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The Aptima HPV Assay Fulfills the Cross-Sectional Clinical and Reproducibility Criteria of International Guidelines for Human Papillomavirus Test Requirements for Cervical Screening

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The Aptima HPV assay (Hologic Gen-Probe, San Diego, CA) is an FDA-approved assay for detecting human papillomavirus (HPV) E6/E7 mRNA from 14 high-risk HPV types. This study evaluated the clinical performance of the Aptima HPV assay for cervical intraepithelial neoplasia of grade 2 or worse (CIN2+), relative to the high-risk HPV GP5+/GP6+ PCR, in a cross-sectional clinical equivalence analysis using the noninferiority score test with cervical samples from population-based screening, i.e., 69 cervical scraping samples from women with CIN2+ and 843 from women without evidence of CIN2+. In addition, intralaboratory reproducibility over time and interlaboratory agreement of the Aptima HPV assay results were assessed with another set of 548 cervical samples. The Aptima HPV assay showed a clinical sensitivity for CIN2+ of 94.2% (95% confidence interval [CI], 85.5 to 97.8%) and a clinical specificity for CIN2+ of 94.5% (95% CI, 92.8 to 95.9%); by comparison, these figures were 97.1% (95% CI, 89.1 to 99.3%) (67/69 samples) and 93.6% (95% CI, 91.7 to 95.0%) (785/839 samples), respectively, for GP5+/GP6+ PCR. The clinical sensitivity and specificity of the Aptima HPV assay were noninferior to those of GP5+/GP6+ PCR ($P = 0.039$ and 0.00016, respectively). In addition, high reproducibility of the Aptima HPV assay, as reflected by the intralaboratory reproducibility over time of 96.0% (95% CI, 94.4 to 97.3%) (526/548 samples; kappa = 0.89) and interlaboratory agreement of 96.7% (95% CI, 95.4 to 98.1%) (531/548 samples; kappa = 0.91), was found. Altogether, these data show that the Aptima HPV assay meets the cross-sectional clinical and reproducibility criteria of the international guidelines for HPV test requirements for cervical screening. Longitudinal data are needed to ensure that the long-term negative predictive value of this mRNA assay is similar to those of validated HPV DNA tests.

The Aptima HPV assay (Hologic Gen-Probe, San Diego, CA, USA) is an *in vitro* nucleic acid amplification test designed to detect human papillomavirus (HPV) E6/E7 mRNA from 14 high-risk HPV types (i.e., types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) as a pool in cervical samples collected in preservative medium (1, 2). The Aptima HPV assay is based on target capture following cell lysis, with subsequent transcription-mediated amplification and probe hybridization protection for the detection of E6/E7 mRNA expression in one measurement. The assay can be run on the fully automated Panther and TIGRIS direct tube sampling (DTS) systems (Hologic Gen-Probe, San Diego, CA) or the semiautomated DTS system (3). The Aptima HPV assay has been approved by the FDA for testing of women ≥21 years of age whose Pap tests show atypical squamous cells of undetermined significance (ASC-US) and for screening of women ≥30 years of age as an adjunct to Pap testing (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm278520.htm).

There is widespread interest in HPV testing as a primary tool for cervical screening given its higher sensitivity versus cytology for the detection of high-grade cervical intraepithelial neoplasia and cervical cancer (i.e., cervical intraepithelial neoplasia of grade 2 or worse [CIN2+]), consequently offering better protection against cervical precancer/cancer (4, 5). It is imperative, however, that HPV assays used for primary cervical screening have proven sufficient clinical performance for the detection of CIN2+ to allow effective detection of women with clinically meaningful cervical disease while minimizing the detection of women with transient HPV infections, to reduce unnecessary follow-up procedures (6). Based on the available data from large prospective screening studies, specific clinical standards for HPV DNA tests for primary cervical screening have been formulated by an international consortium (7). These guidelines can be used to assess the clinical performance of a candidate HPV test, relative to one of the two prototype HPV tests with proven good clinical performance in cervical screening (i.e., high-risk HPV Hybrid Capture 2 [HC2] or GP5+/GP6+ PCR), by a cross-sectional clinical equivalence analysis with a well-defined sample series collected in a screening setting (6). Resulting noninferiority analyses for clinical sensitivity and specificity subsequently allow clinical validation of candidate assays for screening purposes without the need for a large prospective screening study. Furthermore, since screening tests should perform robustly and technically accurately, assessment of the candidate tests for intralaboratory reproducibility over time and interlaboratory agreement is also an intrinsic part of the international guidelines (7).

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This study set out to clinically evaluate the Aptima HPV assay according to these international guidelines, with samples originating from a cervical screening cohort. The clinical performance of the Aptima HPV assay was determined relative to high-risk HPV GP5+/GP6+ PCR, and the intralaboratory reproducibility and interlaboratory agreement of the Aptima HPV assay were analyzed.

MATERIALS AND METHODS

Study population. A total of 912 archived cervical samples collected in PreservCyt liquid-based cytology medium (Hologic, Marlborough, MA) were used. These scraping samples were collected during the course of routine population-based screening and a screening trial (trial NTR2606 at http://www.trialregister.nl/) in the Utrecht and North Holland regions of The Netherlands. These samples were stored at −80°C prior to use. The series included a set of samples for clinical sensitivity analysis, with 69 scraping samples from women (median age, 39 years [range, 30 to 60 years]) who had histologically confirmed CIN2+ (i.e., 32 CIN2 cases, 33 CIN3 cases, and 4 squamous cell carcinoma cases). These cases were considered to be representative of CIN2+ detected by combined HPV and cytology screening; as such, 19 (28%) had a high-risk HPV GP5+/GP6+ PCR-positive normal Pap smear and 50 (72%) an abnormal Pap smear either positive or negative in a high-risk HPV GP5+/GP6+ PCR assay, including 7 cases with borderline or mild dyskaryosis and 43 with greater than mild dyskaryosis. The series also included a set of samples for clinical specificity analysis, including 843 consecutive scraping samples from women (median age, 41 years [range, 31 to 60 years]) who had normal cytology findings and were without evidence of CIN2+ within a 2-year follow-up period (referred to as controls). Informed consent was obtained from all study participants, and this study followed the ethical guidelines of the medical center.

In addition, 548 PreservCyt samples, of which 155 were positive by the high-risk HPV HC2 assay (Qiagen, Hilden, Germany), were used for intralaboratory reproducibility and interlaboratory agreement analyses. Three portions of the original scraping samples were independently subjected to the Aptima HPV assay. The first two portions were tested within the same laboratory (VU University Medical Center, Amsterdam, The Netherlands), with an interval of 4 weeks, and the third was tested in a different laboratory (University of Tübingen, Tübingen, Germany). Different assay lots were used in the different laboratory settings.

Aptima HPV assay. Cervical scraping samples were tested with the Aptima HPV assay according to the recommendations of the manufacturer (3). Briefly, a 1-ml aliquot of each PreservCyt sample was transferred to 2.9 ml of buffered detergent solution, and a 400-μl aliquot of the mixture was then tested on a semiautomated direct tube sampling (DTS) system (Gen-Probe). Assay results were interpreted on the basis of the signal/cutoff ratio for the analyte, and specimens with signal/cutoff ratios of ≥0.5 were considered positive.

GP5+/GP6+ PCR. DNA was extracted from the PreservCyt samples using magnetic beads (Macherey-Nagel, Düren, Germany) on a Microlab STARlet robotic system (Hamilton Robotics, Martinsried, Germany), according to the manufacturer's instructions. DNA was subjected to GP5+/GP6+ PCR followed by enzyme immunoassay (EIA) using an oligonucleotide probe cocktail for 14 high-risk HPV types (i.e., types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), as described previously (8). As a quality control for the presence of amplifiable DNA and the absence of PCR inhibitors in the isolated material, we performed a separate PCR for β-globin.

Genotyping assays. Genotyping of EIA-positive GP5+/GP6+ PCR products was performed with a reverse line blot (RLB) assay as described previously (9). The RLB assay uses type-specific oligonucleotide probes to detect and to differentiate high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and low-risk genotypes 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 67, 69, 70, 71 (CP8061), 72, 73, 81 (CP8304), 82 (MM4), IS39, 83 (MM7), 84 (MM8), 85, 86, Jc9710, and CP6108.

The linear array HPV genotyping test was performed with leftover DNA extracts according to the instructions of the manufacturer (Roche Molecular Diagnostics, Pleasanton, CA, USA). The linear array HPV genotyping test detects 37 mucosal HPV types, including high-risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 and low-risk genotypes 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), 82 (IS39), and CP6108.

Statistical analysis. The Aptima HPV assay analyses were performed in a blinded fashion, and results were then compared to GP5+/GP6+ PCR and histopathology/cytopathology data. Clinical sensitivity and specificity values of the Aptima HPV assay were compared with those of the GP5+/GP6+ PCR assay by noninferiority score testing using previously defined thresholds for noninferiority, i.e., relative sensitivity for CIN2+ of ≥90% and relative specificity for CIN2+ of ≥98% (7). For intralaboratory and interlaboratory agreement, a lower confidence limit of not less than 87% was used as the threshold (7). The level of agreement was determined by using the kappa statistic. R software was used for noninferiority score analysis. For other statistical computations, SPSS 15.0 software was used. The level of statistical significance was set at 0.05.

TABLE 1 Aptima HPV assay findings among cervical scraping samples collected in population-based screening, stratified by case-control status

Sample type and Aptima HPV assay result	No. with GP5+/GP6+ PCR result of:		Total no.
	Negative	Positive	
Control			
Negative	771	22	793
Positive	14	32	46
Total	785	54	839
Case (CIN2+)			
Negative	1[a]	3	4
Positive	1	64	65
Total	2	67	69

[a] Positive for HPV70 by the linear array HPV genotyping test.

RESULTS

Clinical sensitivity and specificity analyses. Valid Aptima HPV assay results were obtained for all 69 (100%) scraping samples from CIN2+ case subjects used for the sensitivity analysis and 839 (99.5%) of the scraping samples from control subjects without evidence of CIN2+ used for the specificity analysis. Of the 69 CIN2+ cases, 65 (94.2%) had positive Aptima HPV assay results (Table 1). This resulted in clinical sensitivity of the Aptima HPV assay for CIN2+ of 94.2% (95% confidence interval [CI], 85.5 to 97.8%). By comparison, the clinical sensitivity of GP5+/GP6+ PCR was 97.1% (95% CI, 89.1 to 99.3%) (67/69 cases). The four cases that did not score positive in the Aptima HPV assay included two CIN2 cases and one CIN3 case identified by a GP5+/GP6+ PCR-positive/cytology-negative screen test and one CIN3 case identified by a cytology-positive/GP5+/GP6+ PCR-negative screen test. The additional case that was negative by GP5+/GP6+ PCR but positive by the Aptima HPV assay involved a woman with CIN2 identified by abnormal cytology findings.

Of the 839 controls without evidence of CIN2+, 46 (5.5%) were Aptima HPV assay positive (Table 1). This corresponds to a clinical specificity for CIN2+ of 94.5% (95% CI, 92.8 to 95.9%). The CIN2+ specificity of GP5+/GP6+ PCR in this series was 93.6% (95% CI, 91.7 to 95.0%) (785/839 controls). Both clinical

TABLE 2 Discrepancy analysis among GP5+/GP6+ PCR-positive and Aptima HPV assay-negative cases and GP5+/GP6+ PCR-negative and Aptima HPV assay-positive cases by linear array HPV genotyping testing and genotyping of GP5+/GP6+ PCR products

Discrepant case no.	Results for:		HPV type(s) found by genotyping of:	
	GP5+/GP6+ PCR	Aptima HPV assay	Linear array HPV	GP5+/GP6+ PCR products
1	Positive	Negative	39, 58, 66	39, 58, 66
2	Positive	Negative	66	66
3	Positive	Negative	16	16
4	Negative	Positive	52	ND[a]

[a] ND, not done.

TABLE 3 Discrepancy analysis among GP5+/GP6+ PCR-positive and Aptima HPV assay-negative controls and GP5+/GP6+ PCR-negative and Aptima HPV assay-positive controls by linear array HPV genotyping testing and genotyping of GP5+/GP6+ PCR products

Discrepant control no.	Results for:		HPV type(s) found by genotyping of:	
	GP5+/GP6+ PCR	Aptima HPV assay	Linear array HPV	GP5+/GP6+ PCR products[a]
1	Positive	Negative	16	16
2	Positive	Negative	31	31
3	Positive	Negative	39	39
4	Positive	Negative	51	51
5	Positive	Negative	56, 70	56, 70
6	Positive	Negative	58	58
7	Positive	Negative	59	59
8	Positive	Negative	66	66
9	Positive	Negative	56, 73	56
10	Positive	Negative	18, 59, 83	18
11	Positive	Negative	35, 54, 62	35
12	Positive	Negative	39, 40, 53, 59	39, 40
13	Positive	Negative	45, 53	45
14	Positive	Negative	16, 53, 54	33
15	Positive	Negative	31, 58	51
16	Positive	Negative	61	16
17	Positive	Negative	61	16
18	Positive	Negative	HPVCP6108, 6, 51, 84, 52	45
19	Positive	Negative	Negative	31
20	Positive	Negative	Negative	59
21	Positive	Negative	Negative	66
22	Positive	Negative	Negative	66
23	Negative	Positive	Negative	ND
24	Negative	Positive	Negative	ND
25	Negative	Positive	Negative	ND
26	Negative	Positive	Negative	ND
27	Negative	Positive	Negative	ND
28	Negative	Positive	Negative	ND
29	Negative	Positive	Negative	ND
30	Negative	Positive	Negative	ND
31	Negative	Positive	Negative	ND
32	Negative	Positive	31	ND
33	Negative	Positive	39, 67	ND
34	Negative	Positive	45	ND
35	Negative	Positive	59	ND
36	Negative	Positive	62	ND

[a] ND, not done.

sensitivity and specificity values of the Aptima HPV assay were noninferior to those of the reference assay, i.e., relative clinical sensitivity for CIN2+ of 0.97 (95% CI, 0.90 to 1.04; $P = 0.039$) and relative clinical specificity for CIN2+ of 1.01 (95% CI, 0.99 to 1.04; $P = 0.00016$).

Discrepancy analysis. For discrepancy analysis of samples with discordant Aptima HPV assay versus GP5+/GP6+ PCR outcomes (i.e., 4 cases and 36 controls), we first performed genotyping of the GP5+/GP6+ PCR products of GP5+/GP6+ PCR-positive/Aptima HPV assay-negative samples using the reverse line blot genotyping method. In addition, to verify the GP5+/GP6+ PCR genotyping results and to further evaluate the samples that were Aptima HPV assay positive but GP5+/GP6+ PCR negative, leftover DNA extracts of all samples with discrepant results were subjected to the linear array HPV genotyping test. Among the case series (Table 2), all 3 Aptima HPV assay-negative but GP5+/GP6+ PCR-positive specimens showed genotypes in the linear array HPV genotyping test that were fully concordant with genotypes found by genotyping the GP5+/GP6+ PCR products (i.e., HPV16 [$n = 1$], HPV66 [$n = 1$], and HPV39/HPV58/HPV66 [$n = 1$]). E6/E7 mRNAs of all of these types are targets in the Aptima HPV assay. The one Aptima HPV assay-positive but GP5+/GP6+ PCR-negative case showed HPV52 in the linear array HPV genotyping test, and the single case that was negative in both the Aptima HPV assay and GP5+/GP6+ PCR showed HPV70 in the linear array HPV genotyping test.

Among the controls (Table 3), 22 were Aptima HPV assay negative and GP5+/GP6+ PCR positive and 14 were Aptima HPV assay positive and GP5+/GP6+ PCR negative. Of the 22 Aptima HPV assay-negative but GP5+/GP6+ PCR-positive specimens, the linear array HPV genotyping test found 16 samples (73%) positive for one or more high-risk HPV types, 2 were positive for only low-risk HPV types, and 4 were HPV negative by the linear array HPV genotyping test. The linear array HPV genotyping test and RLB assay showed concordant data (i.e., full matches in genotypes detected) for 8 controls, compatible data (i.e., at least one genotype in common) for 5 controls, and discordant data (i.e., no match in genotypes found) for 9 controls (including the 4 that were negative in the linear array HPV genotyping test). Of the 14 Aptima HPV assay-positive but GP5+/GP6+ PCR-negative specimens, 9 were negative in the linear array HPV genotyping test, 4 were positive for types that could have been detected by GP5+/GP6+ PCR (i.e., HPV31 [$n = 1$], HPV39 [$n = 1$], HPV45 [$n = 1$], and HPV59 [$n = 1$]), and 1 was positive for a genotype that is not targeted by the Aptima HPV assay and GP5+/GP6+ PCR (i.e., HPV62).

Intralaboratory reproducibility and interlaboratory agreement analyses. The intralaboratory reproducibility and interlaboratory agreement were assessed with another set of 548 scraping samples, of which 155 (28.2%) were high-risk HPV positive by HC2. Valid Aptima HPV assay results were obtained for 542 (98.9%), 529 (96.5%), and 548 (100%) of the specimens in laboratory 1/run 1, laboratory 1/run 2, and laboratory 2, respectively. After retesting of the specimens with initial invalid results, all samples generated valid test results, implying that the internal control

TABLE 4 Intralaboratory reproducibility over time[a]

Aptima HPV assay run 1 result	No. with run 2 result of:		Total no.
	Negative	Positive	
Negative	400	13	413
Positive	9	126	135
Total	409	139	548

[a] The overall HPV test agreement was 96.0% (95% CI, 94.4 to 97.3%) (526/548 samples), with a kappa value of 0.89.

TABLE 5 Interlaboratory agreement[a]

Aptima HPV assay laboratory 1 (run 1) result	No. with laboratory 2 result of:		Total no.
	Negative	Positive	
Negative	410	3	413
Positive	14	121	135
Total	424	124	548

[a] Laboratory 1 was VU University Medical Center (Amsterdam, The Netherlands), and laboratory 2 was University Hospital Tübingen (Tübingen, Germany). The overall HPV test agreement was 96.7% (95% CI, 95.4 to 98.1%) (531/548 samples), with a kappa value of 0.91. Similar overall agreement was observed when data from laboratory 1, run 2, were compared with data from laboratory 2, i.e., 95.1% (95% CI, 93.3 to 96.6%) (521/548 samples), with a kappa value of 0.87.

at initial testing was indicating assay failure rather than sample failure. The Aptima HPV assay displayed high reproducibility, as reflected by intralaboratory reproducibility over time of 96.0% (95% CI, 94.4 to 97.3%) (526/548 samples; kappa = 0.89) (Table 4) and interlaboratory agreement of 96.7% (95% CI, 95.4 to 98.1%) (531/548 samples; kappa = 0.91) (Table 5).

DISCUSSION

In this study, we compared the clinical performance of the Aptima HPV assay with that of GP5+/GP6+ PCR in a cohort of screening participants. The clinical sensitivity and specificity for CIN2+ of the Aptima HPV assay were noninferior to those of GP5+/GP6+ PCR using the predetermined thresholds of 90% and 98%, respectively, as set out by an international consortium (7). Furthermore, the assay displayed sufficient intralaboratory reproducibility over time and interlaboratory agreement, with both lower confidence limits of percent agreement being much higher than 87% and the corresponding kappa values being greater than 0.5, in line with the proposed guidelines (7).

In the current study, the reference test was the GP5+/GP6+ PCR. Both GP5+/GP6+ PCR and HC2 have been clinically validated in randomized trials (7), and, like HC2, the GP5+/GP6+ PCR can be used for cross-sectional clinical validation of candidate HPV tests (10, 11). Collectively, our data indicate that the Aptima HPV assay meets the clinical and reproducibility criteria for HPV test requirements for primary cervical screening. It should be noted, however, that it has been stated that the clinical equivalence criteria based on relative cross-sectional accuracy are for HPV DNA tests and may not necessarily be valid for other molecular markers, such as overexpressed proteins or viral RNA (4). Since no long-term natural history data exist for such biomolecules, longitudinal data on the low-risk period after a negative Aptima HPV assay result are desirable to define the optimal screening interval for this assay.

Our findings are fully in line with those of other studies that compared the clinical performance of the Aptima HPV assay with that of a variety of commercially available HPV assays for ASC-US/low-grade squamous intraepithelial lesion triage or primary screening (12–25). The current study adds because it addresses all aspects of the guidelines, including intralaboratory reproducibility over time and interlaboratory agreement of the Aptima HPV assay.

Among the case samples, one specimen was negative with both the Aptima HPV assay and GP5+/GP6+ PCR. The linear array HPV genotyping test revealed HPV70, a genotype that is not targeted by either assay. In addition, four discordant results between the Aptima HPV assay and GP5+/GP6+ PCR were seen in the case series (two CIN2 cases and two CIN3 cases). These mostly included cytologically negative for intraepithelial lesion or malignancy samples (3/4 cases), and the findings were not particularly related to a specific HPV type. The controls that were not detected by the Aptima HPV assay but were positive in the GP5+/GP6+ PCR assay may be explained by the absence of detectable mRNA while HPV DNA is measurable. The GP5+/GP6+ PCR and linear array HPV genotyping test revealed a lower level of agreement in genotyping results among discordant control samples, compared to case samples. This suggests that these controls harbor lower viral loads, which in part may also underlie the negative assay results with the Aptima HPV assay. Previous studies have shown that lower viral loads are less likely to coincide with detectable E6/E7 mRNA levels (25). Most of the controls that were detected by the Aptima HPV assay over the GP5+/GP6+ PCR were also negative by the linear array HPV genotyping test, which may reflect higher viral mRNA loads than DNA loads. One instance of discrepant findings for the Aptima HPV assay versus GP5+/GP6+ PCR among controls may be explained by cross-reactivity of the Aptima HPV assay with a nontargeted HPV type (e.g., HPV62). In total, our study indicates that the inherent diversity in assay chemistry and related possible minor differences at the analytical level between the Aptima HPV assay and GP5+/GP6+ PCR have no significant effects on their clinical performances.

In conclusion, our data indicate that the Aptima HPV assay, in a cross-sectional setting, performs clinically comparably to GP5+/GP6+ PCR and meets the cross-sectional criteria for clinical sensitivity and specificity for CIN2+, intralaboratory reproducibility over time, and interlaboratory agreement of the international guidelines for HPV test requirements for primary cervical screening (7). Longitudinal data are needed to ensure long-term negative predictive values similar to those for the validated HPV DNA tests GP5+/GP6+ PCR and HC2.

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