A Comparison of the Roche Cobas HPV Test With the Hybrid Capture 2 Test for the Detection of High-Risk Human Papillomavirus Genotypes

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• ***Context.***—**All Food and Drug Administration–approved methods in the United States for human papillomavirus testing including the Hybrid Capture 2 human papillomavirus assay and the Roche cobas human papillomavirus test are approved for cytology specimens collected into ThinPrep media but not for specimens collected into SurePath solution.**

Objective.—**To compare the performance of the Roche cobas and Hybrid Capture 2 tests for the detection of high-risk human papillomavirus using both ThinPrep and SurePath preparations as part of a validation study.**

Design.—**One thousand three hundred seventy-one liquid-based cytology samples, including 1122 SurePath and 249 ThinPrep specimens, were tested for high-risk human papillomavirus DNA using the Roche cobas human papillomavirus test and the Hybrid Capture 2 human papillomavirus assay. For cases with discrepant results, confirmatory testing was performed using Linear Array human papillomavirus testing.**

Results.—**One hundred and fifty-six (11.38%) and 184 (13.42%) of the 1371 specimens tested positive for high-risk human papillomavirus DNA using the Hybrid Capture 2 human papillomavirus assay and Roche cobas human papillomavirus assay, respectively. In addition, 1289 (94.0%) of 1371 specimens demonstrated concordant high-risk human papillomavirus results with a κ value of 0.72 (95% confidence interval, 065–0.78). There was no statistically significant difference in the percentage of positive high-risk human papillomavirus results between the 2 liquid-based preparations with either assay. Discordant results between the 2 assays were noted in 82 of 1371 cases (6%). Twenty-seven of 82 cases (32.9%) were Hybrid Capture 2 positive/Roche cobas negative and 55 of 82 cases (67.1%) were Roche cobas positive/Hybrid Capture 2 negative. Two of 20 Hybrid Capture 2–positive/Roche cobas–negative cases (10%) and 26 of 37 Roche cobas–positive/Hybrid Capture 2–negative cases (70%) tested positive for high-risk human papillomavirus by Linear Array.**

Conclusions.—**Both assays showed good agreement and excellent specificity with either ThinPrep or SurePath preparations. The number of discordant results was relatively small. The performance of both assays was similar for ThinPrep specimens, but the Roche cobas test demonstrated higher sensitivity with SurePath specimens.**

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The causal relationship between human papillomavirus (HPV) and cervical cancer and its precursors has been well established.[1] To date, more than 150 HPV genotypes have been identified, and approximately 60 of them are known to infect the human genital tract including the uterine cervix.[2] Among the latter, 12 HPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are considered carcinogenic or high risk.[3] In addition, 5 genotypes (HPV 26, 53, 66, 68, and 72) are considered possibly carcinogenic as their role in cervical carcinogenesis is unclear.[4,5] The reported clinical sensitivity of high-risk HPV (hrHPV) DNA testing for the detection of cervical intraepithelial neoplasia 2 or greater is approximately 95% in a screening population.[6,7] This has led to the increased use of hrHPV DNA testing in conjunction with cervicovaginal cytology. In the United States, hrHPV DNA testing is currently recommended for the triage of all women with equivocal cytology, and in conjunction with cytology testing for women 30 years of age or older.[8]

The Hybrid Capture 2 (HC2) HPV assay (Qiagen Corporation, Gaithersburg, Maryland) was the first commercially available assay approved by the Food and Drug Administration for the detection of 13 hrHPV genotypes using ThinPrep liquid-based (LB) Papanicolaou (Pap) test specimens (Hologic, Boxborough, Massachusetts). In April 2011, the Roche cobas HPV test (Roche Molecular Systems, Pleasanton, California) was approved by the Food and Drug Administration for detecting hrHPV. Based on real-time polymerase chain reaction technology, the cobas assay identifies HPV 16 and HPV 18 genotypes separately, as well

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as detecting a pool of hrHPV genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and also HPV 66. The cobas assay is based on 2 major processes: (1) automated specimen preparation to simultaneously extract HPV and cellular DNA; and (2) polymerase chain reaction amplification of target DNA sequences using both HPV- and β-globin–specific primer pairs and real-time detection of cleaved fluorescent-labeled HPV- and β-globin–specific oligonucleotide detection probes. The cobas test has been validated in several studies using either ThinPrep LB Pap specimens or specimens collected in specimen transport medium (Qiagen), but neither cobas nor HC2 has been approved for use with SurePath LB Pap specimens (BD Diagnostic, Burlington, Massachusetts).[9–14] As part of the validation process, we compared the performance of the cobas HPV test with our in-house validated HC2 assay for the detection of hrHPV DNA using both SurePath and ThinPrep preparations.

MATERIALS AND METHODS

The protocol of this study was approved by Yale University's Institutional Review Board (New Haven, Connecticut).

Specimen Collection and Storage

During a 2-week period, all LB Pap tests with a concurrent request for hrHPV DNA testing were included in this study. The request for hrHPV DNA testing was either reflex testing triggered by abnormal cytologic interpretations or cotesting with the Pap test regardless of the cytologic result. The specimens consisted of both ThinPrep and SurePath specimens. The specimens were collected using PreservCyt solution (Hologic) and SurePath preservative solution (BD Diagnostic) for ThinPrep and SurePath specimens, respectively. The standard protocol used for obtaining samples for cytologic evaluation only was used for both cytologic evaluation and hrHPV DNA testing. All samples, stored at room temperature for up to 21 days, were tested using both HC2 and cobas HPV tests. The requests for hrHPV DNA testing included both reflex testing (approximately 40%) for atypical squamous cells of undetermined significance in women of all ages, and cotesting (approximately 60%) with cervical cytology in women aged 30 or older. More than 90% of the women in this study were aged 21 or older.

Cobas 4800 Assay

Before the cytology specimen was prepared, 1-mL aliquots of SurePath or PreservCyt fluid were transferred to 13-mL bar-coded round-bottom tubes provided by the manufacturer. The cobas test was carried out according to the manufacturer's protocol.[15] DNA extraction was accomplished using the fully automated cobas x 480 instrument. Briefly, specimens were digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids, along with the β-globin DNA serving as process control, were purified through adsorption to magnetic glass particles, washed, and finally separated from these particles, making them ready for polymerase chain reaction amplification and detection.

The amplification plates were then manually transferred to the cobas z 480 analyzer for real-time polymerase chain reaction amplification of hrHPV and β-globin DNA. The cobas HPV test uses primers that define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the master mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Fluorescent oligonucleotide probes bind to the polymorphic regions within the sequence defined by these primers. An additional primer pair and probe targeting the human β-globin gene (330-bp amplicon) was included as an internal control to provide a measure of specimen adequacy as well as to monitor the quality of the extraction and amplification process. Interpretation of the amplification results was carried out using proprietary software provided with the cobas z 480 analyzer. The cycle threshold cutoffs were set at 40.5 for HPV 16 and at 40 for HPV 18 as well as the remaining 12 hrHPV genotypes.[14] Positive and negative controls were included in each run.

HC2 Assay

The assay identifies 13 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). It uses signal amplification technology to detect DNA-RNA hybrids and does not include any DNA amplification process. An aliquot of 2 or 4 mL of SurePath or PreservCyt fluid, respectively, was obtained from the residual fluid after cytology preparation and mixed with a denaturing agent. The denatured samples plus both positive and negative controls were then processed using the Rapid Capture System (Qiagen) for all steps including hybridization before signal detection. The latter was accomplished using a DML 2000 luminometer. Cutoff values for each run were calculated based on the relative light units (RLUs) of the positive and negative controls and the results were reported as relative light unit to cutoff ratios. Positive and negative results were defined by relative light unit to cutoff ratios greater than or equal to 2.5 and less than 1, respectively. Samples with relative light unit to cutoff ratios greater than or equal to 1 and less than 2.5 were classified as equivocal and retesting of the samples in duplicate was required. The samples were considered positive if the results were greater than or equal to 1 in 2 out of the 3 tests (the initial and the 2 retests).

Linear Array HPV Test

For cases with discrepant test results between the cobas and HC2 assays, confirmatory testing and HPV genotyping were performed using Linear Array (LA) HPV testing (Roche Molecular Systems). Briefly, specimen preparation was performed by loading 1 mL of the cytology sample onto the cobas x 480 instrument for automated extraction; samples collected in SurePath preservative fluid were pretreated with high heat and an equal volume of proprietary buffer to reverse any potential DNA cross-linking prior to loading onto the cobas x 480. A 10-μL 1 M tris-HCL buffer, pH 7.4, was added to the eluted extract from the cobas x 480. The HPV LA was carried out according to the manufacturer's protocol available within the package insert and scored per manufacturer's recommendation. To reduce the chance of a user read error, a research software program, HPV StripScan (Roche Molecular Systems), was used to confirm HPV LA genotypes.

Statistical Analysis

Statistical analysis was performed by using the χ^2 test; statistical significance was set at a level of .05 or less. To determine the level of agreement between the 2 tests, the κ coefficients were estimated; a κ coefficient of 0.00 to 0.20 indicates poor agreement, 0.21 to 0.40 indicates fair agreement, 0.41 to 0.6 indicates moderate agreement, 0.61 to 0.80 indicates good agreement, and 0.81 to 1.00 indicates excellent agreement.[16] We also calculated the sensitivity, specificity, and positive and negative predictive values of both cobas and HC2 assays based on the following criteria. A sample was considered a true negative if results of both the cobas and HC2 assays were negative, or if the results of the LA test were negative for cases with discordant results. Similarly, a sample was considered positive if the results of both cobas and HC2 assays were positive, or if the results of the LA test were positive for cases with discordant results.

RESULTS

During the study period, a total of 1371 LB gynecologic preparations, including 1122 SurePath and 249 ThinPrep specimens, were tested for hrHPV DNA using both cobas and HC2 assays (Table 1). Overall, 156 (11.38%) and 184 (13.42%) of 1371 specimens tested positive for hrHPV DNA using HC2 and cobas assays, respectively. The cobas test

Table 1. Comparison of Cobas 4800 and Hybrid Capture 2 (HC2) Assays for the Detection of High-Risk Human Papillomavirus According to Type of Liquid-Based Preparations

	SurePath	ThinPrep	Total
Cobas, No. (%)			
Positive	149 (13.3)	35 (14.1)	**184** (13.4)
Negative	973 (86.7)	214 (85.9)	**1187** (86.6)
HC2, No. (%)			
Positive	127 (11.3)	29 (11.7)	**156** (11.4)
Negative	995 (88.7)	220 (88.4)	**1215** (88.6)
Total	**1122**	**249**	**1371**

identified 149 (13.3%) of 1122 SurePath specimens and 35 (14.1%) of 249 ThinPrep specimens as hrHPV positive, whereas the HC2 assay identified 127 (11.3%) of 1122 SurePath and 29 (11.7%) of 249 ThinPrep specimens as hrHPV positive. There were no statistically significant differences in the percentages of positive hrHPV results between the 2 LB preparations with either assay ($P = .17$).

Overall, 1289 (94.0%) of 1371 specimens demonstrated concordant hrHPV results (Table 2). Although a higher percentage of ThinPrep specimens than SurePath specimens demonstrated concordant hrHPV results (95.2% versus 93.8%), the difference was not statistically significant ($P = .63$). Therefore, both assays performed similarly, with no significant difference in the number of positives and negatives identified by each assay for both LB preparations. For LB preparations overall, the κ coefficient was 0.72 (95% confidence interval [CI], 0.67–0.78). The individual κ coefficients were 0.79 (95% CI, 0.67–0.90) for ThinPrep and 0.71 (95% CI, 0.65–0.78) for SurePath, indicating a good agreement between the 2 preparations.

Discordant results between the 2 assays were noted in 82 of 1371 cases (6%); of these 82 cases, 27 (32.9%) were HC2 positive/cobas negative, and 55 (67.1%) were cobas positive/HC2 negative. Linear Array HPV test results were available in 57 cases; the remaining 25 cases did not have adequate material available for testing. Of the samples tested by LA, 10% (2 of 20) of HC2-positive/cobas-negative discrepant cases and 70.2% (26 of 37) of cobas-positive/HC2-negative cases were positive for hrHPV by LA (Figure). In contrast, 35% (7 of 20) and 55% (11 of 20) of HC2-positive/cobas-negative discrepant cases resulted in the detection of only low-risk HPV genotypes or HPV-negative results by LA, respectively, compared with only 13.5% (5 of 37) and 16.2% (6 of 37) for the cobas-positive/HC2-negative samples. Of the 7 HC2-positive/cobas-negative and 5 cobas-positive/HC2-negative cases that were positive for low-risk HPV by LA, some were positive for more than 1 low-risk HPV genotype, but there was not one low-risk HPV genotype captured in the majority of either HC2-positive/cobas-negative or cobas-positive/HC2-negative groups (Table 3).

Overall, for both LB preparations, the prevalence for hrHPV infection in the study population was 12.3% (168 of 1371; 95% CI, 10.6%–14.2%); specifically, 12.2% (137 of 1122; 95% CI, 10.4%–14.4%) and 12.6% (31 of 249; 95% CI, 8.9%–17.6%) among women with SurePath and ThinPrep preparations, respectively. Table 4 summarizes the performance of cobas and HC2 assays in the detection of hrHPV according to different LB preparations. Both cobas and HC2 assays demonstrated similar and excellent analytical specificity and negative predictive value for detecting hrHPV DNA with both LB preparations. Both cobas and HC2 assays demonstrated similar sensitivity for hrHPV with ThinPrep preparation. However, with SurePath preparation, the cobas assay demonstrated a higher sensitivity and positive predictive value for detecting hrHPV compared with the HC2 assay.

COMMENT

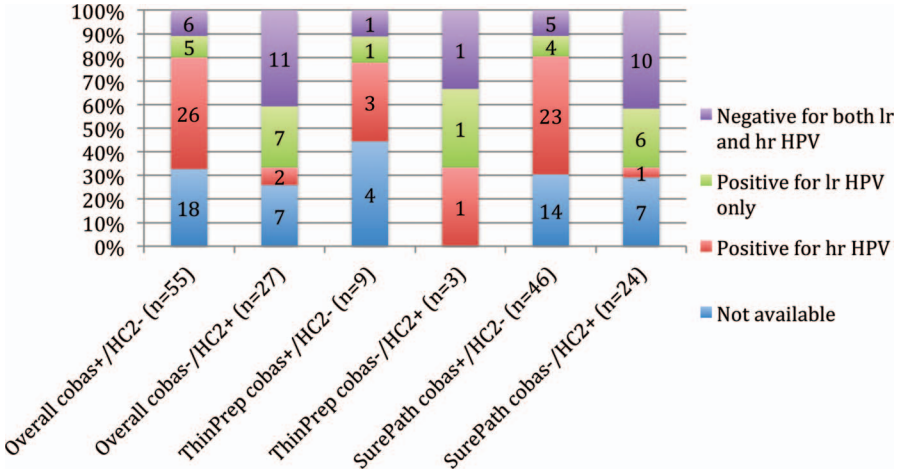
In the summer of 2012, our laboratory decided to replace the HC2 assay with the cobas HPV test for the detection of hrHPV DNA. Before implementing any new testing platform, both Clinical Laboratory Improvement Amendments[17] and the College of American Pathologists regulations require the laboratory to demonstrate that the new platform performs as well as, if not better than, the existing one.[18] Therefore, a validation study was performed to compare the performance of the 2 assays in detecting hrHPV DNA using both SurePath and ThinPrep LB preparations. The prevalence of hrHPV was 12.3% (168 of 1371) in the current study. Although our result is similar to the prevalence of hrHPV infection reported in the ATHENA study (12.6%), in which the study population consisted of women age 21 years or older undergoing routine screening,[19] it is not directly comparable, as we included cases that were cotested during routine screening in women 30 years and older, as well as cases with reflex testing triggered by other abnormal cytologic interpretations in addition to atypical squamous cells of undetermined significance.

Overall, both assays were comparable, with 94% agreement and a κ coefficient of 0.72. Although a higher percentage of ThinPrep specimens demonstrated concordant hrHPV results compared with SurePath specimens (95.2% versus 93.8%, respectively) with both assays, the difference was not statistically significant.

The cobas assay detected a higher rate of hrHPV than the HC2 assay for both LB preparations. The HC2 assay detects 13 hrHPV genotypes, whereas the cobas assay detects the same 13 hrHPV genotypes plus HPV 66. However, this alone would not explain the higher positive hrHPV rate with the cobas assay observed in the current study, as only 2 cobas-positive/HC2-negative cases tested positive for HPV 66 by the LA assay (result not shown). A plausible explanation could be that prealiquot specimens were used in the cobas assay whereas postaliquot specimens were used in the HC2 assay. However, it has been shown that the

Table 2. Concordance Analysis for the Detection of Human Papillomavirus by Hybrid Capture 2 (HC2) and Cobas 4800 Assays Based on Type of Liquid-Based Preparation

Type of Specimen	Both Tests Negative, No. (%)	Both Tests Positive, No. (%)	Discordant Results, No. (%)	HC2 Positive/Cobas Negative, No. (%)	Cobas Positive/HC2 Negative, No. (%)
SurePath (n = 1122)	949 (84.6)	103 (9.2)	70 (6.2)	24 (34.3)	46 (65.7)
ThinPrep (n = 249)	211 (84.7)	26 (10.4)	12 (4.8)	3 (25)	9 (75)
Total (N = 1371)	1160 (84.6)	129 (9.4)	82 (6.0)	27 (32.9)	55 (67.1)



Genotype distribution of discordant samples with the Linear Array genotyping test. Abbreviations: HC2, Hybrid Capture 2 assay; HPV, human papillomavirus; hr, high risk; lr, low risk; +, positive for HPV; −, negative for HPV.

sensitivity for detecting hrHPV using postaliquot specimens obtained from residual fluids after processing for SurePath cytology is comparable with the sensitivity using specimens collected at the time of cytology specimen processing with the Standard Transport Media kit (Qiagen).[20] Based on the ATHENA study, a slightly higher positive hrHPV rate was also noted with the cobas assay compared with the HC2 assay (32.6% versus 31.5%) among women with atypical squamous cells of undetermined significance,[14] whereas other studies observed a higher positive hrHPV rate with the HC2 assay compared with the cobas assay in specimens collected with Preservcyt Solution (Hologic).[10–12]

Approximately 80% (7 of 9) of HC2-positive/cobas-negative cases tested positive for low-risk HPV only by LA whereas 13.3% (4 of 30) of cobas-positive/HC2-negative cases did. This difference was statistically significant. Lindemann et al[11] also reported that significantly higher percentage of HC2-positive/cobas-negative cases tested positive for low-risk HPV genotypes only with the LA assay when compared with that of cobas-positive/HC2-negative cases (58% versus 14%). These observations suggest that the cobas test demonstrates less cross-reactivity with low-risk HPV genotypes than the HC2 assay. Cross-reactivity with low-risk HPV genotypes may result in false positives in women infected with only low-risk HPV genotypes and introduces the potential for unnecessary referral to colposcopy. Cross-reactivity of the HC2 test with low-risk HPV, including genotypes 42, 53, 54, 61, 67, 70, 73, and CP6108, has been reported previously.[21–24] Unlike the study of Lindemann et al,[11] which reported that more than one-third and more than one-fifth of LA low-risk HPV–positive results for the HC2-positive/cobas-negative cases were due to cross-reactivity with the low-risk HPV genotypes 53 and 42, respectively, the current study did not identify a subset of more common low-risk HPV genotypes.

Table 3. Frequency of Low-Risk Human Papillomavirus Genotypes Identified by the Linear Array Assay in Samples With Discordant Test Results Between the Hybrid Capture 2 (HC2) and Cobas 4800 Assays

Low-Risk HPV by Linear Array	HC2 Positive/ Cobas Negative	Cobas Positive/ HC2 Negative
42	1	1
53	1	0
54	1	1
61	1	0
62	0	1
67	1	0
70	1	0
73	1	2
83	1	2
CP6108	1	1
Total No. of specimens	**7[a]**	**5[a]**

[a] Some specimens demonstrated more than one genotype.

Overall, 70% (26 of 37) of cobas-positive/HC2-negative cases and 10% (2 of 20) of HC2-positive/cobas-negative cases tested positive for hrHPV by the LA assay. Similar results were also noted with either ThinPrep (60% [3 of 5] versus 33% [1 of 3]) or SurePath (72% [23 of 32] versus 6% [1 of 17]) preparations. The differences were statistically significant for SurePath cases but not for ThinPrep, indicating that the cobas HPV test identified more cases with hrHPV genotypes than the HC2 assay with SurePath specimens. These observations were also reflected in a higher sensitivity of the cobas assay to detect hrHPV compared with the HC2 assay with SurePath preparation. This is in contrast to a previous study, which compared the HC2 and cobas assay using ThinPrep preparation and reported a higher sensitivity with the HC2 assay.[12] One difference in the prior study is that all specimens were collected in PreservCyt transport medium, whereas only a minority of cases were collected in PreservCyt solution in the present study. Another notable difference between the previous and our current study is that the specimens were stored at –70°C for unknown durations before being tested in the former study, whereas the specimens in the present study were stored at room temperature and were analyzed within 1 week after collection. Another plausible explanation for the differing study results is that the presence of formalin in the SurePath preservative solution may have resulted in cross-linking of DNA, thereby interfering with the polymerase chain reaction.

The relatively high number of cobas-positve/HC2-negative/LA-positive cases may be due to the absence of any hrHPV genotype at the level of detection for the HC2 assay or sample inadequacy of the samples used for HC2. Unlike the cobas HPV test, which uses the human β-globin gene as an internal control, the HC2 test lacks any measure of specimen adequacy, so the cause of a false-negative HC2 test result cannot be determined. This introduces the potential for the HC2 assay to report false-negative results.

Table 4. Operating Performance for High-Risk Human Papillomavirus Detection With Cobas 4800 and Hybrid Capture 2 (HC2) Assays

Type of LBP	Assay Method	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
SurePath	Cobas	93.3 (87.4–96.7)	99.9 (99.3–99.9)	99.2 (95.0–100.0)	99.0 (98.2–99.5)
	HC2	86.7 (79.0–92.0)	98.6 (97.5–99.2)	88.1 (80.6–93.1)	98.4 (97.3–99.0)
ThinPrep	Cobas	93.5 (77.1–98.9)	99.5 (97.0–100.0)	96.7 (80.9–99.8)	99.1 (96.3–99.8)
	HC2	93.1 (75.8–98.8)	98.6 (95.7–99.6)	90.0 (72.3–97.4)	99.0 (96.3–99.8)
Total	Cobas	93.4 (88.2–96.5)	99.8 (99.3–99.9)	98.7 (95.0–99.8)	99.1 (98.3–99.5)
	HC2	87.9 (81.3–92.5)	97.8 (96.8–98.5)	83.4 (76.5–88.7)	98.4 (97.6–99.1)

Abbreviations: CI, confidence interval; LBP, liquid-based preparation; NPV, negative predictive value; PPV, positive predictive value.

For the HC2 assay, each kit had the capacity to test up to 94 samples. If fewer than 88 samples were run, the remaining unused wells were discarded. For the cobas assay, 2 kit sizes were available, one for 94 samples and one for 22 samples. Similar to the HC2 assay, if fewer than 94 or 22 samples were run, the remaining unused wells were discarded. However, the availability of 2 different-sized test kits offered by the cobas assay allowed some degree of flexibility. The total time from sample preparation to processing and reporting results for the HC2 assay was 7 hours regardless of batch size, whereas the total time for the cobas assay was 5 hours and 3.5 hours for 94 and 22 samples, respectively. The actual hands-on time was 3.5 hours for the HC2 assay and 45 minutes for the cobas assay. After switching from the HC2 assay to the cobas assay, our turnaround time for hrHPV testing decreased by 40%, from 48 to 30 hours (results not shown).

In conclusion, our findings demonstrated a high degree of concordance between the cobas HPV test and the HC2 HPV assay. The analytical performance for detecting hrHPV genotypes between the cobas and HC2 assays with ThinPrep preparation was comparable; however, the cobas assay demonstrated higher sensitivity than the HC2 assay in the SurePath preparation with comparable specificity. The cobas assay can be readily adopted in a clinical laboratory setting for detecting hrHPV DNA using both LB preparations. Although this is outside the scope of the current study, the cobas assay has the added ability to identify individual HPV 16 and 18 genotypes, which may prove clinically useful for improving management of hrHPV 16- and 18-positive women.

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