A Comparison of Linear Array and Hybrid Capture 2 for Detection of Carcinogenic Human Papillomavirus and Cervical Precancer in ASCUS-LSIL Triage Study

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Abstract

Background: **We were interested in comparing the performance of Linear Array (LA; Roche Molecular Systems) to Hybrid Capture 2 (hc2; Digene) for the detection of carcinogenic human papillomavirus (HPV) and cervical precancer.**
Methods: **LA and hc2 results were compared on baseline specimens collected from women with an atypical squamous cells of undetermined significance (ASCUS) Pap referred into ASCUS and Low-Grade Squamous Intraepithelial Lesion Triage Study (n = 3,488). hc2 was conducted at the time of the study on liquid cytology specimens. LA was conducted retrospectively on aliquots from a second, stored cervical specimen masked to the hc2 results and clinical data. Paired LA and hc2 results (n = 3,289; 94%) were compared for the detection of carcinogenic HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 2-year cumulative cervical intraepithelial neoplasia (CIN) grade ≥3 as diagnosed by the quality-control pathology review.**
Results: **LA was more likely to test positive for carcinogenic HPV than hc2 (55% versus 53%; P = 0.001). For 2-year cumulative ≥CIN3, LA and hc2 had similar sensitivities (93.3% versus 92.6%, respectively; P = 1), and LA was marginally less specific than hc2 (48.1% versus 50.6%, respectively; P = 0.05). LA and hc2 had similar negative predictive values (98.70% versus 98.64% respectively; P = 0.4), and LA had a slightly lower positive predictive value than hc2 (14.6% versus 15.1%, respectively; P < 0.0001).**
Conclusion: **We observed that LA and hc2 performed similarly in the detection of carcinogenic HPV and identification of CIN3 among women with an ASCUS Pap.** (Cancer Epidemiol Biomarkers Prev 2008;17(5):1248–54)

Introduction

It is now generally recognized and accepted that ~15 cancer-associated (carcinogenic) human papillomavirus (HPV) genotypes cause virtually all cervical cancer and its immediate precursor lesions, most importantly cervical intraepithelial neoplasia (CIN) grade 3 (''cervical precancer''). HPV infections are common and typically resolve within 1 to 2 years. Uncommonly, carcinogenic HPV infections can persist, and this persistence is strongly associated with cervical precancer, which if left untreated can lead to invasive disease.

Based on knowledge of the central role for persistent, carcinogenic HPV in the development of cervical cancer, carcinogenic HPV testing offers several advantages over cytology, including (*a*) greater sensitivity for the detection of CIN3 or cancer (≥CIN3; refs. 1-7); (*b*) as a consequence of greater sensitivity, higher negative predictive value (NPV), that is, testing negative for carcinogenic HPV DNA implies an extremely low risk of prevalent or incipient cancer/CIN3 (1, 2, 8), permitting an extension of screening intervals; and (*c*) greater reproducibility (9, 10). Accordingly, carcinogenic HPV testing has now been approved in the United States as an adjunct to cytology for triage of equivocal cytology at all ages and for general screening in women ≥30 years old (11).

One pooled probe test for detection of the carcinogenic genotypes of HPV DNA (as a group), Hybrid Capture 2 (hc2; Digene), has already been approved by the Food and Drug Administration. Other tests are in development or being validated and are expected to become widely available soon. One of these tests, Linear Array (LA; Roche Molecular Systems), is a PCR-based assay that detects 37 HPV genotypes individually, including the main 14 carcinogenic HPV genotypes. LA is the commercialized (research use–only) version of the Line Blot Assay (Roche) that has been used in numerous epidemiologic and clinical studies (12-17), including the Atypical Squamous Cells of Undetermined Significance (ASCUS) and Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS; refs. 14, 17, 18). Using baseline specimens from women referred into ALTS

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because of an ASCUS (equivocal) Pap, we sought to evaluate the performance of LA, compared with the reference standard of hc2, for detection of carcinogenic HPV and triage of ASCUS Pap for identifying women with ≥CIN3.

Subjects and Methods

Study Design and Population. ALTS was a randomized trial comparing three management strategies for 5,060 women with ASCUS (n = 3,488) or LSIL (n = 1,572) Pap (19): (*a*) immediate colposcopy (IC arm; referral to colposcopy regardless of enrollment test results), (*b*) HPV triage [HPV arm; referral to colposcopy if enrollment HPV result was positive by hc2 or missing or if the enrollment cytology was high-grade squamous intraepithelial lesion (HSIL) for patient safety], or (*c*) conservative management (CM arm; referral to colposcopy at enrollment if cytology was HSIL). At enrollment, all women received a pelvic examination with collection of two cervical specimens: the first specimen in PreservCyt for ThinPrep cytology (Cytyc) and hc2 testing and the second in specimen transport medium (STM; Digene). Women in all three arms of the study were reevaluated by cytology every 6 months for 2 years of follow-up and sent to colposcopy if cytology was HSIL. An exit examination with colposcopy was scheduled for all women, regardless of study arm or prior procedures, at the completion of the follow-up. We refer readers to other references for details on randomization, examination procedures, patient management, and laboratory and pathology methods (19-23). The National Cancer Institute and local institutional review boards approved the study and all participants provided written informed consent. This evaluation was restricted to women referred for an ASCUS Pap because previous work indicated that carcinogenic HPV testing was not clinically useful in young women with LSIL Pap, as more than two thirds of the women were positive (20).

HPV DNA Testing. Residual PreservCyt specimens, after being used for liquid-based cytology, were tested by hc2 (23), a pooled probe, signal amplification DNA test that targets a group of 13 HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; ref. 24). Per the manufacturer's instructions, a protocol for converting the liquid cytology samples into a STM equivalent was used. In addition, hc2 cross-reacts strongly with a 14th carcinogenic HPV genotype, HPV66 (25-27), as well as other untargeted, noncarcinogenic HPV genotypes, including HPV53. hc2 signal strength [relative light units per positive control (RLU/CO)] was used as semiquantitative measure of HPV viral load (''HPV semiquantitative viral load''; ref. 28). An equivalent of 10% of each original specimen was used for hc2 testing.

Aliquots of the archived, enrollment STM specimens were retrospectively tested by LA (29), which employs L1 consensus primers PGMY09/11 for amplification, and amplicons were subjected to reverse-line blot hybridization for detection of 37 HPV individual genotypes (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-56, 58, 59, 61, 62, 64, 66-73, 81, 82, 82v, 83, 84, and 89). Because of intellectual property rights, LA does not directly detect HPV52 but combines a set of probes that detects HPV 33, 35, 52, and 58 combined (HPVmix). Specimens that test negative for HPV 33, 35, and 58 individually, but are positive for the HPVmix, are considered to be HPV52 positive. The specimens that test positive for the HPVmix and HPV 33, 35, and/or 58 have an uncertain HPV52 status, and for this analysis, these specimens were considered to be HPV52 negative. LA was used according to the manufacturer's instructions in the product insert, which includes DNA extraction using the QIAamp MinElute Media Kit (Qiagen, Valencia, CA). The only deviation from the LA product insert protocol was to implement an automated sample preparation for extraction of up to 96 specimens at a time on the Qiagen MDx platform (using the MinElute Media MDx Kit and manufacturer's instructions) rather than processing 24 specimens per batch with the manual vacuum method (29, 30). An equivalent of 2.8% of each original specimen was used for LA testing. Specimens were tested by LA a median of 104 months after collection, masked to the hc2 results and clinical data.

HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered the primary carcinogenic genotypes (31, 32). We included HPV66 in our definition because it was recently reclassified as a carcinogenic HPV genotype (32), and it is well known that hc2 strongly detects HPV66, although it is not one of the 13 genotypes directly targeted by hc2 (25, 26, 27). In fact, in a previous analysis, we found hc2 to be more likely to be positive in the presence of HPV66 than in the presence of HPV68, one of the hc2-targeted HPV genotypes. LA results were assigned a HPV risk group according to *a priori* established cervical cancer risk (e.g., HPV16 causes 50-60% of all cancers, HPV18 causes 15-20% of all cancers, and the other 12 carcinogenic HPV genotypes cause the remaining cases of cancer): (*a*) positive for HPV16, (*b*) else positive for HPV18 and negative for HPV16, (*c*) else positive for any carcinogenic HPV genotypes and negative for HPV 16 and 18 (carcinogenic HPV excluding HPV 16 and 18), (*d*) else positive for any noncarcinogenic HPV genotypes and negative for all carcinogenic genotypes, or (*e*) LA negative (HPV16 > HPV18 > carcinogenic HPV excluding HPV 16 and 18 > noncarcinogenic HPV > PCR negative).

Pathology and Treatment. Clinical management was based on the clinical center pathologists' cytologic and histologic diagnoses. In addition, all referral smears, ThinPrep, and histology slides were sent to the Pathology Quality Control Group (QC Pathology) based at the Johns Hopkins Hospital for independent review and diagnosis. CIN2 or worse (≥CIN2) diagnosis based on the clinical center pathology or a ≥CIN3 diagnosis based on the QC Pathology review triggered treatment by Loop Electrosurgical Excision Procedure. In addition, women with persistent LSIL or carcinogenic HPV-positive ASCUS at the time of the exit from the study were offered Loop Electrosurgical Excision Procedure.

Statistical Analysis. Detection of carcinogenic HPV by LA and hc2 was compared by calculating κ values, percent total agreement, and percent positive agreement (that is, agreement among paired tests in which at least one was positive). Differences in detection were tested for statistical significance ($P < 0.05$) using an exact McNemar's χ^2 (two categories). Results of paired tests were also compared following stratification on enrollment ThinPrep cytology results (negative versus ASCUS

Table 1. Comparison of and agreement for detection of carcinogenic HPV by LA and hc2 for all paired tests, stratified by cytologic interpretations, by hc2 status (negative, positive), and by log units of hc2 signal strength (RLU/CO), semiquantitative measure of viral load, for women referred into ALTS for an ASCUS Pap

	n	hc2+ n(%)	LA+ n(%)	LA-/hc2- n(%)	LA-/hc2+ n(%)	LA+/hc2- n(%)	LA+/hc2+ n(%)	% Agreement	% Positive agreement	κ	*P*
All hc2	3,289	1,749 (53)									
All LA	3,446	1,914 (56)									
All pairs	3,289	1,749 (53)	1,824 (55)	1,238 (38)	227 (7)	302 (9)	1,522 (46)	84	74	0.68	0.001
Cytology negative	1,535	465 (30)	580 (38)	862 (56)	93 (6)	208 (14)	372 (24)	80	55	0.57	<0.0001
Cytology positive (≥ASCUS)	1,707	1,266 (74)	1,222 (72)	354 (21)	131 (8)	87 (5)	1,135 (66)	87	84	0.68	0.003
ASCUS	822	456 (55)	465 (57)	295 (36)	62 (8)	71 (9)	394 (48)	84	75	0.67	0.4
LSIL	581	535 (92)	493 (85)	37 (6)	51 (9)	9 (2)	484 (83)	90	89	0.50	<0.0001
HSIL (including ASC-H)	304	275 (90)	264 (87)	22 (7)	18 (6)	7 (2)	257 (85)	92	91	0.59	0.03
hc2+ (1.00-9.99 RLU/CO)	409		287 (70)		122 (30)		287 (70)				
hc2+ (10.00-99.99 RLU/CO)	478		418 (87)		60 (13)		418 (87)				
hc2+ (100.00-999.99 RLU/CO)	621		582 (94)		39 (6)		582 (94)				
hc2+ (≥1,000 RLU/CO)	241		235 (98)		6 (2)		235 (98)				

NOTE: Differences were tested for statistical significance using an exact McNemar's χ^2 test.

or more severe; alternatively, negative, ASCUS, LSIL, and HSIL, including atypical squamous cells, cannot rule out HSIL; because of the variability of cytologic interpretation (33), the enrollment ThinPrep cytology results differed from the referral cytology). LA results were also stratified on hc2 status (negative, positive) and hc2-derived log units of the HPV semiquantitative viral load (1.00-9.99, 10.00-99.99, 99.99-999.99, and ≥1,000 RLU/CO).

Sensitivity, specificity, positive predictive value (PPV), NPV, and Youden's index (YI) were calculated for histologically confirmed ≥CIN3 as diagnosed by QC Pathology at enrollment restricted to the IC arm in which all women were referred to colposcopy (and therefore there was no bias in referral) and over the 2-year duration of ALTS in all arms combined because the 2-year cumulative risk ≥CIN3 did not differ significantly by study arm, only in the timing of diagnosis. In secondary analyses, the clinical performance was also calculated for histologically confirmed ≥CIN2 as diagnosed by the clinical center pathologist at enrollment (restricted to the IC arm) and over the 2-year duration of ALTS, restricted to IC and HPV arms of the trial because of the evidence of regression of CIN2 in the CM arm of the trial (21, 22). Differences in test sensitivity and specificity for ≥CIN3 and ≥CIN2 were tested for statistical significance using an exact McNemar's χ^2. Differences in PPV and NPV were tested for statistical significance using a method developed by Leisenring et al. (34). Differences in YI were tested for statistical significance as described previously (3). Finally, the 2-year cumulative absolute risks of histologically confirmed ≥CIN3 as diagnosed by QC Pathology were calculated for testing positive or negative at enrollment for carcinogenic HPV as detected by LA or hc2. These risks were also calculated for LA-defined subgroups of HPV genotypes and for log-unit categories of semiquantitative viral load as measured by hc2.

Table 2. Clinical performance of carcinogenic HPV detection by LA and hc2 for enrollment ≥CIN3 as diagnosed by QC Pathology and ≥CIN2 as diagnosed by clinical center pathologists in women referred into ALTS for an ASCUS Pap and restricted to women participating in the IC arm (*n* = 1,089)

	LA		hc2		*P*
	Estimate (%)	95% CI	Estimate (%)	95% CI	
QC Pathology ≥CIN3 (*n* = 55)					
Sensitivity	89.1	77.8-95.9	90.9	80.0-97.0	1
Specificity	46.5	43.4-49.6	47.8	44.7-50.9	0.3
PPV	8.1	6.1-10.6	8.5	6.4-11.0	0.0009
NPV	98.77	97.34-99.55	99.00	97.68-99.67	0.8
Referral	55.3	52.3-58.3	54.2	51.2-57.2	0.4
CC Pathology ≥CIN2 (*n* = 113)					
Sensitivity	87.6	80.1-93.1	89.5	82.3-94.4	0.8
Specificity	48.2	45.0-51.3	50.0	46.8-53.2	0.3
PPV	16.3	13.4-19.5	17.3	14.3-20.6	<0.0001
NPV	97.12	95.22-98.42	97.60	95.84-98.75	0.8
Referral	55.5	52.5-58.5	54.1	51.1-57.1	0.4

NOTE: Differences in sensitivity, specificity, and referral were tested for statistical significance using an exact McNemar's χ^2 test. Statistical differences in PPV and NPV were determined by a method developed by Leisenring et al. (36), a score statistic derived from a marginal regression model and bears some relation to McNemar's statistic.

Results

Carcinogenic HPV Detection. Among the women referred into ALTS because of an ASCUS Pap, 3,289 (94%) had valid baseline results for hc2 using PreservCyt specimens [missing hc2 results were primarily the result of insufficient specimen volume (<4 mL) for testing], 3,446 (99%) had valid results for LA testing of archived, baseline STM specimens, and 3,289 (94%) had valid results for both tests. Restricting our analyses to paired results, LA was more likely to test positive for carcinogenic HPV than hc2 (55% versus 53%; $P = 0.001$), with a percent agreement of 84%, a percent positive agreement of 74%, and a κ of 0.68 (Table 1). Both tests were more likely to test positive for carcinogenic HPV among women with an enrollment cytology of ASCUS or worse (72% for LA versus 74% for hc2; $P = 0.003$) than women with negative cytology (38% for LA versus 30% for hc2; $P < 0.0001$). There was also better agreement between tests among women with an enrollment cytology of ASCUS or worse compared with women with negative cytology. LA was less likely to test positive than hc2 among women with LSIL ($P < 0.0001$) or HSIL cytology ($P = 0.03$). Among hc2-positive women, the proportion of LA tests positive for carcinogenic HPV increased with increasing semiquantitative viral load categorized in log units ($P_{trend} < 0.0001$).

Clinical Performance. There were no significant differences between LA and hc2 in clinical sensitivity (89.1% versus 90.9%, respectively; $P = 1$) and specificity (46.5% versus 47.8%, respectively; $P = 0.3$) for detection of enrollment QC Pathology–diagnosed ≥CIN3 ($n = 55$) in the IC arm (Table 2). Using YI (sensitivity + specificity - 100%) as a measure of accuracy, LA appeared very slightly less accurate than hc2 (35.6% versus 38.7%), but the difference was not significant ($P = 0.4$). LA had a slightly lower PPV than hc2 (8.1% versus 8.5%; $P = 0.0009$), which was statistically but perhaps not clinically significant. The NPV for both assays were similar (98.77% for LA versus 99.00% for hc2). Similar estimates for clinical performance variables for both assays and similar differences between assays were observed when diagnoses of ≥CIN2 by the clinical center pathologists restricted to IC arm were used as the endpoint.

We also considered the clinical performance of both assays for 2-year cumulative QC Pathology–diagnosed ≥CIN3 (in all arms; Table 3), thereby correcting for the differences in sensitivity by study arm and initial clinical management (35). LA and hc2 had similar sensitivities (93.3% versus 92.6%, respectively; $P = 1$) and LA was marginally less specific (48.1% versus 50.6%, respectively; $P = 0.05$). The YI for LA (42.2%) was similar to the YI for hc2 (43.2%; $P = 0.4$). The PPV for LA was lower than for hc2 (14.6% versus 15.1%, respectively; $P < 0.0001$) and there was no statistically significant difference in NPV between LA and hc2 (98.70% versus 98.64%, respectively; $P = 0.4$). LA was less sensitive (87.8% versus 91.0%; $P = 0.09$) and less specific (50.4% versus 53.1%; $P = 0.007$) than hc2 for clinical center pathology-diagnosed ≥CIN2. The YI was lower for LA (38.2%) than for hc2 (44.1%; $P = 0.003$) for 2-year cumulative clinical center pathology diagnosed ≥CIN2.

The 2-year cumulative risk of ≥CIN3 diagnosed by QC Pathology for women with and without carcinogenic HPV for each test is shown in Table 4. The risk of ≥CIN3 was <1.5% for women who tested negative for carcinogenic HPV and ~15% for women who tested positive by either test. Women who tested positive for HPV16 by LA ($n = 563$) were at a higher 2-year cumulative risk (28.6%) of ≥CIN3 than women with HPV18 (9.5%) or other carcinogenic HPV genotypes (8.2%). Women positive for any α9 genotype, excluding HPV16 ($n = 729$; HPV 31, 33, 35, 52, 58, and 67), were at an 11.4% 2-year cumulative risk of ≥CIN3; women positive for any α7 genotype, excluding HPV18 ($n = 732$; HPV 39, 45, 59, 68, and 70), were at a 12.4% 2-year cumulative risk of ≥CIN3 (data not shown).

For comparison, we consider risk stratification for 2-year cumulative risk of ≥CIN3 using hc2-measured semiquantitative HPV viral load. Women with higher semiquantitative viral loads (≥10 RLU/CO) were at ~2-fold higher 2-year cumulative risk for ≥CIN3 than

Table 3. Clinical performance of carcinogenic HPV detection by LA and hc2 for 2-year cumulative ≥CIN3 as diagnosed by QC Pathology ($n = 3,289$) and ≥CIN2 as diagnosed by clinical center pathologists ($n = 2,189$, restricted to women in the IC and HPV arms) referred into ALTS for an ASCUS Pap

	LA		hc2		*P*
	Estimate (%)	95% CI	Estimate (%)	95% CI	
QC Pathology ≥CIN3 ($n = 285$)					
Sensitivity	93.3	89.8-95.9	92.6	89.0-95.4	1
Specificity	48.1	46.3-49.9	50.6	48.8-52.4	0.05
PPV	14.6	13.0-16.3	15.1	13.4-16.9	<0.0001
NPV	98.70	97.98-99.22	98.64	97.92-99.15	0.4
Referral	55.5	53.7-57.2	53.2	51.5-54.9	0.001
CC Pathology ≥CIN2 ($n = 354$)					
Sensitivity	87.8	83.9-91.1	91.0	87.5-93.8	0.09
Specificity	50.4	48.1-52.7	53.1	50.8-55.4	0.007
PPV	24.8	22.4-27.3	26.5	24.0-29.2	<0.0001
NPV	95.68	94.20-96.87	96.93	95.67-97.91	0.8
Referral	55.6	53.5-57.7	53.9	51.7-56.0	0.04

NOTE: Differences in sensitivity, specificity, and referral were tested for statistical significance using an exact McNemar's χ^2 test. Statistical differences in PPV and NPV were determined by a method developed by Leisenring et al. (36), a score statistic derived from a marginal regression model and bears some relation to McNemar's statistic.

women with the lowest viral load (1-9.99 RLU/CO; 7.6%), but there was no trend with increasing viral load (17.4% for 10-99.99 RLU/CO, 18.2% for 100-999.99 RLU/CO, and 15.4% for ≥1,000 RLU/CO).

Discussion

We compared by LA to hc2, as a benchmark of performance, and found the two assays very similar for detection of carcinogenic HPV and ≥CIN3. Each assay offers different advantages over the other. hc2 showed slightly greater clinical specificity and therefore marginally better PPV than LA. Conversely, LA offers the advantage of HPV genotyping for identifying women at the greater risk of ≥CIN3 among carcinogenic HPV-positive women. These high-risk groups include women with HPV16 infection as shown here and in previous reports (18, 36-39). Additionally, LA may confer an advantage of potentially identifying those women with genotype-specific persistent carcinogenic HPV infections that are at the greatest risk of having or getting cervical precancer or cancer (40-42).

When we used 2-year cumulative, clinical center pathology diagnoses of ≥CIN2 as our endpoint, the differences between hc2 and LA were more pronounced, with hc2 showing marginally greater sensitivity than LA due to the detection of CIN2 caused by noncarcinogenic HPV genotypes. Eleven of the 22 CIN2 diagnoses that tested hc2 positive and LA negative for carcinogenic HPV were positive for noncarcinogenic HPV genotypes as detected by LA, and 10 of 11 were confirmed as having noncarcinogenic HPV genotypes (and no carcinogenic HPV genotypes) by Line Blot Assay, the prototype assay for LA (refs. 29, 17; data not shown). This is consistent with previous reports that hc2 cross-reacts with some noncarcinogenic HPV genotypes (25, 26), and as shown here, these genotypes occasionally cause CIN2. In addition, some of these untargeted HPV genotypes, such as HPV 73 and 82, are possibly weekly carcinogenic (43). Although CIN2 is the threshold diagnosis for treatment, CIN2 is equivocal precancer (44), representing a mix of HPV infections of carcinogenic and noncarcinogenic HPV that cause histopathologic changes, such as CIN1, most likely to regress, and CIN3, which is precancerous and may invade if left undetected and untreated. It is also a poorly reproducible diagnosis (33) partly as a consequence of its ambiguous nature and because of the technical difficulties in mounting biopsies. It is therefore important when interpreting clinical performance for detection of ≥CIN2 to account for clinically irrelevant diagnoses of CIN2. For these reasons, we emphasized ≥CIN3 as our primary and more rigorous endpoint.

We have shown previously that LA was more analytically sensitive for any carcinogenic HPV and HPV risk groups compared with its prototype assay, Line Blot Assay (45). This is the likely explanation of why LA was more likely to test positive and was less clinically specific than hc2, the converse of what we observed in our comparison of Line Blot Assay with hc2 (17). LA was more likely to test positive among women with normal cytology, which led to greater overall likelihood of testing positive compared with hc2. Conversely, hc2 was more likely than LA to test positive among women with LSIL and HSIL cytology. It was observed previously that hc2 was more likely than another PCR assay, $SPF_{10}LiPA_{25}$, to test positive among women with LSIL cytology, which is indicative of a productive HPV infection producing higher viral loads compared with normal cytology. We suggest that viral load of the cervical specimen may have a greater influence on hc2 test performance than on PCR-based assay performance.

One additional advantage of LA has over hc2 is the internal β-globin control to verify the adequacy of the specimen. Although specimen adequacy is probably an uncommon occurrence, given the performance of hc2 (1.7) the perception is that lack of internal control is a concern (46). It is also noteworthy that LA uses a smaller fraction of the specimen than hc2 so that its use as a co-test with liquid cytology specimens is also less apt to have a missing test result due to insufficient specimen volume.

One limitation of our analysis was LA and hc2 were conducted on different specimens and in different manners. LA was conducted on STM specimens, a specimen genotype for which it has not been optimized, a median of 104 months after collection, whereas hc2 was conducted on PreservCyt specimens in real time after cytology slides were made. We did not conduct the LA testing on the archived PreservCyt specimens, which had been stored at ambient temperature since 1997 to 1998, because we found LA that performed less sensitively on the PreservCyt specimens than the STM specimens in a pilot study (data not shown). Yet, the performance of LA

Table 4. Two-year cumulative absolute risk of histologically confirmed ≥CIN3 as diagnosed by QC Pathology for carcinogenic HPV detection by LA versus hc2 referred into ALTS because of an ASCUS Pap

	n	QC Pathology	
		N (≥CIN3)	% ≥CIN3
LA			
Carcinogenic HPV			
Negative	1,465	19	1.3
Positive	1,824	266	14.6
Total	3,289	285	8.7
HPV risk group			
Negative	1,030	11	1.1
Noncarcinogenic	435	8	1.8
Carcinogenic	1,103	90	8.2
HPV18	158	15	9.5
HPV16	563	161	28.6
Total	3,289	285	8.7
hc2			
Carcinogenic HPV			
Negative	1,540	21	1.4
Positive	1,749	264	15.1
Total	3,289	285	8.7
Semiquantitative viral load			
1-9.99 RLU/CO	410	31	7.6
10-99.99 RLU/CO	477	83	17.4
100-999.99 RLU/CO	621	113	18.2
≥1,000 RLU/CO	241	37	15.4
Total	3,289	285	8.7

NOTE: LA results were also stratified on HPV risk group, and hc2 results were stratified on log units of signal strength (RLU/CO), a semiquantitative measure of viral load. Carcinogenic, positive for any carcinogenic HPV genotype other than HPV 16 or 18; Noncarcinogenic, positive for any of noncarcinogenic HPV genotypes.

was very similar to hc2, indicating that LA may be a robust assay as performed in an expert laboratory.

Although we found similar clinical performance between the two assays, we point out that clinical performance is only one of several important considerations for introducing a molecular test into clinical practice. Other criteria include reproducibility and capacity for high throughput. hc2 testing has been shown to be reproducible (9, 10, 47, 48) and using the automation provided by Rapid Capture System (Digene; http://www.digene.com/labs/labs_rapid_01.html) can achieve high-volume throughput. LA has shown neither reproducibility nor high-volume capacity, both of which will be needed before LA can be widely used in cervical cancer screening programs.

LA has been approved for use in Europe and is currently in clinical trials, and it will be important to verify its clinical performance and establish reproducibility as criteria for clinical use. We compared LA with hc2, which has repeatedly showed both good clinical performance (1, 4, 7, 49) and reliability (9, 10, 47, 48), and found the two tests comparable. If and when it is approved by the Food and Drug Administration and has high throughput capacity, LA may be another valuable screening tool for the secondary prevention of cervical cancer.

Disclosure of Potential Conflicts of Interest

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