Journal of Clinical Microbiology

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J. Clin. Microbiol. **2012, 50(7):2359. DOI: 10.1128/JCM.00337-12.**
Published Ahead of Print 18 April 2012.

JCM
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Comparison of the Abbott RealTi*me* High-Risk Human Papillomavirus (HPV), Roche Cobas HPV, and Hybrid Capture 2 Assays to Direct Sequencing and Genotyping of HPV DNA

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Infection with high-risk (HR) human papillomavirus (HPV) genotypes is an important risk factor for cervical cancers. We evaluated the clinical performances of two new real-time PCR assays for detecting HR HPVs compared to that of the Hybrid Capture 2 test (HC2). A total of 356 cervical swab specimens, which had been examined for cervical cytology, were assayed by Abbott RealTi*me* HR and Roche Cobas HPV as well as HC2. Sensitivities and specificities of these assays were determined based on the criteria that concordant results among the three assays were regarded as true-positive or -negative and that the results of genotyping and sequencing were considered true findings when the HPV assays presented discrepant results. The overall concordance rate among the results for the three assays was 82.6%, and RealTi*me* HR and Cobas HPV assays agreed with HC2 in 86.1% and 89.9% of cases, respectively. The two real-time PCR assays agreed with each other for 89.6% of the samples, and the concordance rate between them was equal to or greater than 98.0% for detecting HPV type 16 or 18. HC2 demonstrated a sensitivity of 96.6% with a specificity of 89.1% for detecting HR HPVs, while RealTi*me* HR presented a sensitivity of 78.3% with a specificity of 99.2%. The sensitivity and specificity of Cobas HPV for detecting HR HPVs were 91.7% and 97.0%. The new real-time PCR assays exhibited lower sensitivities for detecting HR HPVs than that of HC2. Nevertheless, the newly introduced assays have an advantage of simultaneously identifying HPV types 16 and 18 from clinical samples.

Persistent cervical infection of human papillomavirus (HPV) is a well-known risk factor for developing cervical cancer, which is the second most common malignancy in women, causing approximately 250,000 deaths each year worldwide (35, 36). More than 150 HPV genotypes have been identified, and approximately 50 of them are known to infect cervical epithelia. Recently, the International Agency for Research on Cancer (IARC) defined 12 high-risk (HR) HPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) as group 1 carcinogens (3). In particular, HPV16 is known as the most common HPV type, contributing to approximately half of all invasive cervical cancer cases. This genotype is also known to demonstrate a biological advantage in transmission, persistence, and transformation (2). HPV18 has been reported as the second most common cause of HPV-associated invasive cervical cancers, and the proportion of cervical cancers related to HPV16 and/or HPV18 has been reported to be between 68% and 82% (19).

Morphological examination of cervix cytology has been used as a screening test for the prevention and early detection of cervical cancers over the past 50 years (12, 13). A major target of the cervical screening is to detect cases of cervical intraepithelial neoplasia grade 3 (CIN3), which can be treated before it progresses to invasive cancer. However, cytology tests are limited in predicting HPV infections and cervical cancers. Abnormal findings in cervical cytology, i.e., atypical squamous cells of undetermined significance (ASCUS) or worse results, were present for only a quarter of the patients who were infected with a single prevalent HR HPV type (17). Meanwhile, the Bethesda system, which is widely used to classify cervical cytology, can provide essential information on the history of an HPV infection (22). For instance, a low-grade squamous intraepithelial lesion (LSIL) would reflect microscopic findings of acute HPV infections, and a high-grade squamous intraepithelial lesion (HSIL) could indicate the possibility of current CIN3 or uncertain precancerous lesion (CIN2) (29). When the Bethesda system was used to classify cytologic findings, approximately two-thirds of LSILs as well as the majority of HSILs were associated with HR HPV types (6, 31).

Compared with changes in cervical cytology, HPV DNA can be detected earlier and is identifiable for a longer time (30). Previous randomized trials also reported the usefulness of HPV-based screening tests in cervical screening programs (9, 28). Various assays for detecting and genotyping HPV have been introduced. Among them, the Hybrid Capture 2 HPV test (HC2) was the first assay approved for detecting 13 HR HPVs by the U.S. Food and Drug Administration (FDA), and the usefulness of this assay for screening HPV infections has been extensively studied (1, 7, 14, 30). This assay also has been used as a reference test in many studies to evaluate newly developed HPV detection assays (11, 21, 25–27, 32). HPV detection assays utilizing the real-time PCR method have also been developed; these are able to produce results for HPV types 16 and 18 and other HR genotypes at once (5, 15). Among them, the Abbott RealTi*me* HR HPV assay was introduced to clinical laboratories, demonstrating performances comparable with those of HC2 (4, 16, 24, 33). In April 2011, the Roche Cobas

Received 6 February 2012 **Returned for modification** 20 March 2012
Accepted 30 March 2012
Published ahead of print 18 April 2012
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doi:10.1128/JCM.00337-12
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HPV assay, which also utilizes the real-time PCR principle, was approved by the U.S. FDA for detecting HR HPVs, and a few studies compared the results obtained by Cobas HPV with those of HC2 (10, 20, 34). However, there are currently no published studies in the literature which concurrently evaluated HC2, RealTi*m*e HR, and Cobas HPV assays.

In this study, we evaluated the performances of these three assays for detecting HPV DNA using cervical swab specimens obtained from Korean subjects for cytologic exam. We also compared the results of the three tests with each other and with those of genotyping using a liquid bead microarray (LBMA) as well as direct sequencing.

MATERIALS AND METHODS

Samples. Cervical swab specimens, which were obtained for cytologic exam, were collected from a total of 356 women at Severance Hospital and Green Cross Reference Laboratory between August and October 2011. All specimens were collected, placed in ThinPrep PreservCyt solution (Hologic Inc., Marlborough, MA), and stored at −70°C until assayed for HPV tests. All the specimens were classified into the four following groups according to their cytologic results: normal ($n = 100$), ASCUS ($n = 100$), LSIL ($n = 100$), and HSIL ($n = 56$). Nucleic acids from the samples were prepared using cobas x 480 (Roche Molecular Diagnostics, Pleasanton, CA), an automated instrument for nucleic acid extraction. Using 1 ml of PreservCyt solution per subject, the instrument yielded 150 μl of nucleic acid eluted sample, and this sample was applied for detecting HPV DNA using two real-time PCR assays as well as the HC2 HPV test (Qiagen GmbH, Hilden, Germany).

Real-time PCR assays. Both the RealTi*m*e HR HPV assay (Abbott Molecular Inc., Abbott Park, IL) and the Cobas HPV test (Roche Molecular Diagnostics) utilize a mixture of multiple primers and probes for amplifying and detecting human beta-globin gene from cervical cells, as a target for internal quality control (QC), as well as HPV DNA from cervix swab samples. The RealTi*m*e HR HPV test was performed using an m2000rt automated analyzer (Abbott Molecular Inc.) for PCR amplification and detection, and an assay cutoff of 32.0 for cycle threshold (C_T) as well as an internal QC target cutoff of 35.0 for C_T was used to interpret the results. The Cobas HPV assay was carried out using the cobas 4800 system (Roche Molecular Diagnostics), comprising the cobas x 480 instrument and the cobas z 480 analyzer, which are fully automated instruments for nucleic acid preparation and real-time PCR, respectively. The results were regarded as positive when the C_T from a sample was less than 40.0.

The two real-time PCR assays are suggested by their respective manufacturers to be able to detect 14 HR HPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The results of the two assays are reported as either positive or negative for HPV types 16 and 18 and a pooled result for the remaining 12 HR genotypes. All assay procedures except preparation of nucleic acids, described above, were carried out by following the manufacturers' instructions. Both real-time PCR assays also detect the aforementioned internal control target gene to ensure the quality of prepared nucleic acids. The preparation of nucleic acids was carried out again when the assays did not detect the amplified internal control gene.

HC2 HPV test. HC2 is a sandwich capture hybridization assay with chemiluminescence using unlabeled single-stranded RNA probes (23). After denaturation of nucleic acids, the single-stranded HPV DNA from the specimen was hybridized with mixed RNA probes specific for 13 HR HPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). RNA-DNA hybrids were captured on the surface of antibody-coated microplates. Then, a solution containing alkaline phosphatase-conjugated antibody specific to RNA-DNA hybrids was added to the immobilized hybrids. These hybrids were coupled with chemiluminescent substrates, which were cleaved by alkaline phosphatase to produce light signals. The emitted signals were measured as a ratio of relative light units to cutoff (RLU/CO) using a luminometer. The samples with an RLU/CO greater than or equal to 1.0 were regarded as positive.

Sequencing and genotyping. For the samples which showed discrepant results among the three HPV detection assays, their HPV genotypes were identified using PCR and sequencing. The following primers were used for PCR: forward, TTT GTT ACT GTT GTR GAT ACY AC, and reverse, GAA AAA TAA ACT GYA AAT CAW AYT C. After PCR amplification, the products were electrophoresed and the DNA of samples with a positive band was purified. Sequencing PCR was performed using the general primers GP5 and GP6, and the respective sequences of the HPV DNA regions corresponding to the two primer sets were read using the Applied Biosystems 3730xl DNA analyzer (Life Technologies Co., Carlsbad, CA). Resulting DNA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) database on the website of the NCBI to confirm specific genotypes.

In addition, the same samples were assayed for genotyping using GeneFinder HPV liquid beads microarray (LBMA) (Innomeditech Inc., Seoul, South Korea). This assay utilizes 5.6-μm polystyrene microspheres (beads), which are internally dyed with specific intensities of red and infrared fluorophores. Using different amounts of the two dyes for batches of microspheres, microsphere sets with up to 100 different addresses can be formed. A microsphere for each address is attached with 1 among 32 kinds of oligonucleotides specific to respective genotypes of HPV. The microspheres were reacted with HPV DNA, which had been amplified during PCR, and were detected in a fluid stream using the Luminex 200 system (Luminex Co., Austin, TX), so that HPV genotypes were defined by the unique address of each microsphere.

Specific HPV genotypes concurrently detected by both direct sequencing and the liquid bead microarray were regarded as valid results, and HPV genotypes were classified into HR, low-risk (LR), and uncertain-risk (UR) groups according to previously described criteria (8).

Data analysis. Analyse-it Method Evaluation Edition, version 2.22, software (Analyse-it Software Ltd., Leeds, United Kingdom) was used for all statistical analyses. Chi-square test was performed to compare proportions of positive samples among the groups classified according to the cytology results. Concordance rates and kappa coefficients (k) with 95% confidence intervals (CIs) were calculated to estimate the concordance between the results of the different assays. Sensitivities, specificities, and 95% CIs of the HC2 and the two real-time PCR assays were calculated based on the diagnostic accuracy criteria, which were determined based on the results of the genotyping and direct sequencing as well as those of the HC2 and the two real-time PCR assays. When the results of the three assays were all concordant, they were regarded as true positive or negative, whereas when there were discrepancies among the results by the three assays, including the results for HPV types 16 and 18, the diagnostic accuracy criteria were determined from the results of the HPV genotyping using LBMA and direct sequencing, all of which were in agreement by both methods. A P value less than 0.05 was considered to be significant.

RESULTS

Positive rates of HPV DNA. The positive rates of cervical HPV DNA detected by HC2 and the two real-time PCR assays are summarized in Table 1. For all three assays, positive rates were greater for higher cytologic grades than for the lower grades ($P < 0.05$), except in the case of HPV 18 detected by the RealTi*m*e HR assay. In all of the 356 samples, overall positive rates ranged from 27.0% for the RealTi*m*e HR to 39.7% for the HC2, and HC2 produced more positive results regardless of cytologic findings than the other two assays. HPV16 was detected in more samples by RealTi*m*e HR in the groups with cytologic findings of ASCUS, LSIL, and HSIL, while the positive rates of HPV18 were higher for Cobas HPV than for RealTi*m*e HR in the same cytology groups.

Concordance among the results for the HC2 and two real-time PCR assays. Regardless of HPV genotype, the results for the

TABLE 1 Positive rates of cervical HPV tested by different assays according to genotypes and cytologic results[a]

Assay	HPV genotype	Positive rate (%) for samples with indicated cytology					
		Normal ($n = 100$)	ASCUS ($n = 100$)	LSIL ($n = 100$)	HSIL ($n = 56$)	All ($n = 356$)	*P* value
HC2[b]	All detectable	6.3	30.2	52.0	92.7	39.7	<0.0001
RealTi*me* HR	All detectable	3.0	23.0	30.0	71.4	27.0	<0.0001
	16	1.0	3.0	4.0	33.9	7.6	<0.0001
	18	0.0	2.0	4.0	7.1	2.8	0.0575
	Other than 16 and 18	2.0	21.0	24.0	37.5	19.1	<0.0001
Cobas HPV	All detectable	2.0	27.0	41.0	83.9	32.9	<0.0001
	16	1.0	2.0	6.0	30.4	7.3	<0.0001
	18	0.0	2.0	5.0	10.7	3.7	0.0046
	Other than 16 and 18	1.0	25.0	33.0	58.9	25.8	<0.0001

[a] Abbreviations: ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; HC2, Hybrid Capture 2.
[b] Only 96, 96, 98, and 55 (a total of 345) samples with cytologic results of normal, ASCUS, LSIL, and HSIL, respectively, were assayed by HC2 due to sample quantities.

three HPV detection assays agreed in 285 cases (82.6%) out of a total of 345 samples tested by all the three assays. Discrepant results between the assays are presented in Table 2. Among the 24 samples which were positive by HC2 and Cobas HPV but negative by RealTi*me* HR, 19 (79.2%) were positive for HR HPV genotypes upon sequencing and genotyping. Among the other 22 cases which were positive only by HC2, 3 and 8 samples were positive, respectively, for HR and LR HPVs by sequencing and genotyping, but the other 6 specimens (27.3%) were negative according to PCR for sequencing and genotyping. Most of the samples (6/7 [85.7%]) which were positive by HC2 and RealTi*me* HR but negative by Cobas HPV were positive for HR HPVs by sequencing and genotyping.

The concordance of the results of HC2 with those of the two real-time PCR assays is displayed in Table 3. The results of Cobas HPV agreed with those of HC2 in 89.9% of all samples ($k = 0.78$), and the results of RealTi*me* HR corresponded with those of HC2 in 86.1% of the specimens ($k = 0.69$). HC2 produced positive results in 137, which was more than those for RealTi*me* HR (93 positives) and Cobas HPV (114 positives).

Concordance between the results of the two real-time PCR assays. Agreement between the results of the real-time PCR assays according to the detected genotypes is summarized in Table 4. Regardless of HPV genotype, the results of the RealTi*me* HR and Cobas HPV assays agreed in 89.6% of all cases ($k = 0.75$). Among the 8 samples with positive results only by RealTi*me* HR, 6 (75.0%) were positive for HR genotypes by sequencing and genotyping, while 22 of 29 (75.9%) specimens which were positive only by Cobas HPV were positive for HR genotypes by sequencing and genotyping.

For the HPV16 genotype, the results of the two assays corresponded well with each other (concordance rate, 98.0%; kappa coefficient, 0.86). Among the specimens with discrepant results, four were positive only by RealTi*me* HR, and HPV16 was identified upon sequencing and genotyping in three (75.0%) of them, whereas the three which were positive only by Cobas HPV were all negative for HPV16 by sequencing and genotyping.

In addition, the two assays also produced good agreement for HPV18 results, with a concordance rate of 99.2% and a kappa coefficient of 0.87. RealTi*me* HR showed no false-positive results but one false-negative result for HPV18, while Cobas HPV produced no false-negative result but two false-positive results for HPV18, compared to the results of sequencing and genotyping.

When considering the HR genotypes other than HPV types 16 and 18, the results of the two real-time PCR assays agreed with each other at a rate of 89.9%, which is similar to the overall con-

TABLE 2 Concordance among the results for the three assays for any detectable HPV genotype[a]

HC2 result	RealTi*me* HR result	Cobas HPV result	*n* (%) (total = 356)	Results for sequencing and genotyping (*n*)
Negative	Negative	Negative	201 (56.5)	NT
Positive	**Positive**	**Positive**	84 (23.6)	NT
Positive	Negative	**Positive**	24 (6.7)	**18 (1)**, **HR (18)**, LR(2), UR (2), negative (1)
Positive	Negative	Negative	22 (6.2)	**OHR (3)**, LR (8), UR (5), negative (6)
Positive	**Positive**	Negative	7 (2.0)	**16 (2)**, **HR (4)**, LR (1)
Negative	Negative	**Positive**	5 (1.4)	**HR (2)**, **OHR (1)**, negative (2)
Negative	**Positive**	**Positive**	1 (0.3)	**16 (1)**
Negative	**Positive**	Negative	1 (0.3)	Negative (1)
NT	Negative	Negative	8 (2.2)	**OHR (1)**, LR (1), negative (6)
NT	**Positive**	**Positive**	3 (0.8)	**HR (3)**

[a] Positive findings are indicated by bold type. HR includes HPV genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. OHR indicates HPV genotypes 61, 62, 67, and 72. LR represents HPV genotypes 3, 6, 10, 11, 27, 30, 32, 34, 40, 42, 43, 44, 53, 55, 69, and 73. UR corresponds with HPV genotypes 26, 54, 57, 70, 71, 74, 81, 83, 84, 86, 90, and 97. Abbreviations: HC2, Hybrid Capture 2; NT, not tested; HR, high-risk genotypes which can be detected with the assays; LR, low-risk genotypes; UR, genotypes of uncertain risk; OHR, other high-risk genotypes which are not included in the genotypes detectable by the three assays.

TABLE 3 Concordance of the results between the Hybrid Capture 2 assay and the other real-time PCR assays[a]

Comparative assay	Result	HC2 result (*n*)		Total (*n*)	Concordance rate (%)	Kappa coefficient[b]	95% CI
		Positive (*n* = 137)	Negative (*n* = 208)				
RealTi*me* HR	Positive	91	2	93	86.1	0.69	0.62–0.77
	Negative	46	206	252			
Cobas HPV	Positive	108	6	114	89.9	0.78	0.71–0.85
	Negative	29	202	231			

[a] Abbreviations: HC2, Hybrid Capture 2; CI, confidence interval.
[b] All *P* values for the kappa coefficients were less than 0.0001.

cordance rate between the two assays, regardless of genotype. Cobas HPV also produced 30 more positive results for the HR genotypes other than HPV types 16 and 18. Among these specimens, 20 (66.7%) and 25 (83.3%) showed positive results for HR genotypes other than HPV type 16 or 18 and for any detectable HR genotype, respectively. On the other hand, 6 were positive for HR genotypes other than HPV types 16 and 18 only by RealTi*me* HR, and 4 (66.6%) of them were positive for HR genotypes upon sequencing and genotyping.

Sensitivities and specificities of the HPV detection assays. When the agreeing results between the assays and the results of sequencing and genotyping were regarded as true findings for the detection of HPV DNA, HC2 showed the highest sensitivity (96.6%) but the lowest specificity (89.1%) for detecting HR HPVs, among the three assays (Table 5). On the other hand, RealTi*me* HR showed the lowest sensitivity (78.3%) and the highest specificity (99.2%) for the detection of HR HPVs. The sensitivity and specificity of Cobas HPV for HR HPVs were 91.7% and 97.0% and were between those of the other two assays. In addition, RealTi*me* HR showed higher sensitivity for HPV16 (100.0%) than Cobas HPV (88.5%), while Cobas HPV demonstrated higher sensitivity for HPV18 (100.0%) than RealTi*me* HR (90.9%). The specificities of the two real-time PCR assays for detecting HPV types 16 and 18 were all above 99.0%.

The sensitivity and specificity of HC2 for predicting cytology of HSIL were 92.7% (95% CI, 82.4% to 98.0%) and 70.3% (95% CI, 64.7% to 75.5%), and those of RealTi*me* HR were 71.4% (95% CI, 57.8% to 82.7%) and 81.3% (95% CI, 76.5% to 85.6%). Cobas HPV showed a sensitivity of 83.9% (95% CI, 71.7% to 92.4%) and a specificity of 76.7% (95% CI, 74.5% to 81.3%) for detecting HSIL.

Detection of coinfection with different HPV genotypes. The two real-time PCRs are able to produce specified results for HPV types 16 and 18 and the pool of the other 12 HR HPVs at once; thus, coinfections with HPV types 16 and 18 and other HR HPVs can be detected. As a result, coinfection was detected in 0.0% to 16.1% of the cases according to the different cytologic results (Table 6). Overall, the Cobas HPV assay produced more positive results for coinfection than RealTi*me* HR.

TABLE 4 Concordance between the results of the two real-time PCR HPV detection assays according to HPV genotype

HPV genotype	RealTi*me* HR result	No. of specimens with Cobas HPV result		Total (*n*)	Concordance rate (%)	Kappa coefficient[b]	95% CI[a]
		Positive	Negative				
All detectable	Positive	88	8[c]	96	89.6	0.75	0.68–0.83
	Negative	29[d]	231	260			
	Total	117	239	356			
16	Positive	23	4[e]	27	98.0	0.86	0.75–0.96
	Negative	3[f]	326	329			
	Total	26	330	356			
18	Positive	10	0	10	99.2	0.87	0.71–1.02
	Negative	3[g]	343	346			
	Total	13	343	356			
Other than 16 and 18	Positive	62	6[h]	68	89.9	0.71	0.62–0.80
	Negative	30[i]	258	288			
	Total	92	264	356			

[a] CI, confidence interval. See Table 2 for HR, OHR, LR, and UR. In the footnotes which follow, positive findings are indicated by bold type.
[b] All *P* values for the kappa coefficients were less than 0.0001.
[c] Genotyping results (*n*): **16 (2)**, **HR (4)**, LR (1), and negative (1).
[d] Genotyping results (*n*): **18 (1)**, **HR (20)**, **OHR (1)**, LR (2), UR (2), and negative (3).
[e] Genotyping results (*n*): **16 (3)** and HR (1).
[f] Genotyping results (*n*): HR (1) and UR (2).
[g] Genotyping results (*n*): **18 (1)** and HR (2).
[h] Genotyping results (*n*): **HR (4)**, LR (1), and negative (1).
[i] Genotyping results: 16 (4), 18 (1), **HR (19)**, **OHR (1)**, LR (2), and negative (3).

TABLE 5 Sensitivities and specificities of the HPV detection assays for high-risk genotypes[a]

Assay	HPV genotype	Sensitivity (%)	95% CI (%)	Specificity (%)	95% CI (%)
HC2	All HR	96.6	91.4–99.1	89.1	84.3–92.8
RealTi*me* HR	All HR	78.3	69.9–85.3	99.2	97.0–99.9
	16	100.0	86.8–100.0	99.7	98.3–100.0
	18	90.9	58.7–99.8	100.0	98.9–100.0
Cobas HPV	All HR	91.7	85.2–95.9	97.0	94.0–98.8
	16	88.5	69.8–97.6	99.1	97.4–99.8
	18	100.0	71.5–100.0	99.4	97.9–99.9

[a] Abbreviations: CI, confidence interval; HC2, Hybrid Capture 2; HR, high risk.

DISCUSSION

In this study, we compared the performances of two recently introduced real-time PCR assays for detecting HR HPVs with those of HC2. Accordingly, the positive rates of HPV DNA increased according to the grades of cervical cytology. Particularly, all three assays detected HR HPVs from HSIL samples in approximately 71% to 93% of cases, concurring with previous studies (6, 31). However, the positive rates of HPV detected in our data varied according to the assays, and HC2 produced more positive results for HR HPVs than the other two assays regardless of cytology results. There also was a significant number of discrepant results between the assays, and the concordance rate between the results for HC2 and the two real-time PCR assays ranged roughly from 86% to 90%, with kappa coefficients of 0.69 to 0.78. Thus, we performed genotyping using LBMA and direct sequencing to resolve the discrepancies. HC2 showed the highest sensitivity (96.6%) for detecting HR HPVs but the lowest specificity (89.1%) (Table 5). On the other hand, the RealTi*me* HR assay presented the lowest sensitivity (78.3%) and the highest specificity (99.2%). Similarly, HC2 also presented a slightly better sensitivity (97.6%) and specificity (92.6%) than did the RealTi*me* HR assay (sensitivity, 96.4%; specificity, 92.3%), with a concordance rate of 96.5% ($k = 0.86$) between the two assays in a previous study (4). In contrast to these results, RealTi*me* HR showed better sensitivities (93.1% to 100.0%) for the detection of HR HPVs or CIN2 and worse-grade CINs than HC2 (88.2% to 97.4%) in other previous studies (16, 24, 33). In one other study, the accuracy for detection of HR HPVs was also significantly higher with the Abbott RealTi*me* HR (98.7%) than with HC2 (92.9%) (15).

In addition, the Cobas HPV test demonstrated a sensitivity (91.7%) and specificity (97.0%) between those of the other two assays. In recently published studies, Cobas HPV also showed comparable performances to HC2, with kappa coefficients from 0.69 to 0.87, but there also were discrepant results between HC2 and Cobas HPV in approximately 6% to 15% of the samples (10, 20, 34). In another recent study, Cobas HPV showed a clinical sensitivity of 89.7% and a specificity of 66.7% for detecting cases with CIN2 or worse-grade CINs, whereas HC2 demonstrated a better clinical sensitivity of 93.1% and a specificity of 72.2% (18). Differences in concordance rates between the HPV detection assays among the studies might result from differences in the analytical sensitivities for different HPV genotypes and differences in HPV genotypes among the constituent of subjects with HPV infections. In the detection of HPV types 16 and 18, the results of two real-time PCR assays presented good agreement with each other in 98.0% or more of the samples. Therefore, the discrepancies among the results for the three assays could have been caused by differences in the analytical sensitivity for detecting HR HPVs other than HPV types 16 and 18.

In our study, HC2 produced more positive results than the other two real-time PCR assays, and there were 60 cases in which the results of HC2 were discrepant with those of the other two assays. Among the discrepant cases, HC2 presented 25 false-positive results with the median RLU/CO of 2.8 and 28 true-positive cases with that of 45.6. Therefore, weakly positive results with a low RLU/CO for HC2 could be falsely positive and would need to be retested with other assays when clinical findings and results of cervical cytology exam do not correspond with the result of HC2. However, true- and false-negative cases were not able to be discriminated by their RLU/CO alone.

Regarding HPV 16 detected by the two real-time PCR assays, there were 7 discrepant results between the two assays (Table 4). Three of them were true positive for HPV16, and those three were also positive by RealTi*me* HR (C_Ts = 24.6, 24.9, and 25.8; cutoff < 32.0) but negative by Cobas HPV. The other three were true negative, but Cobas HPV reported false-positive results with C_Ts

TABLE 6 Detection of coinfection with different HPV genotypes according to the assays and cervical cytology results[a]

Cytology (*n*)	Cases positive for HPV DNA [*n* (%)]		HPV coinfection [*n* (%)]		Coinfection rate[b] (%)	
	RealTi*me* HR	Cobas HPV	RealTi*me* HR	Cobas HPV	RealTi*me* HR	Cobas HPV
Normal (100)	3 (3.0)	2 (2.0)	0 (0.0)	0 (0.0)	0.0	0.0
ASCUS (100)	23 (23.0)	27 (27.0)	3 (3.0)	2 (2.0)	13.0	7.4
LSIL (100)	30 (30.0)	41 (41.0)	2 (2.0)	3 (3.0)	6.7	7.3
HSIL (56)	40 (71.4)	47 (83.9)	4 (7.1)	9 (16.1)	10.0	19.1
All (356)	96 (27.0)	117 (32.9)	9 (2.5)	14 (3.9)	9.4	12.0

[a] Abbreviations: ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.
[b] Coinfection rate was calculated by dividing the number of coinfection cases by the number of cases positive for HPV DNA detected by the respective assays.

around the cutoff C_T of 40.0, except for one with a C_T of 28.7. The remaining one case was true negative, but RealTi*m*e HR reported a false-positive result with a C_T of 31.0. Thus, RealTi*m*e HR seems to have better sensitivity and similar specificity for detecting HPV 16 compared to Cobas HPV (Table 5), and the results with C_Ts near 40.0 for Cobas HPV could have been false positive. In the case of HPV18, results for the two real-time PCR assays agreed well with each other, and there were only three discrepant results. Two of the three were true negative for HPV18, but Cobas HPV reported false-positive results with C_Ts of 36.1 and 38.1. Another sample was true positive, and the result for this sample on Cobas HPV was positive, with a C_T of 27.0, but RealTi*m*e HR showed false-negative results for the same case. Therefore, Cobas HPV could have better sensitivity for detecting HPV18, while RealTi*m*e HR might have better specificity.

In addition, there were 36 (10.1% of all 356 cases) discordant results between the two real-time assays for HR HPV other than HPV types 16 and 18. Among the 36 cases, 24 were true positive upon sequencing and genotyping, while only 20 and 4 of them were positive by Cobas HPV (median C_T of 33.2) and RealTi*m*e HR (median C_T of 27.8), respectively. The remaining 12 cases were true negative, but RealTi*m*e HR and Cobas HPV showed false-positive results in 2 (median C_T of 30.2; cutoff < 32.0) and 10 (median C_T of 37.7; cutoff < 40.0) cases, respectively. Thus, C_Ts around 30.0 for RealTi*m*e HR and 38.0 for Cobas HPV also could be false positive. Particularly, the cutoff C_T of Cobas HPV might need to be adjusted to an optimal value in order to reduce false-positive results while retaining its sensitivity, because this assay produced more false-positive results in detecting HR HPVs regardless of genotype.

Considering the cost-effectiveness and the sample quantity, we unified the methods for nucleic acid preparation. Thus, the nucleic acid preparations for HC2 and RealTi*m*e HR assays were performed against the recommendations of the respective manufacturers, and this might produce biased results. However, both real-time PCR assays also amplify human beta-globin gene from cervical cells as the internal control to ensure the quality of the DNA preparation, and we repeated the whole assay procedures, including the nucleic acid preparation, when the internal control was negative. Therefore, the DNA preparation step used in our evaluation might not have significantly influenced our results.

In conclusion, HC2 showed the highest sensitivity for detecting HR HPVs, and RealTi*m*e HR and Cobas HPV demonstrated higher specificities than HC2. There were discrepancies among the results for HR HPVs by the three assays in a significant proportion of samples, and these discrepancies arose mainly in the samples positive for HR HPVs other than HPV types 16 and 18. The two real-time PCR assays presented good agreement with each other for detecting HPV types 16 and 18, although the sensitivities and specificities for detecting HPV type 16 or 18 were different between the two real-time PCR assays. These differences could result from the small number of samples which were positive for HPV type 16 or 18. The clinical utility of the newly introduced real-time assays would need to be further evaluated. Nevertheless, they have an advantage of identifying HPV types 16 and 18 simultaneously from clinical samples.

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