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Human Papillomavirus (HPV) DNA Triage of Women with Atypical Squamous Cells of Undetermined Significance with Amplicor HPV and Hybrid Capture 2 Assays for Detection of High-Grade Lesions of the Uterine Cervix[▽]

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Up to 20% of women having a cytology smear showing atypical squamous cells of undetermined significance (ASC-US) and infected with high-risk human papillomavirus (HR HPV) have high-grade cervical intraepithelial neoplasia (CIN 2/3). Results obtained with the Amplicor HPV and Hybrid Capture 2 (HC-2) assays for HR HPV DNA detection in women referred to colposcopy for an ASC-US smear were compared. Cervical samples in PreservCyt were tested for the presence of 13 HR HPV types with HC-2, with Amplicor at three cutoffs for positivity (0.2, 1.0, and 1.5 optical density units), and for 36 genotypes with the Linear Array (LA). Of 396 eligible women, 316 did not have CIN, 47 had CIN 1, 29 had CIN 2/3, and 4 had CIN of unknown grade. HR HPV was detected in 129 (32.6%) and 164 (41.4%) samples with HC-2 and Amplicor HPV (cutoff, 0.2), respectively ($P = 0.01$). Overall, 112 specimens were positive and 215 were negative with the HC-2 and Amplicor HPV assays (agreement of 82.6%; 95% confidence interval [CI], 78.5 to 86.0). The clinical sensitivity and specificity of Amplicor HPV at cutoffs of 0.2, 1.0 and 1.5 and of HC-2 for detection of CIN 2/3 were 89.7% (95% CI, 72.8 to 97.2) and 62.5% (95% CI, 57.5 to 52.4), 89.7% (95% CI, 72.8 to 97.2) and 64.5% (95% CI, 59.4 to 69.2), 89.7% (95% CI, 72.8 to 97.2) and 64.7% (95% CI, 59.7 to 69.5), and 93.1% (95% CI, 77.0 to 99.2) and 72.2% (95% CI, 67.4 to 76.5), respectively. Both HR HPV detection tests identified women with ASC-US who would benefit the most from colposcopy. Women with persistent HR HPV infection need further investigation despite a first normal colposcopy.

High-risk human papillomavirus (HR HPV) genotypes are associated with high-grade cervical intraepithelial neoplasia (CIN 2/3) and cancer of the uterine cervix (2, 3). Primary screening of cervical cancer is essentially based on Pap cytology testing. In North America, for each new case of cervical cancer screened by cytology, there are between 50 and 100 cases of abnormal smears consistent with low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL) (9). Additionally, there are at least twice as many cases of equivocal or borderline atypias, referred to as "atypical squamous cells of undetermined significance" (ASC-US). Three options have been proposed by the American Society for Colposcopy and Cervical Pathology to perform triage on women with ASC-US, namely, repeat cytology, immediate referral to colposcopy, and reflex HR HPV testing (28).

The most widely used HR HPV detection assay for the triage of women with ASC-US is the Hybrid Capture 2 assay (HC-2; Qiagen, Inc., Mississauga, Ontario, Canada) (15). The HC-2 and Amplicor HPV (Roche Diagnostics, Laval, Quebec, Canada) tests detect the same 13 HR genotypes and are approved by Health Canada for clinical use. Several studies have evaluated the Amplicor HPV test, but only one analyzed fresh samples collected from women with ASC-US (4, 11, 14, 15, 25, 26, 27). The evaluation of the performance of the Amplicor HPV test in a diagnostic setting is mandatory to assess its utility in the management of women with ASC-US.

We evaluated the clinical performance of two generic HPV assays, Amplicor HPV and HC-2, on fresh clinical specimens obtained prospectively from women referred to colposcopy because of an ASC-US cytology result. HPV genotypic analysis was performed on all samples to assess the cross-reactivity of the HPV generic assays.

MATERIALS AND METHODS

Study design and population. Participants were recruited consecutively if (i) they were referred to colposcopy because of at least one ASC-US cytology, (ii) were ≥24 years old, and (iii) had not received treatment for CIN in the last 2 years. During the study period, HPV DNA testing was not widely available to primary care physicians in the study area and women were referred to colposcopy

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TABLE 1. Amplicor HPV and HC-2 detection of HR HPV DNA in cervical specimens collected from women referred to colposcopy because of an ASC-US cytology[a]

Diagnosis group	Total no. of specimens	No. of specimens with indicated results [(%, 95% CI); *P* value] in indicated assay			
		HC-2	Amplicor with cutoff at:		
			0.2	1.0	1.5
All women	396	129 (32.6, 28.1–37.3)	164 (41.4, 36.7–46.3); 0.01	157 (39.7, 35.0–44.5); 0.05	156 (39.4, 34.7–44.3); 0.05
No CIN	316	81 (25.6, 21.1–30.7)	113 (35.8, 30.7–41.2); 0.01	107 (33.9, 28.9–39.3); 0.03	106 (33.5, 28.6–38.9); 0.04
CIN 1	47	20 (42.6, 29.5–56.7)	23 (48.9, 35.3–62.8); 0.68	22 (46.8, 33.3–60.8); 0.84	22 (46.8, 33.3–60.8); 0.84
CIN 2/3	29	27 (93.1, 72.0–99.2)	26 (89.7, 72.8–97.2); 1.0	26 (89.7, 72.8–97.2); 1.0	26 (89.7, 72.8–97.2); 1.0
CIN ?	4	1 (0.3, 0.0–1.6)	2 (0.5, 0.0–3.8); 1.0	2 (0.5, 0.0–3.8); 1.0	2 (0.5, 0.0–3.8); 1.0

[a] Diagnoses were obtained at colposcopy with biopsy in the presence of cervical lesions. HPV DNA detection rates obtained with Amplicor using different cutoffs were compared with rates obtained with HC-2. HC-2, Hybrid Capture 2 assay; CIN, cervical intraepithelial neoplasia; CIN ?, CIN that could not be graded; CI, confidence interval.

after either a single or a repeat ASC-US smear. Cervical cells were first collected with a cytobrush for a conventional cytology. A second cervical specimen was then collected with a cytobrush prior to colposcopic examination. The cytobrush was washed into PreservCyt collection medium (Hologic, Inc., Marlborough, MA). Samples were kept at room temperature and processed within 1 week for HPV DNA testing. Colposcopy-guided biopsy specimens of lesions were obtained, and histological diagnosis was established without knowledge of HPV results. Biopsy samples with CIN 2/3 were confirmed by a second pathologist. This study was approved by the ethics committee of the Centre Hospitalier de l'Université de Montréal. All participants provided written informed consent.

HPV DNA testing. Technologists performing each HPV DNA test were blinded to the other test results, as well as to the cytology, colposcopy, and histology results. Amounts of 4 ml of samples in PreservCyt were processed in the sample conversion kit (Qiagen, Inc., Mississauga, Canada) and tested with HC-2 according to the manufacturer's recommendations. HC-2 was performed using only probe B for HR genotypes, a pool of full-length HPV RNA probes against 13 HR genotypes, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (10). Sample reactivity was measured in relative light units (RLU). A specimen was considered positive for HR HPV DNA if the ratio of the specimen RLU to the mean RLU of triplicates of a positive control at 1 pg per ml was ≥3.00. Samples with ratios between 1.00 and 3.00 were retested twice and were considered positive if 2 out of 3 results had a ratio of ≥1.00.

For HPV testing with Amplicor HPV (Roche Diagnostics, Laval, Canada), HPV DNA was extracted from PreservCyt samples using the AmpliLute liquid medium extraction kit on a Qiagen MDx platform as described in the manufacturer's instructions. Fifty microliters of extracted sample was added to 50 μl of the Amplicor master mix for HR HPV and β-globin amplification. Amplification was performed in a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA), and detection was accomplished with the Amplicor HPV and β-globin detection kits (Roche Diagnostics, Laval, Canada) according to the manufacturer's specifications (11). The positive cutoff point for Amplicor HPV was an A_{450} value of 0.2, as proposed by the manufacturer. Cutoff points with A_{450} values of 1.0 and 1.5 were also evaluated since they have been shown to increase the specificity of Amplicor HPV (26, 27).

Amounts of 50 μl of DNA extracted with the AmpliLute liquid medium extraction kit were also analyzed for HPV genotyping with the Linear Array HPV genotyping (LA) assay (Roche Diagnostics) according to a standard protocol (6). Samples positive with the cross-reactive probe for HPV-52 and containing one of the cross-reactive types other than 52 (HPV-33, -35, or -58) were further tested with a real-time PCR assay specific for HPV-52 (7). Thirty-six mucosal HPV genotypes are detected with LA (HPV types 6, 11, 16, 18, 26, 31, 33, 34 [formerly known as type 64], 35, 39, 40, 42, 44 [formerly known as type 55], 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82 [including subtype IS39], 83, 84, and 89 [formerly known as CP6108]).

Statistical methods. The crude percent agreement between tests was the percentage of paired tests with identical results. The modified Wald method was used to calculate 95% confidence intervals (CI) around binomial proportions. The unweighted kappa statistic was calculated to ascertain agreement between two HPV assays that was in excess of that due to chance. In general, a kappa value above 0.75 indicates excellent agreement, between 0.40 and 0.75 indicates fair to good agreement, and below 0.40 represents poor agreement beyond chance. Differences in the rates of detection of high-risk HPV types were tested for statistical significance using an exact Chi-square McNemar's test. CIN 2/3 served as the clinical endpoint for the evaluation of the clinical performance of HPV tests. Sensitivity, specificity, negative predictive value (NPV) and positive predictive (PPV) value were calculated using the conventional contingency tables. The distribution of RLUs obtained with HC-2 was compared using the nonparametric Mann-Whitney test. All statistical tests were two-sided. All *P* values of <0.05 were considered statistically significant. Statistical analyses were performed with STATISTICA version 6 software (StatSoft, Tulsa, OK).

RESULTS

Cytological and histological findings. Of the 408 women who agreed to participate from January 2002 to January 2005, 396 were eligible. Of the 12 women excluded, 11 were younger than 24 years of age and one woman did not provide a cervical sample. The mean and median of the ages of participants were 39.1 ± 10.7 years and 38 years (range, 24 to 75), respectively. The results of cytology smears repeated at colposcopy were normal for 245 women and showed ASC-US in 85, LSIL in 9, HSIL in 9, and ASC with possible HSIL (ASC-H) in 7. One hundred eighty-two women had a normal colposcopic examination and did not have a histological assessment. Combining these women with those having a normal histological evaluation, 316 women were CIN free, 47 had CIN 1, 29 had CIN 2/3, and 4 had CIN that could not be graded.

HPV prevalence found by Amplicor and HC-2. Table 1 provides the prevalence of HR HPV DNA stratified by CIN grade using several detection cutoffs for Amplicor HPV. Considering the results for all participants, the detection rate of HR HPV DNA was greater with Amplicor HPV than with HC-2. Fifteen samples had to be retested with HC-2 because the initial RLU results were of borderline reactivity; 11 samples were true positives on retesting, with reactivities ranging from 1.1 to 4.6 RLUs. Samples positive with Amplicor HPV generated optical density (OD) units from 0.230 to 3.246. Seven samples had OD units between ≥0.2 and 1.0, 1 between >1.0 and 1.5, and 156 had OD units of >1.5. Of the 396 samples tested with LA, 216 (54.6%) were positive for one of the 36 genital genotypes, of which 159 (40.2%) were positive for one of the 13 HR types targeted by the generic assays. HPV-positive samples contained a median of 2.0 types (range, 1 to 10). Multiple HPV types were detected in 117 samples.

For each assay, the detection rates increased with the CIN grade. Although fewer women without CIN tested positive for HR HPV DNA using a higher cutoff for Amplicor HPV, the difference between the rates of detection of HR HPV with HC-2 and Amplicor remained significant (Table 1). The dif-

TABLE 2. Agreement between Amplicor HPV and HC-2 assays for the detection of 13 HR HPV genotypes in endocervical specimens collected from women having an ASC-US cytology[a]

Amplicor detection cutoff and diagnosis group	Total no. of samples	No. (%) of samples with indicated test results				% Agreement (95% CI)	Kappa value
		HC-2+/ Amplicor+	HC-2+/ Amplicor−	HC-2−/ Amplicor+	HC-2−/ Amplicor−		
0.2							
All	396	112 (28.3)	17 (4.3)	52 (13.1)	215 (54.3)	82.6 (78.5–86.0)	0.63
No CIN	316	68 (21.5)	13 (4.1)	45 (14.4)	190 (60.1)	81.7 (77.0–85.5)	0.57
CIN 1	47	18 (38.3)	2 (4.3)	5 (10.6)	22 (46.8)	85.1 (72.0–92.1)	0.70
CIN 2/3	29	25 (92.6)	2 (7.4)	1 (3.7)	1 (3.7)	89.7 (72.8–98.2)	0.35
1.0							
All	396	111 (28.3)	18 (4.3)	46 (13.1)	221 (54.3)	83.8 (79.9–87.2)	0.65
No CIN	316	67 (21.2)	14 (4.3)	40 (12.7)	195 (61.7)	82.9 (78.4–86.7)	0.59
CIN 1	47	18 (38.3)	2 (4.3)	4 (8.6)	23 (48.9)	85.1 (72.0–92.1)	0.70
CIN 2/3	29	25 (86.2)	2 (6.9)	1 (3.5)	1 (3.5)	89.7 (72.8–98.2)	0.35
1.5							
All	396	111 (28.0)	18 (4.5)	45 (11.4)	222 (56.1)	84.1 (80.2–87.4)	0.68
No CIN	316	67 (21.2)	14 (4.3)	39 (12.3)	196 (62.0)	83.2 (78.7–87.0)	0.60
CIN 1	47	18 (38.3)	2 (4.3)	4 (8.6)	23 (48.9)	85.1 (72.0–92.1)	0.70
CIN 2/3	29	25 (86.2)	2 (6.9)	1 (3.5)	1 (3.5)	89.7 (72.8–98.2)	0.35

[a] Four women had a diagnosis of CIN that could not be graded: one was positive with HC-2 and two were positive with Amplicor HPV. Results for these four women are not shown in the table. See footnote *a* of Table 1 for abbreviations.

ference in the detection rates of HR HPV between HC-2 and Amplicor HPV did not reach statistical significance for women with CIN 1 or CIN 2/3. Of the 159 samples containing at least one of the 13 HR genotypes plus HPV type 66 with LA, 111 (69.8%) and 121 (76.1%) tested positive with HC-2 and Amplicor HPV, respectively ($P = 0.26$). Of the 33 samples positive for HPV-16 with LA, 31 and 33 samples tested positive with HC-2 and Amplicor HPV, respectively ($P = 0.81$). The detection rates of the other types were similar between the generic assays (data not shown).

Agreement between Amplicor HPV and HC-2. The agreement between generic assays differed when different detection cutoffs for Amplicor HPV were utilized and when all samples or samples from women without CIN were considered (Table 2). The percent agreement between assays was stronger in the presence of CIN 2/3 and lower in the absence of CIN. The difference in the intensity of the signal obtained with the HC-2 test in samples positive (median ratio of 85.0) and negative (median ratio of 20.0) with Amplicor HPV was significant ($P = 0.001$). Considering only the 13 genotypes detected with both generic HPV assays, 111 were positive and 217 were negative with HC-2 and LA, 21 were positive with HC-2 only, and 47 were positive with LA only, for an agreement between these tests of 82.8% (95% CI, 78.8 to 86.2) and a kappa value of 0.63 (95% CI, 0.53 to 0.73). The agreement between the two PCR assays was higher: 143 samples were positive and 217 were negative with Amplicor HPV and LA, 21 were positive with Amplicor HPV only, and 15 were positive with LA only, for an agreement of 90.9% (95% CI, 87.6 to 93.4) and a kappa value of 0.81 (95% CI, 0.71 to 0.81).

Genotyping analysis of discordant results. The cross-reactivity of each generic HPV assay was evaluated based on genotyping data obtained with LA. Twelve genotypes are currently considered to be HR and 8 to be probable/possible HR types (3). Eighteen samples that were positive for HR HPV by HC-2 did not contain any of the 13 HR genotypes targeted by the generic assays. Thirteen of these women had no CIN, 3 had CIN 1, and 2 had CIN 2/3. Of these 18 samples, 11 contained HPV types other than the 13 genotypes targeted: 10 contained one of the probable/possible HR types 53, 66, 70, and 82 and one contained low-risk HPV-42. Twenty samples tested positive with Amplicor HPV but did not contain any of the 13 genotypes targeted by the generic assays. Of these 20 samples, 6 contained types other than the 13 HR types targeted: 3 samples contained either HR genotype 66 or 82, and 3 had a low-risk genotype (HPV-72, HPV-61, or HPV-54). Sixteen of these women did not have CIN, 3 had CIN 1, and 1 had CIN 2/3.

Clinical performance of generic HPV assays. Using CIN 2/3 as our primary endpoint, the clinical performance of Amplicor HPV, HC-2, and LA, considering only 13 HR genotypes, was compared for all women and for women ≥30 years old (Table 3). The sensitivities of the three assays were similar irrespective of the three cutoffs evaluated for Amplicor HPV. Adding 7 genotypes considered to be probable or possible HR types (LA 20 HR) to the 13 HR genotypes slightly improved the sensitivity of LA but decreased its specificity (3). Considering only women with CIN 2/3, only one sample was completely negative for HR HPV DNA in all HPV assays. A follow-up colposcopy and biopsy specimen were normal for this participant. Of the two samples with CIN 2/3 that were negative with HC-2, one tested negative with LA. The other woman was infected with HPV-51 and tested positive with Amplicor HPV. CIN 2/3 was confirmed at a follow-up colposcopy. HC-2 was also positive on follow-up. Of the three CIN 2/3 samples that were negative with Amplicor HPV, one was negative in the three HPV tests and two tested positive with HC-2. One of these women was infected with types 53 and 16 on the first visit. On two following visits, colposcopies with normal biopsy specimens were obtained and HC-2 testing was negative. The third woman was negative with LA at the first visit despite being HC-2 positive. Two follow-up colposcopies were normal, and repeat testing

TABLE 3. Clinical performance of three assays in the detection of HR HPV DNA in cervical specimens collected from women with ASC-US to identify women with CIN 2/3

Group and test[a]	Results [% (95% CI)] for:				
	Sensitivity	Specificity	PPV	NPV	Colposcopy referral rate
All women (n = 392)[b]					
HC-2	93.1 (77.0–99.2)	72.2 (67.4–76.5)	21.1 (14.9–29.0)	99.2 (97.1–100.0)	32.7 (28.2–39.0)
Amplicor 0.2	89.7 (72.8–97.2)	62.5 (57.5–52.4)	16.1 (11.1–22.5)	98.7 (96.1–99.7)	41.3 (36.6–46.3)
Amplicor 1.0	89.7 (72.8–97.2)	64.5 (59.4–69.2)	16.8 (11.7–23.5)	98.7 (96.2–99.7)	39.5 (34.8–44.5)
Amplicor 1.5	89.7 (72.8–97.2)	64.7 (59.7–69.5)	16.9 (11.7–23.6)	98.7 (96.2–99.7)	39.3 (34.6–44.2)
LA 13 HR	89.7 (72.8–97.2)	64.2 (59.1–69.0)	16.7 (11.6–23.4)	98.7 (96.2–99.7)	39.9 (35.2–45.8)
LA 20 HR	93.1 (77.0–99.2)	60.1 (54.9–65.0)	15.7 (11.0–21.9)	99.1 (96.5–99.9)	43.9 (39.1–48.8)
Women over 30 years of age (n = 301)[c]					
HC-2	91.3 (72.0–98.8)	75.2 (69.8–79.9)	23.3 (15.7–33.1)	99.1 (96.4–100.0)	29.9 (25.1–35.3)
Amplicor 0.2	87.0 (67.0–96.3)	68.4 (62.7–73.5)	18.5 (12.2–27.0)	98.5 (95.3–99.7)	35.9 (30.7–40.7)
Amplicor 1.0	87.0 (67.0–96.3)	70.1 (64.5–75.2)	19.4 (12.9–28.2)	98.5 (95.4–99.7)	34.2 (29.1–39.7)
Amplicor 1.5	87.0 (67.0–96.3)	70.5 (64.9–75.6)	19.6 (13.0–28.4)	98.5 (95.5–99.7)	33.9 (28.8–39.4)
LA 13 HR	87.0 (67.0–96.3)	69.1 (63.4–74.2)	18.9 (12.5–27.4)	98.5 (95.4–99.7)	35.2 (30.0–40.7)
LA 20 HR	91.3 (72.0–98.8)	63.3 (57.5–68.8)	17.1 (11.4–24.8)	98.3 (95.0–99.7)	40.5 (35.1–46.1)

[a] Four women (three over 30 years of age) had a diagnosis of CIN that could not be graded and were not included in the table. One was positive by HC-2, and two were positive with Amplicor with a cutoff at 0.2. See footnote *a* of Table 1 for abbreviations not listed here. Amplicor 0.2, 1.0, and 1.5, cutoffs used in Amplicor; LA 13 HR, linear array test considering the 13 genotypes targeted by the generic assays; LA 20 HR, linear array considering the 20 definitive/probable/possible HR types (3).
[b] Includes 29 women with CIN 2/3.
[c] Includes 23 women with CIN 2/3.

with HC-2, Amplicor HPV, and LA at the first follow-up visit was negative. All CIN 2/3 samples negative with one or both generic tests contained enough cellular DNA for testing.

As shown in Table 3, the highest specificity was obtained with HC-2. The specificity of Amplicor HPV was improved by using a higher detection cutoff. The negative predictive value of each assay was very high, at 99%. At any of the three cutoffs for Amplicor HPV, more women would have been referred to colposcopy than with HC-2 (Table 3). Using a newly proposed threshold of ≥2 with HC-2 (18), the sensitivity of HC-2 was similar to that of Amplicor HPV (26 of 29 CIN 2/3 cases [89.7%]; 95% CI, 72.8 to 97.2), the specificity increased (270 of 363 women with CIN of a lower grade than CIN 2/3 [74.4%]; 95% CI, 69.6 to 78.6), and the referral rate decreased to 30.3% (95% CI, 25.6 to 35.0).

The specificity of HPV generic assays improved when testing women over 30 years old (Table 3). In women over 30 years old, 209 (75.2%) of 278 women without CIN 2/3 tested negative with HC-2 compared to 53 (62.4%) of 85 women younger than 30 without CIN 2/3 (P = 0.03). Similarly, 190 (68.4%) of 278 women over 30 years old and without CIN 2/3 tested negative with HC-2 compared to 37 (43.5%) of 85 women younger than 30 and without CIN 2/3 (P = 0.001). The rates of detection of HR HPV DNA in women over 30 years of age without CIN were not significantly different between generic assays: 76 of 250 women (30.4%; 95% CI, 25.0 to 36.4) for Amplicor HPV versus 57 of 250 women (22.8%; 95% CI, 25.0 to 36.4%) for HC-2 (P = 0.07).

Use of generic HPV assays in the follow-up of women with ASC-US. Of the 316 women with a normal colposcopy at accrual, 126 participants had a positive result in at least one of the HPV generic assays (81 with HC-2 and 113 with Amplicor HPV). A colposcopy was repeated in the next 2 years following accrual for 75 of these 316 women, including 67 women with a positive HC-2 test at accrual and 63 women with a positive Amplicor HPV test at accrual. Four of these 75 women had CIN 2/3 on follow-up. Only 47 women had a sample tested with HC-2 at the follow-up colposcopy: 31 (66.0%; 95% CI, 51.6 to 77.9) were positive with HC-2 at both visits at an average of 209 days apart (range, 76 to 372 days). Only 34 women had a sample tested with Amplicor HPV at the follow-up colposcopy: 23 (67.7%; 95% CI, 50.8 to 81.0) tested positive with Amplicor HPV at both visits at an average of 201 days apart (range, 126 to 295 days). There was persistence of at least one genotype for all women testing positive at both visits with one or both generic HPV assays. As shown in Table 4, CIN 2/3 was demonstrated at the second colposcopy for 4 women who tested positive for HR HPV at both visits with HC-2.

Of the 29 women with CIN 2/3 at accrual, two women with CIN 2 were followed prospectively without treatment and two were lost to follow-up. The 25 women who were treated re-

TABLE 4. Presence of CIN 2/3 in women with repeat colposcopy and HPV testing after an initial normal colposcopy and positive HR HPV detection assay

Assay and result at indicated time point	No. (%) of women with indicated histology result at 2nd colposcopy	
	With CIN 2/3	Without CIN 2/3
HC-2		
Positive at accrual	4	43
Positive at 2nd visit	4 (100)	27 (62.8)
Negative at 2nd visit	0 (0)	16 (37.2)
Amplicor HPV		
Positive at accrual	4	32
Positive at 2nd visit	2 (50)	21 (65.6)
Negative at 2nd visit	0 (0)	11 (34.4)

ceived conization (12), cryotherapy (7), loop electrosurgical excision procedure (4), or laser therapy (2). A complete clinical response was recorded for all 25 women with a mean follow-up of 419 ± 172 days (median, 373; range, 175 to 752). Of the 23 women who were HC-2-positive before treatment, two were not retested on follow-up while 16 women tested negative with HC-2 at a mean of 248.5 ± 180.8 days posttreatment. Of the 21 women who were positive by Amplicor HPV before treatment, 9 were not retested and 10 women tested negative with Amplicor at an average of 207.7 ± 111.6 days posttreatment. Women still positive for HR HPV by Amplicor HPV and/or HC-2 did not have residual CIN 2/3.

DISCUSSION

HR HPV DNA testing is a cost-effective triage method that is nearly as sensitive as immediate colposcopy, while keeping referrals low (12, 21). In this report, 80% of women with ASC-US did not have an underlying cervical lesion. In this evaluation, the clinical performance of two generic assays approved in Canada for the triage of women with ASC-US to colposcopy was equivalent. Only Amplicor HPV controls for the cellular content of samples with β-globin amplification. Yet only one sample tested negative for β-globin but was still positive for HR HPV DNA. Others have also reported that most cervical samples collected with a cytobrush could be analyzed for HPV detection (23, 27).

The sensitivity of Amplicor HPV for detection of CIN 2/3 in women with ASC-US obtained in the current study is similar to previous reports of 91% with a 1.5 cutoff (27) and 93% with a 2.2 cutoff (26, 27). It is unlikely that the three women with CIN 2/3 who were negative with Amplicor HPV were misclassified, since a second pathologist confirmed the diagnosis (8, 13). Several groups compared HC-2 and Amplicor HPV in different clinical settings and reported good levels of agreement for HR HPV DNA detection, from 78% to 89%, and kappa values from 0.61 to 0.78 (4, 11, 16, 17, 23, 27). Similar to the results of others, the intensity of the signal generated in HC-2 was significantly higher in samples that were also positive with Amplicor HPV (4). The agreement between Amplicor HPV and HC-2 increased with the CIN grade. The relatively low kappa value calculated in our study between tests on samples collected from women with CIN 2/3, despite a high level of agreement, is related to the small number of women with CIN 2/3 in our study, as well as the rare occurrence of having both assays negative in women with CIN 2/3.

Two studies besides this report focused primarily on the comparison of HC-2 and Amplicor HPV assays for the triage of women with ASC-US (11, 27). In the ASCUS-LSIL triage study (ALTS), Amplicor HPV was applied on archived aliquots of specimens that were contained in sample transport medium after being collected from 3,277 women (27). The overall agreement between tests in that study was comparable to our own results (27). The higher specificity of Amplicor HPV and the lower referral rate obtained in the current report compared to the improvement of specificity and referral rate in ALTS could be related to the greater age of women recruited in our study. Older women without CIN will be less likely than younger women to be infected with HPV (24). A second study of 271 women referred for ASC-US reported an agreement of 86.7%, with a kappa value at 0.73, between Amplicor HPV and HC-2 (11).

The rate of detection of HR HPV DNA with Amplicor HPV in our study was greater than with HC-2, especially among women less than 30 years of age and in women without CIN. In our study, raising the cutoff from 0.2 to 1.0 or 1.5 did not significantly alter the sensitivity of Amplicor HPV to detect CIN 2/3 but did decrease the referral rate of women to colposcopy. In two reports, using a cutoff of from 1.5 to 2.2 instead of the proposed cutoff of 0.2 did not significantly modify the sensitivity of Amplicor HPV for CIN 2/3 but did improve its specificity (26, 27). The smaller improvement of specificity by using an increased cutoff point with Amplicor HPV in our study compared to the improvement of specificity in ALTS may be attributable to the difference of the median age between cohorts (25 years in ALTS versus 39 years in the current study), since HPV infection has been shown to decline with age (24). Using Amplicor HPV in women older than 30 years of age also increased the specificity of Amplicor. Cross-reactivity of HC-2 and Amplicor HPV with several genotypes not targeted by the assay is well known (4, 5, 11, 16, 17, 23, 27). Our study adds genotypes 54, 66, and 72 to the list of cross-reactive types with Amplicor HPV. Since the analytical sensitivity of Amplicor HPV is greater than that of the LA test, we cannot exclude that samples reactive only with Amplicor contain one of the 13 high-risk genotypes.

Repeating HR HPV testing in women initially positive for HR HPV but with a normal colposcopy allows the identification of women with underlying CIN 2/3 that was undetected at the first colposcopy (28). Four women in our study with repeated positive HR HPV tests with HC-2 or Amplicor HPV had an underlying CIN 2/3 lesion that had been missed at the first colposcopic evaluation. We could not evaluate the performance of the generic assays for the screening of residual disease after treatment, as all 25 women treated for CIN 2/3 with adequate follow-up responded to therapy. Up to 20% of these women remain infected with HR HPV after treatment, as reported by others (1, 19, 20, 22).

Our study has several strengths. We recruited prospectively women with well-defined inclusion criteria. All participants were referred to colposcopy because of a previous cytology smear with ASC-US. HPV assays were applied prospectively on the same fresh cytobrush sample and performed in parallel in a diagnostic setting. HPV DNA detection tests were standardized. Our laboratory had experience with the three assays evaluated. Discrepancies between HPV generic assays were evaluated with a standardized genotyping assay applied prospectively on all samples. A prospective follow-up was included for several participants to further ascertain cervical status. Our study was, however, limited by the absence of standardized management of participants for repeat HPV testing and colposcopic evaluation of women without lesions at the initial colposcopy and by loss to follow-up. The assessment of these participants was limited to a retrospective review of the clinical charts. There was also a small number of participants with CIN 2/3. The present study population is a referred group of women with an inflated disease prevalence and a consequent selection bias. For these reasons, conclusions from this study can be applied only to women with ASC-US.

Both the HC-2 and the HPV Amplicor test will help to

reduce the number of unnecessary colposcopies in women with ASC-US. When applied to real-life situations, their performance was equivalent to detect CIN 2/3 in women with ASC-US. Still, HR HPV infection is detected more frequently with PCR than with HC-2, especially in women <30 years of age. HPV-positive women with ASC-US and a normal first colposcopy benefit from repeat HPV testing with either test.

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REFERENCES

1. **Bais, A. G., et al.** 2009. Post-treatment CIN: randomised clinical trial using hrHPV testing for prediction of residual/recurrent disease. Int. J. Cancer. **124:**889–895.
2. **Bosch, F. X., A. Lorincz, N. Munoz, C. J. L. M. Meijer, and K. V. Shah.** 2002. The causal relation between human papillomavirus and cervical cancer. J. Clin. Pathol. **55:**244–265.
3. **Bouvard, V., et al.** 2009. A review of human carcinogens. Part B. Biological agents. Lancet Oncol. **10:**321–322.
4. **Carozzi, F., et al.** 2007. Agreement between the AMPLICOR human papillomavirus test and the Hybrid Capture 2 assay in detection of high-risk human papillomavirus and diagnosis of biopsy-confirmed high-grade cervical disease. J. Clin. Microbiol. **45:**364–369.
5. **Castle, P. E., et al.** 2008. Human papillomavirus genotype specificity of hybrid capture 2. J. Clin. Microbiol. **46:**2595–2604.
6. **Coutlee, F., et al.** 2006. Enhanced detection and typing of human papillomavirus DNA in anogenital samples with PGMY primers and the Linear Array HPV genotyping test. J. Clin. Microbiol. **44:**1998–2006.
7. **Coutlee, F., et al.** 2007. Confirmatory real-time PCR assay for human papillomavirus (HPV) type 52 infection in anogenital specimens screened for HPV infection with the linear array HPV genotyping test. J. Clin. Microbiol. **45:**3821–3823.
8. **Ferenczy, A., D. Choukroun, and J. Arseneau.** 1996. Loop electrosurgical excision procedure for squamous intraepithelial lesions of the cervix: advantages and potential pitfalls. Obstet. Gynecol. **87:**332–337.
9. **Franco, E. L., and D. M. Harper.** 2005. Vaccination against human papillomavirus infection: a new paradigm in cervical cancer control. Vaccine **23:** 2388–2394.
10. **Gravitt, P. E., et al.** 2008. New technologies in cervical cancer screening. Vaccine **26:**K42–K52.
11. **Halfon, P., et al.** 2007. Prospective evaluation of the Hybrid Capture 2 and AMPLICOR human papillomavirus (HPV) tests for detection of 13 high-risk HPV genotypes in atypical squamous cells of uncertain significance. J. Clin. Microbiol. **45:**313–316.
12. **Kim, J. J., T. C. Wright, and S. J. Goldie.** 2002. Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. JAMA **287:**2382–2390.
13. **Mayrand, M. H., et al.** 2007. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N. Engl. J. Med. **357:**1579–1588.
14. **Mo, L. Z., et al.** 2008. Comparison of AMPLICOR and Hybrid Capture II assays for high risk HPV detection in normal and abnormal liquid-based cytology: use of INNO-LiPA genotyping assay to screen the discordant results. J. Clin. Virol. **41:**104–110.
15. **Monsonego, J., et al.** 2005. Performance of the Roche AMPLICOR human papillomavirus (HPV) test in prediction of cervical intraepithelial neoplasia (CIN) in women with abnormal PAP smear. Gynecol. Oncol. **99:**160–168.
16. **Poljak, M., K. Fujs, K. Seme, B. J. Kocjan, and E. Vrtacnik Bokal.** 2005. Retrospective and prospective evaluation of the Amplicor HPV test for detection of 13 high-risk human papillomavirus genotypes on 862 clinical samples. Acta Dermatovenerol. Alp. Panonica Adriat. **14:**147–152.
17. **Sandri, M. T., et al.** 2006. Comparison of the Digene HC2 assay and the Roche AMPLICOR human papillomavirus (HPV) test for detection of high-risk HPV genotypes in cervical samples. J. Clin. Microbiol. **44:**2141–2146.
18. **Sargent, A., et al.** 2010. Optimal threshold for a positive Hybrid Capture 2 test for detection of human papillomavirus: data from the ARTISTIC trial. J. Clin. Microbiol. **48:**554–558.
19. **Sarian, L. O., S. F. Derchain, D. R. Pitta, S. S. Morais, and S. H. Rabelo Santos.** 2004. Factors associated with HPV persistence after treatment for high-grade cervical intra-epithelial neoplasia with large loop excision of the transformation zone (LLETZ). J. Clin. Virol. **31:**270–274.
20. **Sarian, L. O., et al.** 2004. HPV DNA test and Pap smear in detection of residual and recurrent disease following loop electrosurgical excision procedure of high-grade cervical intraepithelial neoplasia. Gynecol. Oncol. **94:** 181–186.
21. **Solomon, D., M. Schiffman, R. Tarone, and ALTS Study group.** 2001. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J. Natl. Cancer Inst. **93:**293–299.
22. **Song, S. H., et al.** 2006. Persistent HPV infection after colonization in patients with negative margins. Gynecol. Oncol. **101:**418–422.
23. **Stevens, M. P., et al.** 2007. Comparison of the Digene Hybrid Capture 2 assay and Roche AMPLICOR and Linear Array human papillomavirus (HPV) tests in detecting high-risk HPV genotypes in specimens from women with previous abnormal Pap smear results. J. Clin. Microbiol. **45:**2130–2137.
24. **Trottier, H., and E. L. Franco.** 2006. The epidemiology of genital human papillomavirus infection. Vaccine **24:**S1–S15.
25. **van Ham, M. A., et al.** 2005. Comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. J. Clin. Microbiol. **43:**2662–2667.
26. **Wahlstrom, C., T. Iftner, J. Dillner, and L. Dillner.** 2007. Population-based study of screening test performance indices of three human papillomavirus DNA tests. J. Med. Virol. **79:**1169–1175.
27. **Wentzensen, N., P. E. Gravitt, D. Solomon, C. M. Wheeler, and P. E. Castle.** 2009. A study of Amplicor human papillomavirus DNA detection in the atypical squamous cells of undetermined significance-low-grade squamous intraepithelial lesion triage study. Cancer Epidemiol. Biomarkers Prev. **18:** 1341–1349.
28. **Wright, T. C. J., et al.** 2007. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am. J. Obstet. Gynecol. **197:**346–355.