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Comparison of the cobas Human Papillomavirus (HPV) Test with the Hybrid Capture 2 and Linear Array HPV DNA Tests

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The cobas human papillomavirus (HPV) test (cobas) was recently approved by the U.S. Food and Drug Administration (FDA) and identifies HPV16 and HPV18 separately as well as detecting a pool of 11 HR-HPV genotypes (HPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) and also HPV66. We compared cobas, Linear Array (LA), and Hybrid Capture 2 (HC2) assays for detection of carcinogenic HPV DNA, and cobas and LA for detection of HPV16 and HPV18 DNA, among the first 1,852 women enrolled in the HPV Persistence and Progression Cohort (PaP Cohort) study. Specimens were tested by all 3 assays 1 year after an HC2-positive result. In 1,824 specimens with cobas results, cobas had an 85.9% agreement with HC2 and 91.0% agreement with LA for carcinogenic HPV detection. When results between cobas and HC2 disagreed, cobas tended to call more women HPV positive ($P < 0.01$). Categorizing cobas and LA results hierarchically according to cancer risk (HPV16, HPV18, other carcinogenic HPV genotypes, or carcinogen negative), there was a 90% agreement for all categories of HPV ($n = 1,824$). We found good agreement between the two U.S. FDA-approved HPV tests, with discrepancies between the two assays due to specific characteristics of the individual assays. Additional studies are needed to compare HC2 and cobas for detecting and predicting CIN3 to understand the clinical implications of the discrepant test results between the two tests.

In the United States, testing for a pool of high-risk genotypes of the human papillomavirus (HR-HPV) is currently used as an adjunct to cervical cytology for general screening. There are data supporting the individual detection of the two most carcinogenic genotypes, HPV16 and HPV18, which might be clinically useful for differentiating HPV-positive, cytology-negative women at higher and lower cancer risk (3, 7). The cobas HPV test (cobas; Roche Molecular Systems, Pleasanton, CA) was recently approved by the U.S. Food and Drug Administration (FDA) and identifies HPV16 and HPV18 separately as well as detecting a pool of 11 HR-HPV genotypes (HPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) and also HPV66. The test has been validated in some initial studies (1, 13), and we sought to further add to the literature by assessing the interassay agreement between cobas and two other well-validated HPV DNA assays using samples collected in specimen transport medium (STM) (Qiagen, Gaithersburg, MD).

Specifically, we compared cobas to (i) Linear Array (LA) (Roche Molecular Systems), an HPV genotyping assay that, while not approved by the FDA, is widely used for research (4, 5, 11, 14) and is CE marked for use in Europe, and (ii) the FDA-approved Hybrid Capture 2 assay (HC2) (Qiagen Corporation, Gaithersburg, MD), which targets the 13 HR-HPV genotypes and cross-reacts with HPV66 (as well as a few other possibly carcinogenic or low-risk types) (2).

MATERIALS AND METHODS

To enrich the study population for HPV positivity, this study was nested within the HPV Persistence and Progression Cohort (PaP Cohort) study (9). Kaiser Permanente Northern California (KPNC) routinely uses HC2 as an adjunct to cytology for cervical cancer screening in women 30 and older ("cotesting") and as a triage prior to colposcopy for women with equivocal Pap results at all ages. After taking a specimen for making a conventional Pap smear, a cervical specimen for HPV testing is taken using a sampling kit composed of a collection brush and specimen transport medium (STM; Qiagen, Gaithersburg, MD) for specimen storage and transportation after collection. STM specimens are sent to a central laboratory for routine HPV testing (1, 6).

To create the PaP cohort, we enrolled approximately 54,767 women aged 30 and older who underwent cotesting, 44,962 (82.1%) who tested HC2 positive, 9,778 (17.9%) who tested HC2 negative, and 27 (0.05%) with missing HC2 results. Women are followed prospectively as part of standard clinical guidelines for women 30 and older undergoing routine cotesting in the United States (15, 16). Selected women were mailed an opt-out letter to inform them of the study. If they did not want to participate, they could indicate their refusal by mailing back the opt-out letter using a prepaid envelope or calling a toll-free number. Women could opt out at any time; 6.7% of women who were selected elected not to participate. Women who did not opt out were considered enrolled into the study. Women were assigned a study ID that was linked to the enrollment and follow-up specimens, the latter of which were identified and flagged via the KPNC patient ID using KPNC's tracking system. Test results were linked to clinical data (cytology and histology) for clinical management and then stripped of personal identifiers for research purposes. NCI and KPNC institutional review boards have approved the study.

Per KPNC protocol, specimens from follow-up visits were already tested in real time by HC2 according to the manufacturer's instructions (9). For this study, specimens were neutralized within 14 h to minimize DNA damage. Specifically, 0.5× volume of neutralization buffer was added to the denatured samples (the exact amount of buffer depended on

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TABLE 1 Comparison of HC2, cobas, and LA for detection of high-risk HPV genotypes[a]

Group and comparison	No. of samples	Result of comparison								*P* value[c]	% agreement	Kappa	95% confidence interval
		Neg/neg		Pos/neg		Neg/pos		Pos/pos					
		No.	%	No.	%	No.	%	No.	%				
Total	1,852												
HC2 vs. cobas	1,824	665	35.9	112	6.1	164	9.0	893	49.0	0.002	85.9	0.692	0.659–0.726
HC2 vs. LA	1,849	653	35.3	121	6.5	178	9.6	897	48.5	0.001	83.8	0.671	0.637–0.705
LA vs. cobas	1,824	681	37.3	86	4.7	78	4.3	979	53.7	0.532	91.0	0.815	0.788–0.842
ASC-US[b] or worse cytology	639												
HC2 vs. cobas	626	57	9.1	48	7.7	20	3.2	501	80.0	0.001	89.1	0.565	0.473–0.656
HC2 vs. LA	637	56	8.8	49	7.7	22	3.5	510	80.1	0.001	88.9	0.549	0.456–0.641
LA vs. cobas	626	84	13.4	21	3.4	17	2.7	504	80.5	0.516	93.9	0.779	0.712–0.846
Normal cytology	1,205												
HC2 vs. cobas	1,190	594	49.9	63	5.3	144	12.1	389	32.7	<0.001	82.6	0.643	0.600–0.687
HC2 vs. LA	1,204	594	49.3	71	5.9	155	12.9	384	31.9	<0.001	81.2	0.615	0.570–0.660
LA vs. cobas	1,190	593	49.8	64	5.4	61	5.1	472	39.7	0.788	89.5	0.788	0.753–0.823

[a] Samples were evaluated for the presence of 1 or more among 14 HPV genotypes (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68, as well as HPV66) among specimens taken at 1-year follow-up visit after a previous HC2-positive test result, stratified by cytologic result. Of 1,852 1-year follow-up specimens, cobas, LA, and cytology results were missing for 28, 3, and 8 samples, respectively.

[b] Atypical squamous cells of undetermined significance.

[c] Exact McNemar's test.

residual volume after HC2 testing), the sample was mixed using a vortex mixer, and the solution was examined for color to determine adequate neutralization (indicator turns yellow). Neutralization buffer was composed of 180 mM 2-morpholinoethanesulfonic acid and 100 mM acetic acid, available from HyClone, part number RR11093.01.

For this analysis, we selected a convenience sample of the first 1,852 women to test HC2 positive and return for a 1-year follow-up visit (median, 12.8 months; range, 9.3 to 15.4). Baseline and 1-year follow-up visit specimens were tested by cobas and LA 2 to 3 years after the first (median, 34 months; range, 26 to 45) and follow-up (median, 22 months; range, 17 to 33) visits. To prepare DNA for both LA and cobas, automated sample extraction was performed on the neutralized STM sample using the ×480 sample extraction module of the cobas 4800 system. A 250-microliter sample of cobas lysis buffer was added to 250 μl neutralized specimen in a secondary tube (Falcon 5-ml polypropylene round-bottom tube, 12- by-75-mm style, nonpyrogenic, sterile). The sample was capped, vortexed, uncapped, and placed on the ×480 specimen rack. The ×480 extraction module of the cobas 4800 system then inputs 400 μl of this material into the specimen preparation process.

The sample extraction for the cobas HPV test is based on lysis and digestion of cells followed by binding of nucleic acid to magnetic glass beads. The bead-bound DNA is then washed to remove impurities and eluted from the beads in 120 μl of buffer at pH 8.7. The ×480 sample preparation module is also used to prepare and aliquot the master mix and to perform sample addition for the cobas HPV testing. Twenty-five microliters of sample is added to 25 μl of master mix in a 96-well PCR plate. This plate is then manually transferred to the z480 real-time amplification and detection module of the cobas 4800.

HPV detection for the cobas HPV test is performed via real-time PCR on the z480 module as per the manufacturer's recommended protocol (10). HPV genotypes 16 and 18 are identified and reported separately. This identification is accomplished by use of spectrally unique dyes to label TaqMan probes for HPV16, HPV18, and the other HR-HPV genotypes.

The HPV linear array test was carried out according to the manufacturer's protocol available within the package insert of the kit (11) with the following exceptions. A 50-μl portion of extracted sample from the ×480 module of the cobas 4800 system was used as a target in the PCR. If extracted samples had to be stored before amplification, they were sealed tightly with foil film (USA Scientific TempPlate sealing foil, part number 2923-0100) and stored at 4°C until use (never frozen). Because the cobas HPV extraction employs a different elution buffer than the recommended sample extraction for HPV LA, an addition to the master mix must be made to adapt it to this altered buffer. Ten microliters of 1 M Tris-HCl, 0.09% sodium azide, pH 7.4, buffer was added to the activated master mix. The solution was mixed by inverting a minimum of five times before dispensing into reaction tubes. Amplified samples were hybridized to the LA oligonucleotide probe strip and scored as per the manufacturer's recommendation. In addition, to reduce the chance of user read error, a research software program, HPV StripScan, was utilized to confirm HPV Linear Array genotypes. In the event of a discrepancy between the manual read and the StripScan result, a second, blinded manual read was performed of the 48-strip run; the consensus result (2 out of 3) was reported.

Restricting our analysis to the first 1,852 1-year follow-up specimens, we compared all three assays at the level of test positivity/negativity for the pool of 14 HPV genotypes (13 HR-HPV and HPV66). Then, cobas and LA results were categorized hierarchically according to cancer risk: HPV16 positive (possibly positive for another HPV genotype), HPV18 positive (HPV16 negative and possibly positive for another HPV genotype), positive for one or more other HR-HPV genotypes (-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 as well as HPV66 but negative for both HPV16 and -18), and negative for all HR-HPV genotypes. We compared the agreement between cobas and Linear Array for this grouping. The paired results for cobas and LA were also stratified by concurrent HC2 and conventional Pap smear results as crude metrics of HPV viral load. Percent agreement, kappa, and weighted kappa values were calculated across the three tests. Statistical significance of differences of positive/negative agreement or multicategory agreement was determined using exact tests of McNemar's chi-square tests (2 by 2) or exact tests of symmetry (4 by 4), respectively.

To assess the genotypes present in nonconcordant cobas and HC2 tests, we further categorized LA HPV genotype results according to phylogenetic hierarchy associated with cervical cancer risk (12): (i) HPV16 positive (possibly positive for another HPV genotype); (ii) positive for one or more high-risk HPV genotypes (HPV16 negative and positive for HPV18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, or -68); (iii) positive for one or more non-high-risk HPV genotypes within the alpha 5, 6, 7, 9, or 11 species (negative for HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 but positive for HPV26, -53, -66, -67, -69, -70, -73, or -82); (iv) positive for one or more non-high-risk HPV genotypes not in the alpha 5, 6, 7, 9, or 11 species (negative for HPV16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -67, -68, -69, -70, -73, and -82

TABLE 2 Hierarchical classification of HPV genotypes detected by cobas and Linear Array[a]

cobas group and subgroup	No. found by Linear Array				
	HPV16	HPV18	Other high-risk genotype	Negative for all high-risk genotypes	Total (column %)
All women[b]					
HPV16	233	**2**	**8**	**10**	*253 (13.9)*
HPV18	**0**	69	4	7	*80 (4.4)*
Other high-risk HPV genotype	**4**	1	658	61	*724 (39.7)*
Negative for all high-risk HPV genotypes	**9**	0	77	681	*767 (42.1)*
Total (row %)	*246 (13.5)*	*72 (4.0)*	*747 (41.0)*	*759 (41.6)*	*1,824 (100)*
HC2 negative[c]					
HPV16	16	0	1	6	*23 (2.8)*
HPV18	0	11	1	4	*16 (2.0)*
Other high-risk HPV genotype	0	1	87	37	*125 (15.2)*
Negative for all high-risk HPV genotypes	8	0	53	594	*655 (80.0)*
Total (row %)	*24 (2.9)*	*12 (1.5)*	*142 (17.3)*	*641 (78.3)*	*819 (100)*
HC2 positive[d]					
HPV16	217	2	7	4	*230 (22.9)*
HPV18	0	58	3	3	*64 (6.4)*
Other high-risk HPV genotype	4	0	571	24	*599 (59.6)*
Negative for all high-risk HPV genotypes	1	0	24	87	*112 (11.1)*
Total (row %)	*224 (22.2)*	*60 (5.9)*	*608 (60.2)*	*118 (11.7)*	*1,005 (100.0)*

[a] Classification of specimens taken at 1-year follow-up visit after a previous HC2-positive test result, stratified by HC2 status at follow-up. LA and cobas results were categorized hierarchically according to cancer risk: HPV16 positive (possibly positive for another high-risk HPV genotype), HPV18 positive (HPV16 negative, HPV18 positive, and possibly positive for another high-risk HPV genotype), positive for one or more other high-risk HPV genotypes (HPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 as well as HPV66 but negative for both HPV16 and -18), and negative for all high-risk HPV genotypes (negative for HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 as well as HPV66). Bold type indicates values that indicate that cobas tended to classify more women as HPV 16 positive than LA when the tests disagreed; underlining indicates values that indicate cobas tended to classify more women as HPV18 positive than LA when the tests disagreed; italics indicate total; shading indicates agreement between cobas and LA. Of 37 specimens that were HPV66 positive by LA and negative for all 13 carcinogenic HPV genotypes, 26 (70.3%) were HC2 positive. Comparatively, 31 (86.1% of 36; one specimen was missing a cobas result) were cobas positive for any of the carcinogenic HPV genotypes.

[b] Agreement, 90.0%; overall kappa, 0.844 (95% CI, 0.822-0.866); weighted kappa, 0.871 (95% CI, 0.850-0.891); exact *P* value for symmetry, 0.029.

[c] Agreement, 86.4%; overall kappa, 0.609 (95% CI, 0.543-0.675); weighted kappa, 0.629 (95% CI, 0.556-0.702); exact *P* value for symmetry, 0.229.

[d] Agreement, 92.8%; overall kappa, 0.875 (95% CI, 0.848-0.903); weighted kappa, 0.901 (95% CI, 0.876-0.925); exact *P* value for symmetry, 0.101.

but positive for HPV6, -11, -40, -42, -52, -54, -55, -61, -62, -64, -71, -72, -81, -83, -84, or -89); and (v) negative for all HPV genotypes. Results were stratified by concurrent conventional Pap smear results classified by the Bethesda 2001 classification system. Differences in genotype distribution for cobas and HC2 concordant versus discordant results were compared using McNemar chi-square tests.

RESULTS

We compared overall positivity for 1 or more of 14 genotypes by the 3 tests (Table 1). Positivity for one or more HPV genotypes was similar for all three tests, as 55.1%, 57.9%, and 58.1% tested positive for HC2, cobas, and LA, respectively (chi-square $P = 0.09$). However, in examining the data in a pairwise fashion, agreement between cobas and LA was superior (91.0% agreement) to that between cobas and HC2 (85.9% agreement). When results between cobas and HC2 disagreed, cobas tended to call more women HPV positive (164 versus 112 women; $P < 0.01$).

After stratifying HC2, cobas, and LA results by cytologic result (normal [$n = 1,205$] versus nonnormal [$n = 639$]) (Table 1), HPV positivity was higher among women with nonnormal cytology (87.8%, 83.2%, and 83.6% for HC2, cobas, and LA, respectively) than women with a normal cytology (37.8%, 44.8%, and 44.8% for HC2, cobas, and LA, respectively). Agreement was also higher among women with a nonnormal cytology than among women with normal cytology.

As shown in Table 2, using the hierarchical classification of HPV genotypes according to cancer risk, the agreement and kappa values between cobas and LA were also high: 90.0% agreement with unweighted and weighted kappas of 0.844 and 0.871, respectively. However, the exact test of symmetry showed that cobas tended to classify women as higher risk ($P = 0.03$). For example, when cobas and LA results disagreed, the cobas test labeled more women as HPV16 positive (20 versus 13; $P = 0.20$; differences highlighted in bold) and HPV18 positive (11 versus 1, $P < 0.01$; differences underlined) compared to LA. Agreement was lower among women testing HC2 negative (presumably because the specimens had lower viral load) than among those testing HC2 positive (86.4% versus 92.8%, respectively, chi-square $P < 0.01$).

Our *post hoc* review of discordant HC2 and cobas test results found that women who tested cobas positive and HC2 negative were more likely to be called positive for HPV16 and carcinogenic HPV genotypes detected by LA while cobas-negative and HC2-positive women were more likely to be called LA positive for one or more low-risk genotypes phylogenetically related to high-risk genotypes (Fisher's exact $P < 0.01$) (Table 3). Similar trends were observed among women with abnormal cytology, although not statistically significant (Fisher's exact $P = 0.09$).

DISCUSSION

Here we presented the largest study of the agreement for carcinogenic HPV DNA detection for two of the three U.S. FDA-approved tests, HC2 and cobas. We found that the agreement between the two tests was modest, with cobas more likely to test

TABLE 3 Hierarchical classification of HPV genotypes according to phylogenicity[a] detected by LA given HC2 and cobas results[c]

LA group and subgroups[a]	cobas positive[b]				cobas negative				Total	
	HC2 positive		HC2 negative		HC2 positive		HC2 negative			
	No.	Column %	No.	Column %	No.	Column %	No.	Column %	No.	Column %
Total[d]										
1	221	24.7	16	9.8	1	0.9	8	1.2	*246*	*13.4*
2	616	69.0	95	57.9	24	21.4	48	7.3	*783*	*42.9*
3	32	3.6	10	6.1	42	37.5	46	7.0	*130*	*7.1*
4	6	0.7	12	7.3	10	8.9	96	14.7	*124*	*6.8*
5	18	2.0	31	18.9	35	31.3	457	69.8	*541*	*29.7*
Total (row %)	*893*	*49.0*	*164*	*9.0*	*112*	*6.1*	*655*	*35.9*	*1,824*	*100.0*
ASC-US or worse cytology[c,e]										
1	139	27.7	1	5.0	0	0.0	2	3.5	*142*	*22.7*
2	338	67.5	11	55.0	14	29.2	5	8.8	*368*	*58.8*
3	16	3.2	4	20.0	21	43.8	5	8.8	*46*	*7.3*
4	3	0.6	2	10.0	2	4.2	13	22.8	*20*	*3.2*
5	5	1.0	2	10.0	11	22.9	32	56.1	*50*	*8.0*
Total (row %)	*501*	*80.0*	*20*	*3.2*	*48*	*7.7*	*57*	*9.1*	*626*	*100.0*
Normal cytology[f]										
1	82	21.1	15	10.4	1	1.6	6	1.0	*104*	*8.7*
2	275	70.7	84	58.3	10	15.9	42	7.1	*411*	*34.5*
3	16	4.1	6	4.2	20	31.7	41	6.9	*83*	*7.0*
4	3	0.8	10	6.9	8	12.7	83	14.0	*104*	*8.7*
5	13	3.3	29	20.1	24	38.1	422	71.0	*488*	*41.0*
Total (row %)	*389*	*32.7*	*144*	*12.1*	*63*	*5.3*	*594*	*49.9*	*1,190*	*100.0*

[a] LA results were categorized hierarchically according to cancer risk: group 1, HPV16 positive (possibly positive for another HPV genotype); group 2, positive for one or more high-risk HPV genotypes (HPV16-negative and positive for HPV18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, or -68); group 3, positive for one or more non-high-risk HPV genotypes within the alpha 5, 6, 7, 9, or 11 species (negative for HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68 but positive for HPV26, -53, -66, -67, -69, -70, -73, or -82); group 4, positive for one or more non-high-risk HPV genotypes not in the alpha 5, 6, 7, 9, or 11 species (negative for HPV16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -67, -68, -69, -70, -73, and -82 but positive for HPV6, -11, -40, -42, -52, -54, -55, -61, -62, -64, -71, -72, -81, -83, -84, or -89); and group 5, negative for all HPV genotypes. Results are for specimens taken at 1-year follow-up visit after a previous HC2-positive test result. Italics indicate totals.

[b] cobas positive for one or more high-risk HPV genotypes (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) as well as HPV66.

[c] Concurrent cytology result was atypical squamous cells of undetermined significance or worse.

[d] Fisher's exact $P < 0.01$ for difference between cobas-positive/HC2-negative and cobas-negative/HC2-positive results.

[e] Fisher's exact $P = 0.09$ for difference between cobas-positive/HC2-negative and cobas-negative/HC2-positive results (for all high-risk genotypes combined).

[f] Fisher's exact $P < 0.01$ for difference between cobas-positive/HC2-negative and cobas-negative/HC2-positive results.

positive. Agreement between the two tests was better among women with abnormal cytology, probably because of the higher HPV viral load (8). Discrepancies between the two assays had two causes: cobas appeared to be more analytically sensitive for carcinogenic HPV than HC2, resulting in the overall increased likelihood of testing positive (versus HC2), while HC2 was more likely to cross-react with certain noncarcinogenic HPV genotypes, as previously documented for HC2 (2), than cobas.

We found agreement between cobas and LA to be very good, better than that between cobas and HC2, albeit lower than previously reported in a smaller study with similar HPV prevalence (1). The reasons for differences in agreement between the two studies are uncertain. One possibility is that the previous study used a different specimen type, PreservCyt liquid-based cytology medium, while this study used STM. Another difference is that the previous study sampled more women with abnormal cytology, consistent with the increased agreement between the two assays among those with abnormal cytology shown here. For those results that differed, cobas tended to call more women positive for HPV16 and especially HPV18, a finding similarly reported by Castle et al. (1). A recent analysis demonstrated that HPV16 and HPV18 detection by cobas in conjunction with cytology is useful in the management of HPV-positive women (3).

The cobas assay was recently U.S. FDA approved and the main trial results published (13), again showing that carcinogenic HPV DNA detection is more sensitive but less specific for identifying women with precancerous lesions, specifically cervical intraepithelial neoplasia grade 3 (CIN3). Here we showed that there is good agreement between the two U.S. FDA-approved HPV tests, with discrepancies between the two assays due to specific characteristics of the individual assays. Additional studies are needed to compare the HC2 and cobas for detecting and predicting CIN3 to understand the clinical implications of the discrepant test results between the two assays.

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