytology samples collected were transferred into PreservCyt solution (Hologic Inc, Bedford, MA), stored at 4°C, and then transferred to Central Lab, Cancer Hospital, Chinese Academy of Medical Sciences for HPV testing. All specimens were classified into five groups according to their pathological results: Normal (n = 125), CIN1 (n = 15), CIN2 (n = 39), CIN3 (n = 31), and squamous cell carcinoma (SCC; n = 4).

The five HR-HPV tests were performed using cobas (Roche Molecular Systems Inc, Roche, Shanghai, China), Tellgen (Nucleic Acid Detection kit for HPV and 16/18 Genotyping; Tellgen, Shanghai, China), Hybribio (14 HR-HPV with 16/18 Genotyping Real-Time PCR kit; Hybribio, Guangdong, China), Liferiver (HPV Genotyping Real-time PCR kit; Liferiver, Shanghai, China), and Sansure (HR-HPV DNA Fluorescence Diagnostic Kit; Sansure, Hunan, China) sequentially across specimens.

The HPV genotyping test was performed using INNO-LiPA HPV genotyping extra (Gent, Belgium) on 124 samples indicating positive diagnosis by either the five HR-HPV tests or abnormal cervical lesions. Women with positive test results were referred directly to colposcopy and if colposcopy confirmed this finding, four-quadrant biopsies were then taken. If the colposcopy was unable to detect lesions, a random biopsy was obtained at the squamocolumnar junction in localized quadrant at 2, 4, 8, or 10 o'clock.

2.2 | Real-time PCR HPV testing

The cobas HPV test was the first real-time PCR technology approved by the U.S. FDA for cervical cancer screening and is commonly available in most settings. The remaining four low-cost HR-HPV tests (Tellgen, Hybribio, Liferiver, and Sansure) have been approved by the CFDA for HPV detection and were commonly used in China.

A sample of 1 mL of liquid cytology was separated for investigation using five real-time PCR HPV tests (see Table 1; cobas, Tellgen, Hybribio, Liferiver, and Sansure), all of which are based on TaqMan technology. The cobas, Tellgen, and Hybribio reportedly detect 14 HPV genotypes (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68). They also differentiate HPV-16 and -18 from the other pooled high-risk HPV groups. In addition to the 14 HPV types mentioned above, the Liferiver and Sansure tests are also capable of detecting HPV -82.

TABLE 1 Comparison of features of the five available HR-HPV tests

Performance specification	Tellgen	Hybribio	Liferiver	Sansure	Cobas
Regulatory status	CFDA approved	CFDA approved	CFDA approved	CFDA approved	FDA approved
Detection target	14 high-risk HPV DNA	14 high-risk HPV DNA	15 high-risk HPV DNA	15 high-risk HPV DNA	14 high-risk HPV DNA
Detection chemistry	TaqMan	TaqMan	TaqMan	TaqMan	TaqMan
Sample processing	Semiautomatic sample extraction	Semiautomatic sample extraction	Semiautomatic sample extraction	Semiautomatic sample extraction	Automated sample extraction
Vol of sample required, mL	1	1	1	1	1
Throughput	~96	~96	~96	~96	~96
Internal control	β-Globin	β-Globin	β-Globin	β-Globin	β-Globin
Cut-off value (C_t)	30.0	36.0	38.0	39.0	40.5
Cost	$6+ per test	$6+ per test	$7+ per test	$7+ per test	$35+ per test

β-Globin monitors for samples quality, extraction, amplification, and detection steps of the test; throughput, suppose that using an instrument, a rough number of samples can be detected in one test. Abbreviations: CFDA, China Food and Drug Administration; C_t, cycle threshold; FDA, Food and Drug Administration; HR-HPV, high-risk human papillomavirus.

The cobas HPV test has a differentiating feature in that the cobas 4800 system is a highly automated instrument for DNA extraction using the Roche HPV DNA kit, PCR amplification on the cobas ×480 instrument, and detection on the cobas z480 analyzer.

They perform part of the manual DNA extraction using related HPV DNA kits and PCR amplification with a mixture of multiple probes and detection on the ABI 7500 or SLAN-96P automated analyzer. The experimental conditions for the five HR-HPV tests follow guidelines provided within the associated protocols. During each run, both positive and negative controls are included to ensure that proper PCR responses are not subjected to carry-over. The resulting fluorescence from the reaction is then measured to determine whether HPV is present in the sample.

2.3 | HPV genotyping testing

INNO-LiPA HPV genotyping extra is based on PCR amplification followed by reverse line hybridization using short PCR fragment (SPF10) primers to achieve amplification of a 65-bp region within the L1 open reading frame (ORF) of multiple HPV types.[17] The obtained biotinylated amplicon is hybridized to the strip, and type-specific oligonucleotides are fixed on the strip, and then an Auto-LiPA48 instrument is used for automatic colorimetric detection. The results are interpreted using a direct-vision method or utilizing the analytical software, LIRAS for LiPA HPV. Due to shorter amplification, this is theoretically more sensitive but possibly less specific for detecting HPV than DNA-based tests.[18] This assay has been widely used in clinical trials on HPV and HPV-related disease research for the identification of specific sequences in the L1 region of 28 HPV types containing 15 HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 probable HR-HPV (26, 53, and 66) and 10 low-risk (10LR) HPV (6, 11, 26, 40, 43, 44, 54, 69, 70, 71, and 74).[19]

2.4 | Statistical analysis

SAS 9.2 software (SAS Institute Inc, Cary, NC) was used for statistical analysis. Agreement and corresponding Kappa coefficients with 95% confidence intervals were calculated to estimate levels of agreement between the four HR-HPV tests and the cobas HPV test. The median score and Mann-Whitney U tests were calculated *P* values for the median four HR-HPV and cobas test cycle threshold (C_t) values for concordant versus discordant positive specimens. McNemar's test chi-square test was used to compare HPV-positive rates, sensitivity, specificity, positive predictive value, and negative predictive value. *P* values less than 0.05 (two-sided) were considered statistically significant.

2.5 | Ethical approval

Informed consent was requested and consequently approved by all participants in this study. This study was formally approved by the institutional review boards of the Beijing Obstetrics and Gynecology Hospital, Capital Medical University and National Health Commission of the People's Republic of China (No. 2015-071).

2.6 | Experimental human participants statement

All methods were performed in accordance with the relevant guidelines and regulations.

3 | RESULTS

3.1 | Prevalence of HPV genotype in all subjects and CIN2+

Of the 214 cytology samples, 4 cases were considered invalid due to lack of remnant DNA and were thereby excluded. The

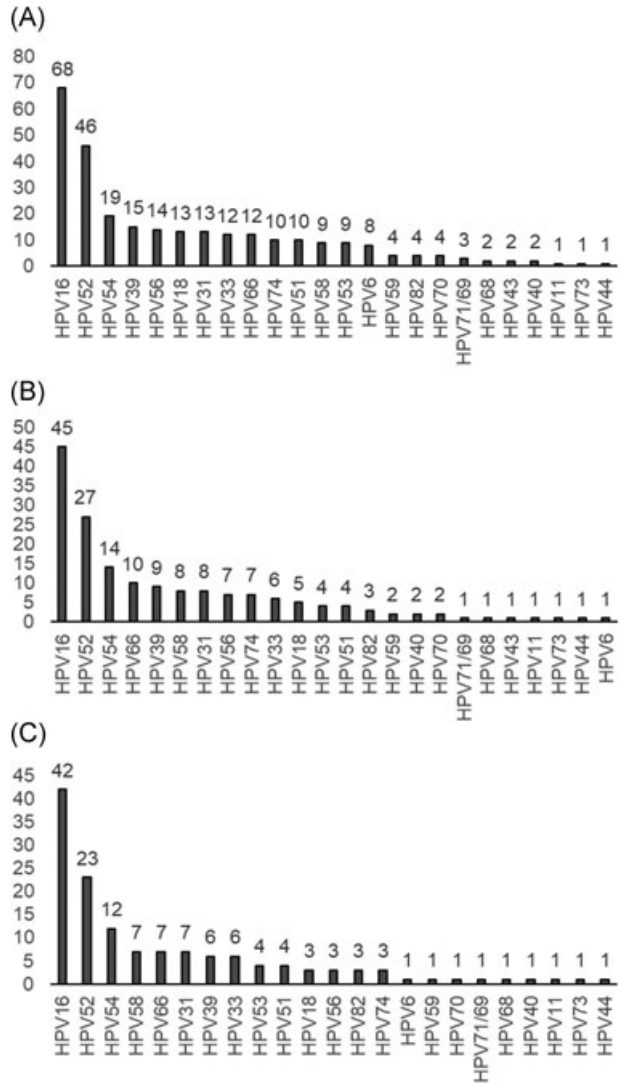

FIGURE 1 Prevalence of different HPV genotype types. A, All the positive subjects (n = 124). B, Cervical intraepithelial neoplasia grade 1 or worse (CIN1+; n = 89). C, Cervical intraepithelial neoplasia grade 2 or worse (CIN2+; n = 74). CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus

remaining 210 samples were included for analysis. The distribution of HPV types among these samples is presented in Figure 1. The INNO-LiPA HPV genotyping test revealed that 124 cases (59.05%) contained 1 or more HPV types. These consisted of 44 cases (20.95%) infected with a single HPV type, 35 cases (16.67%) with 2 HPV types, and 45 cases (21.43%) with 3 or more HPV types. In the HPV infected subjects, HPV-16 was detected in 68 cases, accounting for 54.84% of all HPV-positive subjects, including mixed infections. HPV-16 was the most prevalent genotype across this sample, followed by HPV-52, -54, -39, and -56 (see Figure 1A). Further analysis found that the most common types identified across CIN1+ cases were 16, 52, 54, 66, and 39 (see Figure 1B) and the most common types identified across CIN2+ cases were 16, 52, 54, 58, and 66 (see Figure 1C).

3.2 | Comparison of the HPV detection results by four HR-HPV tests and the cobas HPV test

In 210 cytology samples, overall HR-HPV-positive rates ranged from 50.0% to 53.3%. None of the included tests performed less well in HPV-positive rates overall ($P = 0.964$). Table 2 displays HPV-positive results with corresponding histopathologic grading. Data suggested that there is a positive correlation of HPV with histopathologic grading ($P < 0.0001$), except for HPV-18-positive rates that may be partly due to the relative small number of HPV-18 cases within the sample. Table 3 displays independent levels of agreement for each of the four HR-HPV tests compared with the cobas test. The four HR-HPV tests, compared with the cobas test demonstrated high levels of agreement with 95.24% (Kappa, 0.905), 95.71% (Kappa, 0.914), 95.24% (Kappa, 0.905), and 94.76% (Kappa, 0.895) of all samples.

In cases infected with HPV-16, levels of agreement in the four HR-HPV tests against those analyzed using the cobas HPV test were 96.19% (Kappa, 0.908), 98.10% (Kappa, 0.953), 98.10% (Kappa, 0.954), and 95.24% (Kappa, 0.885). In cases infected with HPV-18, levels of agreement with the cobas HPV test were 99.52% (Kappa, 0.939), 98.10% (Kappa, 0.740), 98.57% (Kappa, 0.835), and 98.57% (Kappa, 0.835). HR-HPVs other than HPV types 16 and 18 were also analyzed and again performed with equally high levels of agreement compared with the cobas HPV test with 90.48% (Kappa, 0.783), 91.90% (Kappa, 0.813), 89.52% (Kappa, 0.768), and 87.62% (Kappa, 0.726). Samples with discordant results had C_t values significantly closer to cut-off values for the four HR-HPV tests or the cobas HPV test. Median C_t values of concordance and discordance in the four HR-HPV tests and cobas test results are presented in Table 4. All concordant cases between the four HR-HPV as well as the cobas HPV tests have significantly lower median cobas C_t values compared with those discordant cases ($P < 0.001$).

3.3 | Clinical performance of the five HR-HPV tests for detection of CIN2+

According to pathological results, CIN2+ lesions were identified in 74 women, including 39 CIN2, 31 CIN3, and 4 SCC cases. Among 74 CIN2+ cases, 1 CIN2 (A030) and CIN3 (A045) cases were undetected by all the 5 HR-HPV tests, 1 CIN2 (A008) case was missed by the Tellgen, Hybribio ,and cobas, 1 CIN3 (A064) case was missed by the Liferiver, Sansure, and cobas, 3 CIN3 cases and 1 CIN2 (A042, A020, A009, and A040) were undetected by the Tellgen, Hybribio, Liferiver, and cobas, respectively (see Table 5). Clinical performances of each of the HR-HPV tests as well as the cobas HPV test to the disease endpoint of CIN2+ were analyzed (see Table 6). Data revealed that the sensitivity (94.59%, 94.59%, 94.59%, 95.59%, and 93.24%) and specificity (72.79%, 73.53%, 71.32%, 69.86%, and 73.53%) of all tests were similar. Overall, there was no significant difference in either sensitivity (McNemar's test; $P = 0.971$) or specificity (McNemar's test; $P = 0.953$) across these HR-HPV tests for detecting CIN2+. For these missing CIN2+ cases, the INNO-LiPA HPV test revealed that

TABLE 2 Positivity rates of the five HPV tests according to histopathology classification, n (%)

HPV test	HPV type	Normal (n = 121)	CIN1 (n = 15)	CIN2 (n = 39)	CIN3 (n = 31)	SCC (n = 4)	All (n = 210)	*P* value
Tellgen	HR-HPV	23 (19.01)	14 (93.33)	37 (94.87)	29 (93.55)	4 (100.0)	107 (50.95)	<0.0001
	HPV-16	13 (10.74)	3 (20.00)	20 (51.28)	20 (64.51)	4 (100.0)	60 (28.57)	<0.0001
	HPV-18	2 (1.65)	2 (13.33)	3 (7.69)	1 (3.22)	0 (0)	8 (3.81)	0.0765
	HPV others	16 (13.22)	12 (80.00)	29 (74.35)	13 (41.94)	0 (0)	70 (33.33)	<0.0001
Hybribio	HR-HPV	24 (19.83)	12 (80.00)	37 (94.87)	29 (93.55)	4 (100.0)	106 (50.48)	<0.0001
	HPV-16	13 (10.74)	2 (13.33)	19 (48.72)	20 (64.51)	4 (100.0)	58 (27.62)	<0.0001
	HPV-18	2 (1.65)	2 (13.33)	2 (5.13)	1 (3.22)	0 (0)	7 (3.33)	0.1014
	HPV others	20 (15.53)	11 (73.33)	26 (66.67)	10 (32.26)	0 (0)	67 (31.90)	<0.0001
Liferiver	HR-HPV	25 (20.66)	14 (93.33)	38 (97.44)	28 (90.32)	4 (100.0)	109 (51.90)	<0.0001
	HPV-16	14 (11.57)	3 (20.00)	19 (48.72)	20 (64.51)	4 (100.0)	60 (28.57)	<0.0001
	HPV-18	3 (2.48)	2 (13.33)	3 (7.69)	2 (6.45)	0 (0)	10 (4.76)	0.1646
	HPV others	22 (18.18)	13 (86.67)	30 (76.92)	13 (41.94)	0 (0)	78 (37.14)	<0.0001
Sansure	HR-HPV	29 (23.97)	12 (80.00)	37 (94.87)	30 (96.77)	4 (100)	112 (53.33)	<0.0001
	HPV-16	15 (12.40)	2 (13.33)	18 (46.15)	21 (67.74)	4 (100)	60 (28.57)	<0.0001
	HPV-18	3 (2.48)	3 (20.00)	3 (7.69)	1 (3.22)	0 (0)	10 (4.76)	0.0517
	HPV others	25 (20.66)	11 (73.33)	30 (76.92)	12 (38.71)	0 (0)	78 (37.14)	<0.0001
Cobas	HR-HPV	25 (20.66)	11 (73.33)	36 (92.31)	29 (93.55)	4 (100.0)	105 (50.00)	<0.0001
	HPV-16	13 (10.74)	4 (26.67)	20 (51.28)	21 (67.74)	4 (100.0)	62 (29.52)	<0.0001
	HPV-18	2 (1.65)	2 (13.33)	4 (10.26)	1 (3.22)	0 (0)	9 (4.28)	0.0433
	HPV others	19 (15.70)	10 (66.67)	25 (64.10)	11 (35.48)	1 (25.00)	66 (31.43)	<0.0001

Abbreviations: CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HR-HPV, high-risk human papillomavirus; SCC, squamous cell carcinoma.

these cases include two low-risk types, one HPV-82, three HPV-16, one HPV-33, and one HPV-52.

4 | DISCUSSION

The present study was designed to evaluate the four readily available and widely used HR-HPV test kits (Tellgen, Hybribio, Liferiver, and Sansure) for advantages over the cobas test, which is relatively cumbersome and costly. The cost of the four HR-HPV tests used to screen cervical cancer in China has been estimated and range between $6 and $7 per test, which was much lower than that of the approved cobas HPV test.

To the best of our knowledge, there are few real-world studies that have evaluated the analytical and clinical performances of four low-cost HR-HPV tests simultaneously compared with the cobas HPV test. Currently, due to the lack of a true gold standard for HPV tests, studies evaluating new HPV assays usually rely on comparisons with established assays and evaluation of associations with disease endpoints.[20] Since approval by the U.S. FDA and validation by numerous large-scale studies,[21,22] such as ATHENA study, the cobas HPV test was regarded as a reference HPV assay with which to compare the efficacy of the newly developed HPV tests.[23,24] In this study, the four HR-HPV tests demonstrated high levels of agreement with the cobas HPV test for HPV detections. Discordant results across all five HPV tests were found in samples with C_t values closer to the test limits of detection than concordant samples ($P < 0.001$), which suggests that these discordant samples may have contained lower viral loads based on high C_t values. Therefore, discrepancies observed in this study may have manifested through varying predetermined cut-off values chosen for optimization around test sensitivity and specificity.

In our study, the most common HPV type was HPV-16, followed by HPV-52, -54, -39, and -56. Further analysis of CIN1+ and CIN2+ cases showed that the most common HPV types were also HPV-16, -52, and -54. However, HPV-16 and -52 were found to be the most common HPV types that were consistent with other research on Chinese populations.[25,26] Interestingly, our data suggest that HPV-54 may be the most common mixed infection, low-risk HPV. This may manifest through the association between infection with multiple HPV types and may increase the risk of cervical cancer although further research is required.

Compared with the cobas HPV test, the four HR-HPV tests performed similarly with positive rates ranging from 50.0% to 53.3%. Such high HPV-positive rates were not surprising because samples were taken from registered patients, receiving treatment. In addition, by using CIN2+ as a threshold, it became possible to compare levels of clinical sensitivity and specificity of all four HR-HPV tests, which ultimately performed equally well when compared with the cobas HPV test. This study suggests that these HR-HPV tests and cervical histopathology correlate positively. All the evaluated HR-HPV tests had the ability to detect HPV from CIN2+ samples in approximately 90.3% to 100.0% of cases. Therefore, this analysis demonstrated that the four simple and low-cost HR-HPV tests performed as equally well at detecting HPV infection in cervical lesions compared with the cobas HPV test.

Further examination of undetected CIN2+ cases highlighted that one CIN2 and one CIN3 case were not detected by any of the five HR-HPV tests. This observation was also unsurprising because HPV-44 and -70 are low-risk types not targeted by these HR-HPV

TABLE 3 Concordance rates between the results of the four HR-HPV tests and the cobas test

				% Agreement (95% CI)				
		Cobas		Overall	Positive	Negative	Kappa (95% CI)	*P* value
		Positive (all)	Negative					
Tellgen	Positive	101	4	95.24 (91.46-97.39)	94.39 (88.30-97.40)	96.12 (90.44-98.48)	0.905 (0.847-0.962)	0.5271
	Negative	6	99					
		Positive for HPV-16	Negative for HPV-16					
	Positive	57	3	96.19 (92.66-98.06)	91.94 (82.47-96.51)	97.97 (94.21-99.31)	0.908 (0.845-0.970)	0.4795
	Negative	5	145					
		Positive for HPV-18	Negative for HPV-18					
	Positive	8	0	99.52 (97.35-99.92)	88.89 (56.05-98.01)	100.00 (98.12-100)	0.939 (0.819-1.000)	0.3173
	Negative	1	201					
		Positive for others	Negative for others					
	Positive	58	12	90.48 (85.75-93.75)	87.88 (77.86-93.73)	91.67 (86.00-95.17)	0.783 (0.692-0.873)	0.3711
	Negative	8	132					
		Positive (all)	Negative					
Hybribio	Positive	101	5	95.71 (92.06-99.73)	96.19 (90.61-98.51)	95.24 (89.33-97.95)	0.914 (0.860-0.969)	0.7389
	Negative	4	100					
		Positive for HPV-16	Negative for HPV-16					
	Positive	58	0	98.10 (95.21-99.26)	93.55 (84.55-97.46)	100.00 (97.47-100)	0.953 (0.908-0.999)	0.0455
	Negative	4	148					
		Positive for HPV-18	Negative for HPV-18					
	Positive	6	1	98.10 (95.21-99.26)	66.67 (35.42-87.94)	99.50 (97.24-99.91)	0.740 (0.496-0.985)	0.3173
	Negative	3	200					
		Positive for others	Negative for others					
	Positive	58	9	91.90 (87.42-94.88)	87.88 (77.86-93.73)	93.75 (88.55-96.68)	0.813 (0.728-0.898)	0.8084
	Negative	8	135					
		Positive (all)	Negative					
Liferiver	Positive	102	7	95.24 (91.46-97.39)	97.14 (91.93-99.02)	93.33 (86.87-96.73)	0.905 (0.847-0.962)	0.2059
	Negative	3	98					
		Positive for HPV-16	Negative for HPV-16					
	Positive	59	1	98.10 (95.21-99.26)	95.16 (86.71-98.34)	99.32 (96.27-99.88)	0.954 (0.909-0.999)	0.3173
	Negative	3	147					
		Positive for HPV-18	Negative for HPV-18					
	Positive	8	2	98.57 (95.88-99.51)	88.89 (56.05-98.01)	99.00 (96.45-99.73)	0.835 (0.651-1.000)	0.5637
	Negative	1	199					
		Positive for others	Negative for others					
	Positive	61	17	89.52 (84.65-92.98)	92.42 (83.46-96.72)	88.19 (81.91-92.50)	0.768 (0.677-0.859)	0.0105
	Negative	5	127					
		Positive (all)	Negative					
Sansure								
	Positive	103	9	94.76 (90.87-97.05)	98.10 (93.32-99.48)	91.43 (84.51-95.43)	0.895 (0.835-0.955)	0.0348
	Negative	2	96					
		Positive for HPV-16	Negative for HPV-16					
	Positive	56	4	95.24 (91.46-97.39)	90.32 (80.45-95.49)	97.30 (93.26-98.94)	0.885 (0.815-0.954)	0.5271
	Negative	6	144					
		Positive for HPV-18	Negative for HPV-18					
	Positive	8	2	98.57 (95.88-99.51)	88.89 (56.50-98.01)	99.00 (96.45-99.73)	0.835 (0.651-1.000)	0.5637
	Negative	1	199					
		Positive for others	Negative for others					
	Positive	59	19	87.62 (82.48-91.41)	89.39 (79.69-94.77)	86.81 (80.31-91.39)	0.726 (0.629-0.824)	0.0189
	Negative	7	125					

Abbreviations: CI, confidence interval; HR-HPV, high-risk human papillomavirus .

TABLE 4 C_t values for concordant and discordant four HR-HPV tests and the cobas test results

	C_t (all) median	C_t (HPV-16) median	C_t (HPV-18) median	C_t (HPV others) median
Tellgen+/cobas+	(20.03, 27.80)	(18.42, 27.90)	(19.44, 28.25)	(20.75, 27.55)
Tellgen−/cobas+	(0, 38.75)	(0, 36.90)	(0, 35.10)	(0, 38.90)
Tellgen+/cobas−	(25.42, 0)	(26.30, 0)	NA	(24.71, 0)
Hybribio+/cobas+	(28.96, 27.75)	(27.06, 28.15)	(35.83, 27.30)	(30.00, 27.55)
Hybribio−/cobas+	(0, 37.80)	(0, 35.90)	(0, 35.1)	(0, 38.78)
Hybribio+/cobas−	(29.41, 0)	NA	(37.42, 0)	(28.54, 0)
Liferiver+/cobas+	(24.24, 27.95)	(23.47, 28.40)	(22.84, 28.25)	(25.05, 27.78)
Liferiver−/cobas+	(0, 38.80)	(0, 36.9)	(0, 35.10)	(0, 39.00)
Liferiver+/cobas−	(34.84, 0)	(37.48, 0)	(37.09, 0)	(34.32, 0)
Sansure+/cobas+	(27.83, 27.80)	(27.76, 27.80)	(30.69, 28.25)	(27.73, 27.60)
Sansure−/cobas+	(0, 38.75)	(0, 38.70)	(0, 35.10)	(0, 38.80)
Sansure+/cobas−	(36.03, 0)	(37.83, 0)	(35.10, 0)	(34.22, 0)

Tellgen cutoff, 30; Hybribio cutoff, 36; Liferiver cutoff, 38; Sansure cutoff, 39; and cobas cutoff, 40.5. Abbreviations: C_t, cycle threshold; HR-HPV, high-risk human papillomavirus; NA, not available.

TABLE 5 The missing CIN2+ samples among the five HR-HPV tests

Subject ID	Histopathology	Tellgen	Hybribio	Liferiver	Sansure	Cobas	INNO-LiPA	
A030	CIN2	NEG	NEG	NEG	NEG	NEG	HPV-44	⋆⋆⋆⋆⋆
A045	CIN3	NEG	NEG	NEG	NEG	NEG	HPV-70	⋆⋆⋆⋆⋆
A008	CIN2	NEG	NEG	POS	POS	NEG	HPV-82	⋆⋆⋆
A064	CIN3	POS	POS	NEG	NEG	NEG	HPV-16	⋆⋆
A042	CIN3	NEG	POS	POS	POS	POS	HPV-16	⋆
A020	CIN3	POS	NEG	POS	POS	POS	HPV-16	⋆
A009	CIN3	POS	POS	NEG	POS	POS	HPV-33	⋆
A040	CIN2	POS	POS	POS	POS	NEG	HPV-52	⋆

⋆, samples for which one HR-HPV test reported a negative result compared with histopathology and LiPA; ⋆⋆, sample for which two HR-HPV tests reported a negative result compared with histopathology and LiPA; ⋆⋆⋆, sample for which three HR-HPV tests reported a negative result compared with histopathology and LiPA; ⋆⋆⋆⋆⋆, sample for which all the five HR-HPV tests reported a negative result compared with histopathology and LiPA. Abbreviations: CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HR-HPV, high-risk human papillomavirus; NEG, negative; POS: positive; underline, undetected samples.

TABLE 6 Clinical performance of these HR-HPV tests for the detection of CIN2+ in women with positive HPV results

Endpoint	HPV test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CIN2+ (n = 74)	Tellgen	94.59 (86.91-97.88)	72.79 (64.77-79.57)	65.42 (56.02-73.76)	96.12 (90.44-98.48)
	Hybribio	94.59 (86.91-97.88)	73.53 (65.54-80.22)	66.04 (56.60-74.35)	96.15 (90.53-98.49)
	Liferiver	94.59 (86.91-97.88)	71.32 (63.22-78.26)	64.22 (54.88-72.59)	96.04 (90.26-98.45)
	Sansure	95.95 (88.75-98.61)	69.86 (61.68-76.93)	63.39 (54.17-71.73)	96.94 (91.38-98.95)
	Cobas	93.24 (85.14-97.08)	73.53 (65.54-80.22)	65.71 (56.23-74.09)	95.24 (89.33-97.95)

Abbreviations: CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HR-HPV, high-risk human papillomavirus; NPV, negative predictive value; PPV, positive predictive value.

tests. Also, HPV-82 is not included within the detection parameters of the Tellgen, Hybribio, or cobas test, and thus it was accepted that CIN2+ cases infected with HPV-82 will not be identified using these kits. These genotyping differences between HPV kits will affect the performance; therefore, we suggest that efforts be made to standardize testing around HPV genotype classification. One CIN2 case with HPV-52 was also undetected using the cobas HPV test, though this may be due to a low sensitivity in detecting HPV-52, as has been demonstrated in previous studies.[27,28] However, HPV-16 in only one of CIN3 cases was missed by all the five HR-HPV tests and only one CIN3 case with HPV-33 was missed using the Liferiver. A possible explanation for these undetected cases is low-copy numbers

of the HPV-16 and -33, which is below the cut-off value; improving detection for these genotypes should be solved readily by the manufacturer. No test is absolutely sensitive or specific, and therefore to minimize the loss of CIN2+ cases, we suggest that cut-off values for HR-HPV tests need to be optimized based on the detection of high-grade cervical lesions but without sacrificing detection specificity.

There were some limitations that ought to be addressed for transparency. First of all, this is a preliminary study with a relatively small sample (n = 210). Results lead to a high-quality appraisal of four HR-HPV tests; however, due to the small sample size, our recommendations remain tentative. Additional research with a large sample size is required to verify these findings. Second, we could not have a reproducibility assessment for HR-HPV tests because there was a limit to sample volumes. Third, the total HR-HPV concordance rates were perhaps overestimated due to the fact that detection methods identify HPV types as a pool rather than for each HPV genotype. Finally, manual operation of the four HR-HPV tests was labor intensive and, therefore, was likely to suffer through a potential sample carry-over effect. We suggest that manufacturers should use an automated DNA extraction system if conditions are available to minimize this effect as well as to reduce hands-on time.

In summary, this initial study suggests that the four included HR-HPV tests have strong levels of agreement and similar clinical performance when compared with the cobas HPV test. The affordability and simplicity of the four HR-HPV test kits show each of these could be implemented in low-resource settings to accelerate the control of cervical cancer. However, we also suggest that efforts be made to further standardize and optimize testing around clinical sensitivity and specificity. Further research with larger samples comparing an increased number HPV tests is of course required.

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CONFLICT OF INTERESTS

The authors declared that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

WC and JS participated in studying conception and design, revision of the manuscript critically for important intellectual content, and provided the final approval of the manuscript version to be published. PX participated in samples collection, analysis, and interpretation of data and the writing and revision of the manuscript. L-LG and JY participated in acquisition, analysis interpretation of data, and revision of the manuscript. L-LH, JZ, and LL participated in samples collection and acquisition data. SS and X-YH participated in revision of the manuscript. T-YL participated in acquisition and analysis of data. YJ participated in administrative support. All authors read and approved the final manuscript.

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REFERENCES

1. Shi JF, Canfell K, Lew JB, Qiao YL. The burden of cervical cancer in China: synthesis of the evidence. *Int J Cancer*. 2012;130(3):641-652.
2. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer*. 2007;121(3):621-632.
3. Sundstrom K, Eloranta S, Sparen P, et al. Prospective study of human papillomavirus (HPV) types, HPV persistence, and risk of squamous cell carcinoma of the cervix. *Cancer Epidemiol Biomarkers Prev*. 2010;19(10):2469-2478.
4. Kjaer SK, Frederiksen K, Munk C, et al. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*. 2010;102(19):1478-1488.
5. Munoz N, Bosch FX, Castellsague X, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer*. 2004;111(2):278-285.
6. World Health Organization. A global call for action towards the elimination of cervical cancer. 2018. https://www.who.int/cancer/cervical-cancer. Accessed May 2018.
7. Alemany L, Saunier M, Alvarado-Cabrero I, et al. Human papillomavirus DNA prevalence and type distribution in anal carcinomas worldwide. *Int J Cancer*. 2015;136(1):98-107.
8. Koliopoulos G, Nyaga VN, Santesso N, et al. Cytology versus HPV testing for cervical cancer screening in the general population. *Cochrane Database Syst Rev*. 2017;8:CD008587.
9. Tracht J, Wrenn A, Eltoum IE. Primary HPV testing verification: a retrospective ad-hoc analysis of screening algorithms on women doubly tested for cytology and HPV. *Diagn Cytopathol*. 2017;45(7):580-586.
10. Giorgi-Rossi P, Franceschi S, Ronco G. HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. *Int J Cancer*. 2012;130(6):1387-1394.
11. Li J, Zhang D, Zhang Y, Wang X, Lin Y, Hu L. Prevalence and genotype distribution of human papillomavirus in women with cervical cancer or high-grade precancerous lesions in Chengdu, western China. *Int J Gynaecol Obstet*. 2011;112(2):131-134.
12. Lorincz AT. Hybrid capture method for detection of human papillomavirus DNA in clinical specimens: a tool for clinical management of equivocal pap smears and for population screening. *J Obstet Gynaecol Res*. 1996;22(6):629-636.
13. Shah SS, Senapati S, Klacsmann F, et al. Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. *Cancers*. 2016;8(9):85.
14. Cui M, Chan N, Liu M, et al. Clinical performance of Roche cobas 4800 HPV Test. *J Clin Microbiol*. 2014;52(6):2210-2211.
15. Wright TC, Jr., Stoler MH, Behrens CM, et al. The ATHENA human papillomavirus study: design, methods, and baseline results. *Am J Obstet Gynecol*. 2012;206(1):46.e1-46.e11.
16. China Food and Drug Administration. *Regulation for the technical review of human papillomavirus (HPV) nucleic acid testing and genotyping reagents*. 2015. http://samr.cfda.gov.cn/WS01/CL1421/136488.html. Accessed November 26, 2015.

17. Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol*. 1999;37(8):2508-2517.
18. Else EA, Ryan S, Yuhua Z, et al. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with INNO-LiPA HPV genotyping extra assay. *J Clin Microbiol*. 2011;49(5):1907-1912.
19. Mollers M, King AJ, Knol MJ, et al. Effectiveness of human papillomavirus vaccine against incident and persistent infections among young girls: results from a longitudinal Dutch cohort study. *Vaccine*. 2015;33(23):2678-2683.
20. Meijer CJLM, Berkhof J, Castle PE, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int J Cancer*. 2009;124(3):516-520.
21. Heideman DA, Hesselink AT, Berkhof J, et al. Clinical validation of the cobas 4800 HPV test for cervical screening purposes. *J Clin Microbiol*. 2011;49(11):3983-3985.
22. Stoler MH, Wright TC, Jr., Sharma A, et al. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. *Am J Clin Pathol*. 2011;135(3):468-475.
23. Chen W, Yu LL, Wang H, et al. [Evaluation of cobas 4800 high-risk HPV test as a tool in cervical cancer screening and cytology triage]. *Zhonghua Zhong Liu Za Zhi*. 2012;34(7):543-548.
24. Jun SY, Park ES, Kim J, et al. Comparison of the cobas 4800 HPV and HPV 9G DNA chip tests for detection of high-risk human papillomavirus in cervical specimens of women with consecutive positive HPV tests but negative pap smears. *PLoS One*. 2015;10(10):e0140336.
25. Sun P, Song Y, Ruan G, et al. Clinical validation of the PCR-reverse dot blot human papillomavirus genotyping test in cervical lesions from Chinese women in the Fujian province: a hospital-based population study. *J Gynecol Oncol*. 2017;28(5):e50.
26. Wang R, Guo XL, Wisman GBA, et al. Nationwide prevalence of human papillomavirus infection and viral genotype distribution in 37 cities in China. *BMC Infect Dis*. 2015;15(1):257.
27. Nakamura M, Nakade K, Orisaka S, et al. Comparison study of BD onclarity HPV with digene HC2 high-risk HPV DNA test and Roche cobas 4800 HPV for detecting high-risk human papillomavirus in Japan. *Am J Clin Path*. 2019;151:263-269.
28. Sasagawa T, Maehama T, Osaka Y, et al. Comparison of the digene hybrid capture 2 and Roche cobas 4800 HPV tests for detection of CIN2+ in a referral population in Japan. *J Med Virol*. 2018;90(5):972-980.

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