Testing and Genotyping of High-Risk Human Papillomavirus by the cobas HPV Test and the Hybrid Capture 2 High-Risk HPV DNA Test Using Cervical and Vaginal Samples

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The accurate detection and typing of high-risk human papillomavirus (HPV) are critical for cervical cancer screening. The Hybrid Capture 2 (hc2) and cobas HPV tests showed high agreement for cervical samples (94.4%, $\kappa = 0.72$, $n = 693$) and moderate agreement for vaginal samples ($\kappa = 0.62$, $n = 108$). The HPV16 and HPV18 results were highly consistent between the cobas and Linear Array tests ($\kappa \geq 0.96$, $n = 197$). Three hc2-negative vaginal samples were repeatedly invalid by the cobas test due to β -globin control failures, highlighting amplification control benefits. No cross-contamination was detected in a challenge experiment.

Cervical cytology screening has significantly decreased the incidence and mortality of cervical cancer. In 2013, an estimated 12,340 new cases of cervical cancer will be diagnosed and 4,030 deaths will occur in the United States (1). Persistent infection with high-risk (HR) human papillomavirus (HPV), particularly genotypes HPV16 and HPV18, is the major cause of cervical cancer. The American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology recommend cotesting (concurrent cytology and HPV screening) every 5 years for cervical cancer screening in women between 30 and 65 years of age (2–4). In women who have undergone a total hysterectomy for cervical precancer or cancer, HPV testing may be used (but has not been cleared by the U.S. FDA) with vaginal cytology specimens to detect vaginal neoplasias.

There are currently four U.S. FDA-approved tests for HPV testing using cervical cytology samples in PreservCyt solution (ThinPrep; Hologic, Bedford, MA), including the Digene Hybrid Capture 2 High-Risk HPV DNA Test (hc2) (Qiagen, Gaithersburg, MD) and the cobas HPV test (cobas) (Roche Diagnostics, Indianapolis, IN). The hc2 test uses nucleic acid hybridization and chemiluminescent signal amplification to detect a pool of 13 HR-HPV types (5). The recently FDA-cleared cobas HPV test is an automated multiplex real-time PCR assay to detect separately HPV16, HPV18, a pool of 12 other-high-risk HPV (HR-HPV) types, and β -globin as a process control (6).

The clinical performance of newer HPV tests is less well understood but almost impossible for most clinical laboratories to assess, since this requires very large prospective clinical trials. However, new tests offer advantages, including processing controls, simultaneous HPV16/18 genotyping, and automated workflow, which are important factors for clinical laboratories faced with choosing an HPV test. In addition, accurate HPV16/18 detection has increasingly important implications for triaging HR-HPV-positive women. We performed a comprehensive analytical performance evaluation with cervical specimens and assessed the genotyping accuracy, testing of vaginal specimens, precision, and carryover risk for the cobas test.

Consecutive residual cervical cytology samples in PreservCyt medium ($n = 693$) from women >30 years of age (range, 30 to 79

years; median, 45 years) with ≥ 8 ml residual volume after hc2 testing, per the package insert (5), were deidentified using a standard Associated Regional and University Pathologists (ARUP) procedure according to the University of Utah institutional review board (IRB), stored at 4°C, and analyzed with the cobas test in “HPV High Risk Panel Plus Genotyping” mode per the manufacturer’s instructions. The median (interquartile range) between hc2 and cobas testing was 28 days (26 to 41 days). Samples positive by any assay were also tested with the Linear Array test according to the manufacturer’s recommendations, except that residual DNA from the cobas test was used as a PCR template after the addition of 10 μ l of 1 M Tris (pH 7.4) to the PCR master mix (7). Different operators performed hc2 and cobas testing, and cobas testing was performed independently of hc2 results and in a random sequence. The Linear Array test results were interpreted without referring to cobas/hc2 test results and per the Linear Array test (LA) (Roche Diagnostics, Indianapolis, IN) package insert. The percent agreement and Cohen’s kappa were calculated to compare the hc2 and cobas test results. McNemar’s test was used to assess the significance of positivity rates for independent assay results. Samples with discordant results were retested in both assays. A composite reference standard was determined for each sample based on the majority result obtained by the original cobas and hc2 tests, repeat cobas and hc2 tests, and the Linear Array test (HR-HPV types only).

Table 1 shows the overall agreement (94.4% [$\kappa = 0.72$]) and positivity rates for the hc2 test (10.4%) and the cobas test (12.3%, $P = 0.055$). Compared to the composite reference standard, the

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TABLE 1 Comparison of cervical samples ($n = 693$) previously tested in the hc2 test tested in the cobas test with the composite results^a

Results of indicated tests	Positive	Negative	Total	Agreement (%)	κ	Positivity rate (%) for:		<i>P</i>
						cobas/hc2	hc2/composite	
hc2 vs cobas				94.4	0.72	12.3 (cobas)	10.4 (hc2)	0.055
Positive	59 (8.5)	13 (1.9)	72 (10.4)					
Negative	26 (3.8)	595 (85.9)	621 (89.6)					
Total	85 (12.3)	608 (87.7)	693 (100)					
Composite vs cobas				97.8	0.89	12.3 (cobas)	10.7 (composite)	
Positive	72 (10.4)	2 (0.3)	74 (10.7)					
Negative	13 (1.9)	606 (87.4)	619 (89.3)					
Total	85 (12.3)	608 (87.7)	693 (100)					
Composite vs hc2				96.5	0.82	10.4 (hc2)	10.7 (composite)	
Positive	61 (8.8)	13 (1.9)	74 (10.7)					
Negative	11 (1.6)	608 (87.7)	619 (89.3)					
Total	72 (10.4)	621 (89.6)	693 (100)					

^a Unless indicated otherwise, the values are the number (%) of isolates with the given results. The percentages do not always total 100% due to rounding.

agreement was 97.8% for the cobas test ($\kappa = 0.89$) and 96.5% for the hc2 test ($\kappa = 0.82$). The positivity rate of the composite reference standard was 10.7%. Fig. S1 in the supplemental material summarizes the original, repeat, Linear Array results, and the interpretation of the original results. Of 39 samples with discordant results, the composite standard agreed with the original results in 24 (for cobas tests) and 15 (for hc2 tests) cases. The detailed results are shown in Table S1 in the supplemental material.

To determine the accuracy of the cobas test for HPV genotyping, an additional 112 unselected samples positive by the cobas test were included (patient age range, 30 to 85 years; median, 38 years). The results for HPV16, HPV18, and “other-HR-HPV” from all ($n = 197$) samples positive by the cobas test were compared to those of the Linear Array test (Table 2). The median (interquartile range) between hc2 and cobas testing for these 197 samples was 43 days (32 to 53 days). An overall agreement of $\geq 98.5\%$ was demonstrated for both HPV16 ($\kappa = 0.96$) and HPV18 ($\kappa = 0.97$), and there was no statistically significant difference in the positivity rates ($P = 1.0$). Four samples had discordant results (three for HPV16 and one for HPV18). Two samples were HPV16-negative/other-HR-HPV positive by the cobas test but HPV16/33 with or without genotype 52 and HPV16/45/56 positive by the Linear Array test, with very weak HPV16 bands in both cases. A third sample was HPV16 positive by the cobas test but had only an HPV CP6108 band in the Linear Array test. The HPV18 discordant

sample was other-HR-HPV positive by the cobas test but HPV18 negative and HPV52/59 positive by the Linear Array test.

We also collected 108 vaginal samples (patient age range, 20 to 91 years; median, 55 years) in PreservCyt medium that were previously tested by the hc2 test and analyzed them with the cobas test in the same manner as for the cervical samples. The median (interquartile range) between hc2 and cobas testing was 123 days (46 to 132 days). The overall results are shown in Table 3, and the details are shown in Fig. S2 in the supplemental material. The total agreement was 88.9% ($\kappa = 0.62$), and the positivity rates for the cobas test and the hc2 test were 13.9% and 18.5%, respectively. Compared to the composite reference standard, the cobas test and the hc2 test showed 99.1% ($\kappa = 0.97$) and 89.8% ($\kappa = 0.65$) total agreement, respectively. The testing details for the 11 discordant samples are shown in Table S2 in the supplemental material. Notably, three samples (2.8%) that were negative by the hc2 test were repeatedly invalid by the cobas test due to β -globin control failures.

The reproducibility of the cobas test was evaluated using 7 samples selected based on the Linear Array genotype, threshold cycle (C_T), and residual volume (see Table S3 in the supplemental material). Three samples were positive only in the other-HR-HPV channel, one each in the HPV16 and HPV18 channels, and one each positive for other-HR-HPV and HPV16 or HPV18. Seven samples were tested in triplicate on each of three runs. Two sam-

TABLE 2 One hundred ninety-seven cervical samples that were positive by the cobas test versus the Linear Array test for HPV16 and HPV18^a

Results of Linear Array vs cobas tests	Positive	Negative	Total	Agreement (%)	κ	Positivity rate (%) for:		<i>P</i>
						cobas	Linear Array	
HPV16				98.5	0.96	21.8	22.3	1.0
Positive	42 (21.3)	2 (1.0)	44 (22.3)					
Negative	1 (0.5)	152 (77.2)	153 (77.7)					
Total	43 (21.8)	154 (78.2)	197 (100)					
HPV18				99.5	0.97	8.1	7.6	1.0
Positive	15 (7.6)	0 (0)	15 (7.6)					
Negative	1 (0.5)	181 (91.9)	182 (92.4)					
Total	16 (8.1)	181 (91.9)	197 (100)					

^a Unless indicated otherwise, the values are the number (%) of isolates with the given results. The percentages do not always total 100% due to rounding.

TABLE 3 Vaginal samples ($n = 108$) previously tested in the hc2 test were tested in the cobas test and compared to the composite results^a

Results of indicated tests	Positive	Invalid	Negative	Total	Agreement (%)	κ	Positivity rate (%) for:	
							cobas/hc2	hc2/composite
hc2 vs cobas					88.9	0.62	13.9 (cobas)	18.5 (hc2)
Positive	13 (12.0)	0 (0)	7 (6.5)	20 (18.5)				
Invalid	0 (0)	0 (0)	0 (0)	0 (0)				
Negative	2 (1.9)	3 (2.8)	83 (76.9)	88 (81.5)				
Total	15 (13.9)	3 (2.8)	90 (83.3)	108 (100)				
Composite vs cobas					99.1	0.97	13.9 (cobas)	13.0 (composite)
Positive	14 (13.0)	0 (0)	0 (0)	14 (13.0)				
Invalid	0 (0)	3 (2.8)	0 (0)	3 (2.8)				
Negative	1 (0.9)	0 (0)	90 (83.3)	91 (84.3)				
Total	15 (13.9)	3 (2.8)	90 (83.3)	108 (100)				
Composite vs hc2					89.8	0.65	18.5 (hc2)	13.0 (composite)
Positive	13 (12.0)	0 (0)	1 (0.9)	14 (13.0)				
Invalid	0 (0)	0 (0)	3 (2.8)	3 (2.8)				
Negative	7 (6.5)	0 (0)	84 (77.8)	91 (84.3)				
Total	20 (18.5)	0 (0)	88 (81.5)	108 (100)				

^a Unless indicated otherwise, the values are the number (%) of isolates with the given results. The percentages do not always total 100% due to rounding.

ples had an invalid result due to a clot error (cobas detected a clot during pipetting, so the sample could not be processed further), so a fourth run was performed with samples in duplicate (or triplicate for the two samples with prior clots detected), for a total of 11 replicates per sample. The total coefficient of variation for C_T ranged from 1.1% to 6.5% in the other-HR-HPV channel, 1.9% to 2.6% in the HPV16 channel, 3.8% to 5.3% in the HPV18 channel, and 1.3% to 2.6% in the β -globin channel.

Contamination during cytology processing and automated pipetting is of concern with HPV testing, especially when samples contain high HPV titers. The cobas product insert describes a cross-contamination rate of 0.71% in a nonclinical study (6). In a previous study, Rao et al. (8) found no evidence of contamination during cytology processing or automated pipetting. We tested the cobas system using a checkerboard method, alternating aliquots of 15 very-high-positive samples (hc2 ratio, >2,000) with blank PreservCyt medium. All four possible checkerboard layouts on the 96-well plate were tested (data not shown), for a total of 4 runs using 94 high-positive aliquots and 282 blank PreservCyt aliquots. The average C_T for the high-positive aliquots was 27.1 (range, 17.5 to 35.2). No signal was detected in any channel for any of the blank samples, indicating a very low cross-contamination potential during automated pipetting.

Although the hc2 and cobas assays showed good agreement for the vast majority of samples, there are some discrepancies. These might be explained by differences in analytical sensitivity, analytical specificity, and clinical cutoffs. Consistently, the cobas assay had higher concordance with the Linear Array test. However, since residual nucleic acids extracted by the cobas test were used for the Linear Array test, a sampling bias cannot be excluded. Since the hc2 test has been used for over a decade, its performance is well documented. Although the hc2 assay design does not target HPV66 directly, this HR-HPV genotype (9) is detected due to cross-reactivity (5). In contrast, the cobas test directly targets HPV66 in the other-HR-HPV channel. Cross-reactions with non-oncogenic HPV genotypes (10, 11) have also been shown for the hc2 assay. Contrary to our results, Park et al. (12) showed a higher

proportion of samples that were positive by the hc2 test and negative by the cobas test, as well as a higher positivity rate for the hc2 test than the cobas test; Wong et al. (13) showed a higher confirmation rate by the Linear Array test for the hc2 test than for the cobas test.

One advantage of the cobas test is the β -globin internal control, which limits false-negative results due to PCR inhibition or insufficient cellularity. This advantage was most evident in 3% (3/108) of the vaginal samples that were invalid by the cobas test but negative by the hc2 test, despite visualization of a cell pellet during hc2 testing. In contrast, no invalid results were obtained for >800 cervical samples.

The accurate detection of HPV16 and HPV18 is important, as the results are used to determine the need for colposcopy in women with normal cytology but a positive HR-HPV test. The concordance of the cobas test with the Linear Array test for HPV16 and HPV18 was very good. Three samples were discordant for HPV16 and one sample was discordant for HPV18. Due to the clinical cutoffs (C_T s of 40.5, 40.0, and 40.0 for HPV16, HPV18, and other-HR-HPV, respectively) established during the ATHENA trial (14), the cobas test might report a negative result, despite the presence of detectable virus, while the Linear Array test has no established clinical threshold and may be analytically more sensitive. Rare results that are positive by the cobas test and negative by the Linear Array test might be interpreted as reflecting cross-reactivity of the cobas test with low-risk types or false-negative Linear Array test results. No consistently cross-reactive genotype was seen in our study. Competition in PCR from other more prevalent virus strains in a sample might mask or repress signal and cause a false-negative result in the cobas test or the Linear Array test.

Despite testing with 94 very high-positive samples, we saw no evidence of contamination in 282 negative samples. While our study was designed only to assess the contamination potential of the cobas instrument, contamination during cytology processing would be expected to affect both hc2 and cobas testing. In addition, a study comparing aliquots removed before or after cytology processing on the ThinPrep 2000 and 3000 systems showed no

evidence of contamination (8), and the cobas system was recently approved for testing postcytology samples. The cobas test was very reproducible, with a maximum % coefficient of variation (CV) of 6.5%. Reproducibility may be influenced by sampling error, since liquid-based cervical cytology samples do not necessarily have a homogenous suspension.

In conclusion, our study illustrates high agreement for the cobas test and the hc2 test for cervical samples and moderate agreement for vaginal cytology samples in PreservCyt medium. We demonstrated a high accuracy for HPV16 and HPV18 genotyping, as in other studies (15, 16), and a low probability of cross-contamination, even from very high-positive samples. In addition, the cobas β -globin control provides assurance that inadequate or inhibited specimens are flagged and not incorrectly reported as negative, which may be of particular concern for vaginal samples.

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